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Potential Immunomodulatory Effect of Anesthetic Drugs on Immune Response in Patients Undergoing Orthopedic Surgery

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بِسْمِ ٱللَّهِ ٱلرَّحْمَٰنِ ٱلرَّحِيمِ

يَرْفَعِ اللَّهُ الَّذِينَ آمَنُوا مِنكُمْ وَالَّذِينَ أُوتُوا الْعِلْمَ دَرَجَاتٍ وَاللَّهُ بِمَا تَعْمَلُونَ خَبِيرٌ

صدق الله العلي العظيم

سورة المجادلة الآية رقْم 11

DEDICATION

I dedicate this modest effort to my wife, the spirit of my parents, my family, my children, spirit of Prof. Dr. Hammadi Al Hilali and to those who taught me patience and success.

Khalid

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To all, please accept my truthful thanks

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Summary

Anesthesia is thought to be the main factor affecting patient postoperative outcomes. Anesthetics, surgical stress and trauma have a modulatory effect on immune response which can increase or decrease of immune mediators either by direct effect or by stimulation of neuroendocrine system. The mechanism of immune modulation is by disturbing the balance between pro-inflammatory and anti-inflammatory and other mediators.

The aim of this study is to evaluate the possible role of anesthetics and surgery on innate and adaptive immunity by measuring the levels of Interleukin -2 (IL-2), Tumor Necrosis Factor-α (TNF-α), Interferon-γ (IFN-γ) which are pro-inflammatory and Interleukin-10 (IL-10) an anti-inflammatory by Enzyme Linked Immune-sorbent assay (ELISA). Flow cytometry was used to determine Cluster of Differention CD4, CD8, CD16 and Monocyte Chemoatractant Protein MCP-1. C-reactive protein and white blood cell count were measured before and after surgery.

This study was conducted on 30 patients with Orthopedic surgeries and Arthroscopy 16 males (53.3%) and 14 females (64.7%) with the age ranged between 10-72 years old, these were recruited from orthopedic and rheumatology department of Al-Diwaniyah Teaching Hospital during period from January of 2018 to the end of April of 2018.

Three types of anesthesia were used, 10 patients anesthetized with general anesthetics, 10 patients with local and the last 10 patients with regional anesthesia with duration of anesthesia at 75 minutes (range 15-90 minutes).

Blood samples were collected at three different timing periods from each volunteer patient; the first is preoperatively 24 hours before surgery, the second sample was during surgery peri-operatively and the third sample 12 hours postoperatively.

In this study, there was a highly significant increase in level of C reactive protein after surgery compared with serum level preoperatively 2.65 (4.99) mg/L and 3.99 (5.64) mg/L respectively (P <0.001).

The results revealed a significant rise in neutrophil count after operation in comparison with its baseline level before operation, 6.74 ±2.89(cell/L) versus 5.62 ±2.75(cell/L) respectively (P < 0.001), while the level of lymphocytes showed significant decline following operation, 2.85 ±1.13(cell/L) versus 3.03 ±1.15(cell/L) respectively, (P <0.001). The level of monocyte and eosinophil also got significantly reduced after operation (P < 0.001), whereas, the level of basophil showed no significant alteration after operation (P = 0.687).

Observation of CD4 lymphocytes counts revealed significant rise during the time of anesthesia (42.23%); however it showed significant reduction post-operatively (37.12%), but the reduction did not reach baseline count. In addition, the observation of CD8 lymphocytes counts revealed a significant rise during the time of anesthesia (28.21%); however it showed significant reduction post-operatively (22.91%), but the reduction did not reach the baseline count.

Moreover, the results of CD16 Natural Killer cells (NK Cell) count revealed a significant rise during the time of anesthesia (21.05%); however, it showed a significant decrease post-operatively (13.35%), but the reduction did not reach the baseline count. Also it has been noticed that the level of MCP-1 got raised significantly in association with

anesthesia induction (13.24%); however it returned back to its baseline level following the surgery.

The current study showed that the count of CD4 lymphocytes, CD8 and CD16 lymphocytes before, perioperative and post-operatively, had no significant association with gender (p value > 0.05). It was also obvious, that the level of the chemotactic chemokine MCP-1 remains insignificantly altered before, within and after operation ($P > 0.05$).

Immune markers have been analyzed in relation to type of anesthesia and the results showed that the count of CD 4 lymphocyte, CD8 and CD16 NK cells did not vary significantly in relation to type of anesthesia, whether local, regional or general, in all situations; whether before, during or after operation ($P > 0.05$)

Regarding the correlation of immune marker with time duration of anesthesia, the results showed that neither immune cells, lymphocytes and natural killer cells, nor MCP-1 showed significant correlation with duration of anesthesia ($P > 0.05$),

The higher level of cytokine was IL-2 mainly post-operative median level (1257.7 pg/ml), and lower level was seen with IL-10 mainly pre-operative median level (36.08 pg/ml). Although the level of interleukin-2 (IL-2 p=0.393), interleukin-10 (IL-10 p=0.131), interferon-gamma (IFN-γ p=0.740) and tumor necrosis factor-alpha (TNF-α p=0.741), all showed no significant change in relation to time of anesthesia whether pre, peri and post-operative ($P > 0.05$)

The results which associate between cytokines serum level pre, peri and post-operatively with gender and age, showed no significant association between male and female, and there was no significant

correlation with age of patients (p>0.05) for all cytokines including IL-2, IL-10, IFN-γ and TNF-α.

The analysis of data to correlate the cytokines level (IL-2, IL-10, IFN-γ and TNF-α) with types of anesthetic drugs (general, local and regional anesthesia) showed no significant association between these cytokine level and type of anesthesia (P > 0.05).

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Abbreviations

Abbreviation	Key
ADCC	Antibody dependent cellular cytotoxicity.
APCs	Antigen Presenting cells.
α	Alpha.
β	Beta
CI	Confidence Interval.
CD $^{+}$ 4	Helper T-cell.
CD^{+}8	Cytotoxic T-cells.
Ch.	Chromosome.
CBP	Complete Blood Picture
CRP	C-Reactive Proteins.
cAMP	Cyclic adenosine monophosphate.
CBR	Central benzodiazepine receptor.
CBC	Complete blood counts.
CD	Cluster of differentiation.
CTL	Cytotoxic T-lymphocyte.
CCL2	Chemokine-C-motive Ligand 2.
CSIF	Cytokine synthesis inhibitory factors.
DM	Diabetes Mellitus.
ELISA	Enzyme-linked immunosorbent assay.

EDTA	Ethylene Diamine Tetra Acetic acid.
FITC	Flouro-isothiocyanate.
Fc	Fragment crystalizable.
FACS	Fluorescent activated cell sorting.
γ	Gamma.
GPI	Glycosyl-phosphotidylinositol.
GABA	Gamma amino-butyric acid.
HSA	Human Serum Albumin.
HLA	Human leukocyte antigen.
HT	Hypertension.
IgV	Immunoglobulin variable.
Ig	Immunoglobulin.
ICAM	Intracellular adhesion molecule.
IL	Interleukin.
iNOS	Inducible nitric oxide synthase.
IFN-γ	Interferon-gamma.
IFN-αR	Interferon alpha receptor.
ITAM	Immune-receptor tyrosine activation motife.
IQR	Inter-quartile rang.
JAK	Janus kinase.
KARs	Killer activated receptors.

KIRs	Killer inhibitory receptors.
LDC	Leukocyte differential counts.
LPS	Lipopolysaccharide.
LcK	Lymphocyte tyrosine kinase.
mRNA	Messenger ribonucleic acid.
MAPK	Mitogen-activated protein kinase.
MCAF	Monocyte chemotactic and activating factor.
MIP	Macrophage inflammatory protein.
MPS	Mononuclear phagocyte system.
MHC	Major Histocompatibility complex.
MCP-1	Monocyte chemoatractant protein-1.
ml	milliliter.
NO	Nitric Oxide.
NF-kB	Nuclear factor kappa.
Na cl	Sodium Chloride.
ng	Nano-gram.
NK	Natural Killer cells.
NMDA	N-methyl-D-aspartate.
OR	Odd Ratio.
OD	Optical Density.
PCR	Polymerase Chain Reaction.

PBR	Peripheral benzodiazepine receptor.
PMNs	Polymorph nuclear cells
pg	Protein gram.
PABA	Para-amino benzoic acid.
PF	Protective Fraction.
PAMPs	Pathogen associated molecular patterns.
PRR	Pattern recognition receptor.
PG	Prostaglandin.
rpm	Rotation per minutes
STAT	Signal transducer and activator of transcription.
SIRS	Systemic inflammatory response syndrome.
SC	Standard Curve.
SD	Standard Deviation.
SPSS	Statistical package for social science.
TARC	Thymus and activation regulating factor.
TCR	T cell receptor.
T cell	T lymphocyte.
TH-1	T helper 1.
TH-2	T helper 2.
TGF	Tumor growth factor.
TLR	Toll-like receptor.

T reg	T regulatory cell.
T m	T memory cell.
TNFR1	Tumor necrosis factor receptor-1.
WBC	White blood cells.
VCAM	Vascular cell adhesion molecules.

Chapter One

Introduction

And

Literature Review

1. Introduction and Literature Review

1.1 Introduction

The immune response is a highly specific reaction carried out by means of specialized cells that belong to the immune system. There are two types of immune response mechanisms aimed towards pathogens: non-specific, innate reactions, and specific, acquired reactions, acquired immunity, characterized by its specificity, is comprised of lymphocytes, including both T cell and B cell populations (Bakaeva , 2016).

The role of B lymphocytes is not limited to the humoral response; though the cellular immune response is carried out mainly by various T lymphocyte subpopulations. The reactions of the humoral and cellular responses complement and stimulate one another mutually; cytokines are their common linking element. The attachment of cytokines to their specific receptors activates a sequence of signals – either intracellular or between the cells of various systems (Lisowska, *et al.* 2013).

Cytokines are essential mediators for the regulation of both innate and acquired immunity and hematopoiesis. They modulate immune cell signaling, activation, adhesion and functioning. They regulate the individual response to several insults such as infection, inflammation, trauma, and pain. Moreover, the balance between pro-inflammatory and anti-inflammatory cytokines is critical for the evolution of surgical complications and tumor progression. Several drugs, including anesthetic agents, influence cytokines secretion. Opioids, inhalational agents, intravenous and local anesthetics have shown different effects on immune system and cytokine expression. Therefore, anesthesia may play an important role in postoperative recovery (Kitamura *et al*. 2015).

The balance between the proinflammatory cytokines (TNFa 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 (Amin and Salah 2011).

The cytokines receptors are formed by one or more transmembrane Proteins, the extracellular portion binds the cytokine, whereas the Cytoplasmic part starts the signal cascade (Kitamura, *et al*. 2015).

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. Along with stress such as surgery, blood transfusion, hypothermia, hyperglycemia and postoperative pain, anesthetics are associated with suppressed immunity during perioperative periods because every anesthetic has direct suppressive effects on cellular and neurohumoral immunity through influence upon the functions of immunocompetent cells and inflammatory mediator gene expression and secretion (Kurosawa and Kato 2008).

Preoperative and postoperative usage of opioids medications may inhibit cellular and humoral immune function in human. Surgical stress and general anesthesia may suppress immunity through suppressing natural killer and cytotoxic T cells and also activating sympathetic nervous system. However, regional anesthesia can reduce the neuroendo-crine stress by blocking sympathetic nervous system, increase natural killer cell function and decrease the release of endogenous opioids, Therefore, epidural anesthesia combined with or without general anesthesia, had lower suppression to immune function than opioid analgesia (Gu, *et al*. 2016).

Aim of the study

- This study aimed to evaluate the impact of type and time of anesthesia on immune response during surgery, by assessment of cytokines, cells count and functions, which can be achieved by the following objectives:

1. Evaluation of CRP serum levels and WBCs count for patients involved in the study.

2. Using ELISA technique to measure serum level of IL-2, IL-10, IFN-γ and TNF-α.

3. Calculation of CD4, CD8, CD16 and MCP-1 percentage via flow cytometry technique.

1.2 Literatures Review

1.2.1 Overview of Immune System:

The immune system plays a vital role in survival by protecting us from the many potentially deadly infectious pathogens in our environment, as well as from cancer cells. The immune system is able to recognize pathogens and trigger their elimination through innate and then adaptive immune responses(Cruz, *et al*. 2017).

Innate immunity, also called natural or native immunity, is the first line of defense and refers to protective mechanisms that are present even before infection. Its principal components are the epithelial membranes (which block pathogen entry), phagocytic cells (neutrophils and macrophages), dendritic cells, natural killer (NK) cells and several plasma proteins, including the complement system. The most important cellular reaction of innate immunity is inflammation – the process, mediated by dendritic and NK cells, whereby phagocytic cells are recruited and activated to eliminate aggressor agents (Colucci, *et al*. 2013; Aamri and Basnawi., 2017).

Adaptive immunity, also called specific or acquired immunity, consists of mechanisms that are induced by the recognition of specific pathogen antigens. The adaptive immune system is mediated primarily by lymphocytes, and its function can be classified into two types: humoral immunity, mediated by B-lymphocytes and their secreted antibodies; and cell-mediated or cellular immunity, mediated mostly by T-lymphocytes and their cytokines, which play an important role in immune cell activation, regulation, and communication (Cruz, *et al*. 2017).

1.2.2 Components of Innate Immunity

The innate immunity contains the following elements such as: The Langerhans cells inside the skin, the submucosal tissues of the respiratory and alimentary tracts, the complement system, the phagocytic cells (granulocytes and macrophages) and the glial cells. Lymphocytes, granulocytes and macrophages are responsible for the elimination of antigens. The initiation of the immune response has its own essential origin in the mononuclear phagocyte system (MPS) which is represented by monocytes and macrophages (both type of cells accumulate in the site of surgery)(Lisowska., 2012).

1.2.2.1 Inflammation Reaction

Besides its role in host defense against infectious agents and tumor cells, the inflammatory response is essential for tissue reconstitution after injury caused by accidental or surgical insults. Dysregulation of this inflammatory process may increase susceptibility to infections, accelerate the growth and metastasis of residual cancer cells, and result in postoperative complications, such as wound healing disturbances and infections leading to sepsis followed by multiple organ failure and death (Schneemilch, 2004).

The pathogen-associated molecular patterns (PAMPs) are the molecules associated with microorganisms which can be recognized by sophisticated presentation to the pattern-recognition receptors (PRR) and the Toll-like receptors (TLRs) situated on the membrane surface or inside of various cells. They have unique ability of the triggering innate immune responses. Toll like receptors (TLR) belong to pattern recognition receptors (PRR) family and play key role whilst the induction of the immune response. The TLRs are also expressed by immune cells and

various other cells such as endothelium, muscle cells and adipocytes. It can be said they participate while both types of immune response especially during the induction and the regulation of T and B lymphocytes activates (Iwasaki, *et al*. 2015).

1.2.3 Components of Adaptive immune response

1.2.3.1Cellular Components

When the innate mechanisms are not enough to inactive microorganisms then the proteins from pathogenic antigens are presented to molecules expressed on B or T cells becoming their activators. The process called the antigen processing and the presentation leads to the proliferation of the activated lymphocytes and is typical for the acquired immunity. As the after-consequence of the response to signals from the mononuclear phagocytes the B lymphocytes differentiate into plasma cells. Moreover, T lymphocytes become active and start to release soluble factors known as cytokines (Iwasaki, *et al*. 2015).

The acquired response is more sophisticated form of the immunity and requires longer activation period. The main cells of the acquired immunity are both type of lymphocytes and also the antigen presenting cells (APC) such as dendritic cell. Chronic inflammatory state can modify these elements and delay the triggering of the both mechanism of the immune response. The primary role of the B lymphocytes/ plasma cells is the synthesis and the secretion of antibodies associated with the components of the humoral response (Baatar, *et al*. 2011).

The T lymphocytes are a part of the cell-mediated immunity and their role is the cytokines production and support of the B cells activation. The T lymphocytes are divided into following subsets: helper (Th1, Th2 and Th17), cytotoxic (CTL), regulatory (T reg) and memory (Tm). Similarly

B cells and the T cells also have the antigen binding receptor on their surface (TCR) but in contrast to the BCR, the TCR interacts with small fragments of antigenic proteins called peptides which are presented on APC in association with class I and class II molecules included the major histocompatibility complex (MHC) (Lisowska, 2012).

1.2.3.1.1Clusters of Differentiations (CD)

Cluster of differentiation (also known as cluster of classification or designation), it's a protocol or system used for detection and identification of cell surface molecules that provide a targets of cell immunophenotyping (Babatope, *et al*. 2018). It have numerous physiological function, its often acts as receptor or ligands that are important to cells in signal cascade which is usually initiated and lead to alter the cell's behavior. Others have another function like cell adhesion (Zola, *et al*. 2007).

Major use of CD system as markers in immunophenotypic, is that allowing identification of cell depending on what molecules are present on their surface. These markers usually used to correlate cells with certain immune functions. CD molecules also used in cell sorting by applying different methods including flow cytometry (Ho, *et al*. 2009).

Commonly used CD molecules are CD8 and CD4, that are generally used as marker for cytotoxic and helper T-cells respectively, which are usually defined in combination with CD+ molecules (Mason, *et al*. 2002).

1. Cluster of Differentiation 4(CD4):

It's a glycoprotein found on immune cell surface like: T-helper cells (essential cell types of human immune system), macrophage, monocyte and dendritic cells. First discovered was known as leu-3 and T4 before named as CD4 in 1984 (Kappes,2007). Like most cell surface receptor/markers, CD4 is a member of immunoglobulin superfamily; it has four immunoglobulin domains (D_1 to D_4) that are exposed on the extracellular surface of the cell (Figure 1-1). CD4 interacts with β2-domain of MHC class II molecules through its D_1 domain. T cells displaying CD4 molecules (and not CD8) on their surface, therefore, are specific for antigens presented by MHC II and not by MHC class I. Main function of CD4 is a co-receptor of the T cell receptor (TCR) and assists the latter in communicating with antigen-presenting cells. The TCR complex and CD4 each bind to distinct regions of the antigen-presenting MHCII molecule - α1/β1 and β2, respectively (Rudd, *et al.* 2010).

In CD4 the interaction involves its extracellular D1domain, and the resulting close proximity between the CD4 and TCR complex allows the tyrosine kinase (Lck) bound to cytoplasmic tail of CD4 to tyrosine-phosphorylate the immune-receptor tyrosine activation motifs (ITAM) on the cytoplasmic domains of CD3 to amplify the signals that is generated by TCR. Phosphorylated ITAM on CD3 will activate and recruit SH2 domain that contain tyrosine kinase protein for further signaling mediation, so leading to activation of transcription factors resulting in consequent T-cell activations (Zeitlmann, *et al*. 2001).

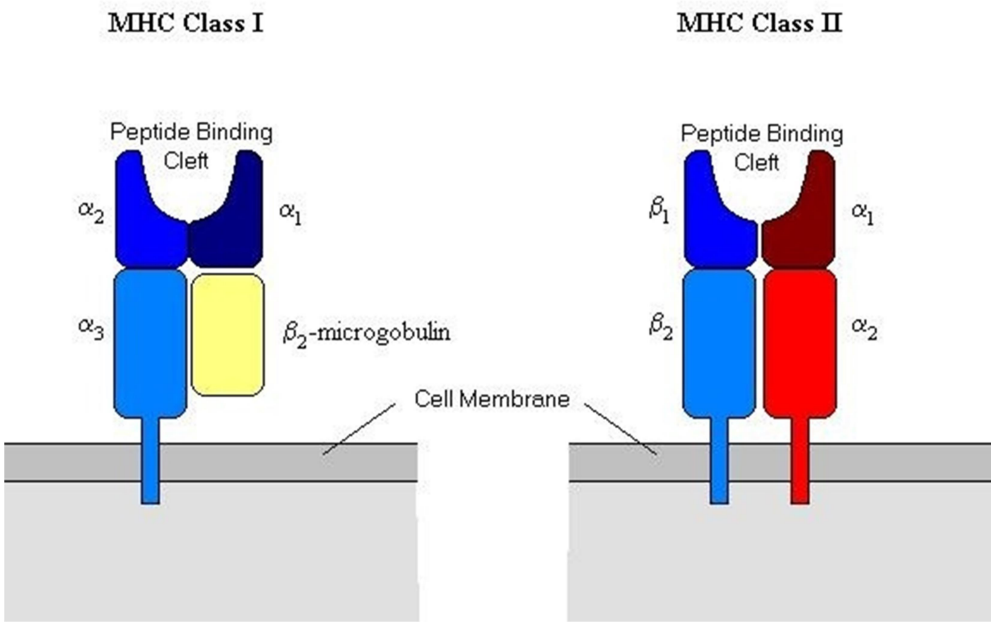

Figure (1-1): Overall Structure of MHC I and II Molecule (Ihsan *et. Al* .,2013)

2. Cluster of Differentiation 8 (CD8):

Is a transmembrane glycoprotein that serves as a co-receptor for the T cell receptor (TCR). Like the TCR, CD8 binds to a major histocompatibility complex (MHC) molecule, but it is specific for class I MHC protein (Gao and Jakobsen, 2000). There are two isoforms of this glycoprotein, alpha and beta (Figure 1-1) each encoded by a different gene. In humans, both genes are located on chromosome 2 in position 2p12. The CD8 co-receptor is predominantly expressed on the surface of cytotoxic T cells, but can also be found on cortical thymocytes, natural killer cells, and dendritic cells. The CD8 molecule is a marker for cytotoxic T cell population (Leong, *et al*. 2003).

CD8 to function, it will form a dimer, consisting of a pair of CD8 chains. The most common form of CD8 is composed of a CD8-α and CD8-β chain, both are members of the immunoglobulin superfamily with an immunoglobulin variable (IgV)-like extracellular domain connected to

the membrane by a thin stalk, and an intracellular tail. The extracellular IgV-like domain of CD8-α interacts with the α_3 portion of the Class I MHC molecule (Cui and Kaech 2010). This affinity keeps the T cell receptor of the cytotoxic T cell and the target cell bound closely together during antigen-specific activation. Cytotoxic T cells with CD8 surface protein are called CD8+ T cells, and the main recognition site is a flexible loop at α3 domain of an MHC I molecule (Osińska, *et al.* 2014).

Once the body infected by certain pathogens, naïve CD8+ cells will recognize and bound to infected cells through MHC I, and start to kill the infected cells by secreting granzymase and perforin onto infected cells resulting in destroying the cells integrity (Voskoboinik, *et al*. 2015).

2. Clusters of Differentiation 16 (CD16):

It also known as FcγRIII, is a cluster of differentiation molecule that found on the surface of natural killer cells, neutrophil polymorph nuclear leukocytes, monocytes and macrophages. CD16 has been identified as Fc receptors FcγRIIIa (CD16a) and FcγRIIIb (CD16b), which participate in signal transduction (Fattouh, *et al*. 2015).

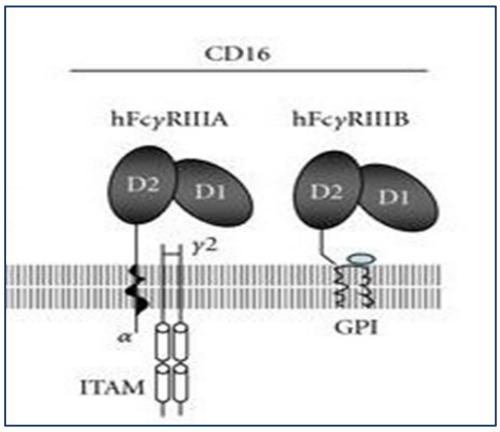

Figure (1-2): Graphic Illustrating The Structure of CD16 (Makaryana and Finleya.,2018).

The most well-researched membrane receptor implicated in triggering lysis by NK cells, CD16 which is a molecule of immunoglobulin superfamily (IgSF) involved in antibody-dependent cellular cytotoxicity (ADCC). It can be used to isolate populations of specific immune cells through fluorescent-activated cell sorting (FACS) or magnetic-activated cell sorting, using antibodies directed towards CD16(Romee, *et al*. 2013).

In humans, it exists in two different forms: FcγRIIIa (CD16a) and FcγRIIIb (CD16b), while FcγRIIIa is expressed on mast cells, macrophages, and natural killer cells as a transmembrane receptor, FcγRIIIb are only expressed on neutrophils (Jing, *et al*. 2015). In addition, FcγRIIIb is the only Fc receptor anchored to the cell membrane by a glycosyl-phosphatidylinositol (GPI) linker, and also plays an important role in triggering calcium mobilization and neutrophil degranulation. FcγRIIIa and FcγRIIIb together are able to activate degranulation, phagocytosis and oxidative burst, which allow neutrophils to clear opsonized pathogens (Zhang, *et al.* 2000). CD16 receptors bind to the Fc portion of IgG antibodies, which then activates antibody-dependent cell-mediated cytotoxicity (ADCC) in human NK cells. It also required for ADCC processes carried out by human monocytes (Yeap, *et al*. 2016).

1.2.3.2 Humoral Components

1.2.3.2.1 Cytokines:

Cytokines are proteins expressed in both innate and acquired immune system and are often named according to the secreting cells (*i.e*., lymphocytes, interleukins, etc.). They are essential mediators of the natural (non-specific, innate) immunity, which is the initial step of the inflammatory response. They also play a pivotal role during the specific immunity, which occurs after exposure to antigens, as they regulate

lymphocyte and leukocyte activation, growth and differentiation, and the immune-mediated inflammation .The cytokines have different structures, but also common aspects (Kitamura, *et al*. 2015).

Cytokines are immensely responsible for the transduction of information among the immune system cells and are the very important components of the bi-directional communication between the immune and the neuroendocrine systems Depending on the prevailing effects of inflammatory response they are called as a pro-inflammatory or anti-inflammatory. The pro-inflammatory cytokines are mainly represented by interleukins: IL-1, IL-6, TNF, IL-2, IL-6, IL-8, and IL-15. The examples of the anti-inflammatory cytokines are interleukins IL-4, IL-10, TGF-β (Lisowska, *et al.* 2009).

Cytokines may act locally or systemically by cell to cell signaling. The action of cytokines can be described as autocrine, paracrine or endocrine. Some cytokines are known to act in either synergistic or antagonistic way. Cytokines may affect the action of each other which may give synergistic or antagonistic effect on the targeted cells. Both the synthesis and activity of cytokines are regarded at different levels. Different types of antagonistic molecules (e.g. soluble receptor, cytokine binding proteins, molecules that compete with cytokine for binding to the receptors) are produced to restrict them activities. The potent pro-inflammatory activities of cytokines are restricted by three major systems that can prevent uncontrolled excessive production of cytokines (Lisowska ,2012).

The potent pro-inflammatory activities of cytokines are restricted by three major systems that can prevent uncontrolled excessive production of cytokines. These relates to their synthesis and release membrane receptors and intracellular signal transduction. Both overproduction and

underproduction of cytokines and their receptors take place in many diseases with prevailed acute or chronic inflammatory state (Axel, 2010).

1. Interferon gamma (IFN-γ):

Interferon-gamma (IFN-γ) is a cytokine that play an important role in modulating and inducing array of immune responses. Cellular response to IFN-γ is mediated by its heterodimeric cell-surface receptor (IFN-γR), which activates downstream signal transduction cascades, ultimately leading to regulation of gene expression. IFN-γ is a soluble cytokine that is the only member of type II class of interferon and its primarily secreted by activated natural killer (NK) cells and T cells and can promote macrophage activation, enhance antigen presentation, regulate activation of innate immune system, mediate antiviral and antibacterial immunity, orchestrate Th1/Th2 balance, coordinate lymphocyte-endothelium interaction and control cellular proliferation and apoptosis (Haring, *et al*. 2005).

The alpha (α) chain of the IFN-γR, also known as IFN-γR1 or CD 119, was the first component of the receptor to be identified and cloned. Although it binds IFN-γ with relatively high affinity, IFN-γR1 alone is unable to mediate the biologic responses to this cytokine (Zhou, Chen *et al*. 2009).

Both chains of the IFN-γ receptor are members of the class II family of cytokine receptors that includes tissue factor, IL-10 ligand-binding component, and both chains of the IFN-α receptor (IFN-αR). Like other family members, the IFN-γR α and β chains lack intrinsic kinase activity. Signaling through the IFN-γ R is mediated through JANUS kinase (JAK1 and JAK2), and members of protein tyrosine kinases family, which are constitutively associated with specific membrane-proximal

residues on the cytoplasmic domains of IFN-γ R, that is finally end up with mediating the biologic functions of IFN-γ (Trifunović, *et al*. 2015).

IFN-γ produced mainly by Th1, CD8 and NK lymphocytes. It activates macrophages and inhibits Th2 differentiation, so it is considered a proinflammatory cytokine. In vitro, propofol increases its concentration, whereas thiopental and remifentanil decrease its concentration. The same effect has been observed during chronic opioid administration, such as morphine (Lisowska *et al*, 2013).

2. Tumor Necrosis Factor alpha (TNF-α):

Human tumor necrosis factor alpha (TNFα) is a type II homotrimeric transmembrane protein that acts as a proinflammatory cytokine. It is produced mainly by macrophages in addition to a variety of other cells including neutrophils, monocytes, B and T-cells. The involvement of TNFα in several signal transduction pathways links the protein to such diverse functions as acute inflammation, apoptosis, septic shock, cellular proliferation, and differentiation, also overproduction of TNF-α preceded shock and death induced by endotoxin or gram-negative bacteria (Feng, *et al*. 2015).

This cytokine favors extravasation with endothelial cell stimuli to produce IL-8 and adhesion molecules. Isoflurane raises mRNA expression and increases TNF-α level, and is the same with fentanyl, remifentanil and bupivacaine (Wu *et al*, 2012). Sevoflurane, enflurane, ketamine, thiopental, propofol and opiates inhibit its liberation from mononuclear cells (Lisowska *et al*, 2015).

Tumor Necrosis Factor have the ability to bind with tow receptor TNFR1, which is expressed in most tissues and can fully activated by

both forms of TNF(soluble and membrane-bounded forms), the second type is TNFR2 that is typically found in immune system cells, and responds to membrane –bound form of TNF (Olszewski, *et al*. 2007).

Maine pharmacological action of TNF is promoting inflammatory response that, in turn, causes many clinical problems mainly that associated with autoimmune disease and refractory asthma (Korneev, *et al.* 2017).

1.2.3.2.2 Interleukins (ILs)

Interleukins are group of cytokines (include secreted protein and signaling protein molecules), that were first seen to be expressed by leukocytes (WBCs), and their effects on immune system depends on several factors like: receptor expression patterns, their local concentration and integration of multiple signaling pathway in response to immune cells (Brocker, *et al.* 2010).

Functions of immune system are largely depending on ILs, and any deficiency in one of these ILs will results in immune deficiency or auto-immune disorders. Interleukins are mostly produced by T-helper (CD4) cells, macrophage, monocyte and endothelial cells. These interleukins have a complex immune-modulatory function, involving: proliferation and maturation of cells as well as migration and cell adhesion. They also play a crucial role in differentiation and activation of immune cells. Also have pro-inflammatory and anti-inflammatory action (Menachem-Zidon, *et al*. 2011).

These functions distinguish ILs from chemokines, which their main function is directing the immune cells to the site of infection via chemotaxis and IFNs, which is mainly modulate cellular response to viral infection. Interleukins are able to induce an immune reactions when bind

with high affinity receptors that located on cell surface (Sokol, Xu et al. 2008, Brocker, Thompson et al. 2010)(Sokol, *et al*. 2008, Brocker, *et al*. 2010) .

1. Interleukin 2 (IL-2):

It is a type of cytokine signaling molecule in the immune system. It is a protein that regulates the activities of white blood cells (leukocytes, often lymphocytes) that are responsible for immunity. IL-2 is part of the body's natural response to microbial infection, and in discriminating between foreign "non-self" and "self" antigen. IL-2 mediates its effects by binding to IL-2 receptors, which are expressed by lymphocytes (Liao, Spolski et al. 2014)(Liao, *et al.* 2014).

Interleukin-2 primarily produced by activated CD4+ T-cells and CD8+ and dendritic cells after antigenic stimuli, it acts as a pro-inflammatory cytokines and play a crucial role in activation of regulatory T-cell to release IFN-γ and TNF-α, also enhance the cytolysis function of NK cells and specific cytotoxicity by CD8 and also increases MHC II molecule expression, thereby it orchestrates the immune response and participate effectively in pathogenesis of most pathological disorders (Marcela *et al*., 2016).

Anaesthetic drugs, through different mechanisms, decrease its effects. Ketamine inhibits its release, morphine diminishes its levels and propofol suppresses IL-2 production (Zura et al, 2012).

2. Interleukin 10 (IL-10):

Interleukin 10 (IL-10), also known as human cytokine synthesis inhibitory factor (CSIF), it’s an important immunoregulatory cytokine (anti-inflammatory cytokine), which is mainly secreted by macrophages, and by T helper 1 (Th1) and Th2 lymphocytes, dendritic cells, cytotoxic

T cells, B lymphocytes, monocytes and mast cells. It can be produced even by human carcinoma cell lines (Mosser and Zhang 2008)(Mosser and Zhang, 2008). It considered as the main anti-inflammatory cytokine because it inhibits the synthesis of IFN-γ, TNF-α, IL-2 and IL-12. IL-10 also induces IgG synthesis. Many anaesthetic drugs such as ketamine, Thiopental, Propofol, Fentanyl, Remifentanil, Isoflurane, Sevoflurane and Bupivacaine increase IL-10 levels (Schneemilch, *et al*. 2005).

IL-10 activity is mediated by the IL-10 receptor (IL-10R) which is a member of the class II cytokine receptor family. IL-10 inhibits the capacity of monocytes and macrophages to present antigen to T cells via an inhibitory effect on expression of major histocompatibility complex (MHC) class II, costimulatory molecules such as CD80 (B7.1) and CD86 (B7.2) and therefore downregulates the expression of IL-1, IL-6, IL-8, IL-12 and TNF-α (Li, *et al.* 2012, Lazear, *et al*. 2015).

In B cells, IL-10 prevents apoptosis, enhances cell proliferation and has a role in immunoglobulin (Ig) class switch. The IL-10 encoded by *IL-10* gene that is located on chromosome 1, and the expression of IL-10 is strongly regulated at both transcriptional and post-transcriptional level (Saraiva and O'garra, 2010).

1.2.3.2.3 Chemokines Protein

Chemokines are family of small cytokines, or signaling proteins, their names derived from its ability to induce directed chemotaxis in nearby responsive cells, they are chemotactic cytokines. They are small hapten-binding protein that constitute a large family of peptide (60-100 amino acid), structurally related to cytokines whose main function is regulating cell trafficking (Deshmane, *et al*. 2009).

Most important two types of chemokines included and tested in this study are C - reactive protein (CRP) and Monocyte chemoattractant protein (MCP-1), these chemokines studied in a detailed manner due to its important role in inflammatory process. Chemokine expression by macrophages, fibroblasts, and osteoblasts exposed to implant debris is also a central innate immune effector reaction to implant debris (Nibbs and Graham, 2013).

The chemokines, particular to implant aseptic loosening pathology, include IL-8, MCP-1 MIP-1α, Chemokine-C motif ligand 17/ Thymus and activation regulated chemokine (CCL17/TARC), and CCL22. Increased expression of MCP-1, macrophage inflammatory protein-1(MIP1)/ (CCL-2), and MIP 1α (CCL3) was observed in periprosthetic tissues from failed arthroplasties and also in macrophages analyzed cell culture after exposure to different types of wear particles (Landgraeber, *et al*. 2014).

1. C-Reactive Proteins (CRP):

C - reactive protein (CRP) found in blood plasma, whose levels rise in response to inflammation. It is an acute-phase protein of hepatic origin that causes increase following interleukin-6 (IL-6) secretion by macrophages and T-cells. Its physiological role is to bind to lysophosphatidylcholine expressed on the surface of dead or dying cells (and some types of bacteria) in order to activate the complement system via C1q. CRP is synthesized by the liver in response to factors released by macrophages and fat cells (adipocytes) (Lau, *et al*. 2005).

It is not related to C-peptide (insulin) or protein C (blood coagulation). C-reactive protein was the first pattern recognition receptor (PRR) to be identified. CRP in blood has a half-life less than 24 hrs. compared with

other acute phase protein half-life that may reach to 4 days. Continuous elevation of CRP level indicates persistent proinflammatory stimuli in the body (Mantovani *et al*. 2008).

2. Monocyte Chemoattractant Protein-1(MCP-1):

Chemokines constitute a family of chemoattractant cytokines and are subdivided into four families on the basis of the number and spacing of the conserved cysteine residues in the N-terminal of the protein. Chemokines 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 (Lu, *et al*. 2016).

Monocyte chemoatractant protein-1 is also known as monocyte chemotactic and activating factor (MCAF) and CCL2. MCP-1 is a CC chemokine which presents as a dimer derived from monocyte and vascular endothelial cells; it exerts chemoatractant activity on a variety of cell types, such as monocyte, basophile, T-cells and NK cells via chemokine receptors such as CCR2, CCR5 and CCR10. Chemokine C-motive ligand-2(MCP-1/CCL2) it is one of the key chemokines that regulate migration and infiltration of monocytes/macrophages to the site of infection. Also its have been demonstrated to be implicated in pathogenesis of various diseases such as rheumatoid arthritis, atherosclerosis and psoriasis (Xia and Sui 2009).

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 leads to kill tumor targets in vitro, but later it have been established that MCP-1 is a major chemoattractant for T-cells and also activate T-cell adhesion fibronectin via stimulation of β1 integrin (Oh, Qian *et al*. 2018).

1.2.4 Immune Receptors:

Immune receptor or immunologic receptor is found on cell membrane and bind to substance (like, cytokine) and causes immune response. The main immune receptors in immune system are: toll like receptor (TLRs), killer activated and killer inhibitor receptor (KARs and KIRs), pattern recognition receptor (PRRs), B-cells and T-cells receptor, complement receptor, fragment crystalizable (Fc) receptor and cytokines receptors (Ansar and Ghosh 2016).

The cytokines receptors are formed by one or more transmembrane proteins: the extracellular portion binds the cytokine, whereas the cytoplasmic part starts the signal cascade. Based on the extracellular portion divided into five categories(Kitamura, Di Biaso et al. 2015).

-Type I Receptors: which have four α-helical stands and contain four cysteine residues (Figure 1-3), and the amino acid motif Tryptophan-Serine-X-tryptophan-serine (WSXWS).

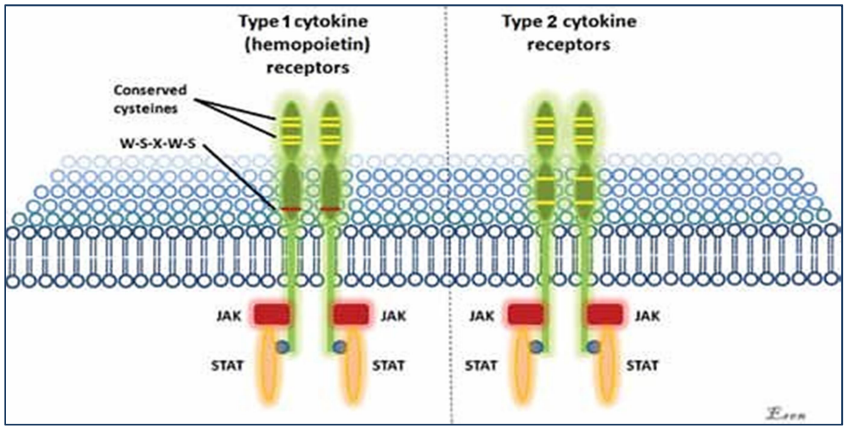

Figure (1-3): Structure of Type 1 and 2 Cytokines Receptors (Wang *et. Al.*, 2009).

-Type II Receptors: structurally similar to Type I receptors, but without the sequence WSXWS (Figure 1-3).

-Interferon Receptors: which have domains rich of cysteine and can induce apoptosis or stimulate gene expression (Figure1-4).

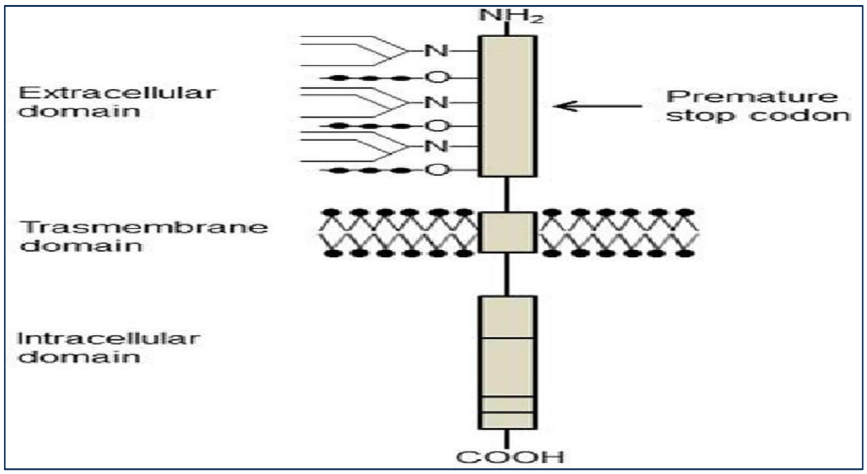

Figure (1-4): Structure of IFN Receptor. (Wang *et. Al.*, 2009).

-Immunoglobulin Receptors: which have extracellular domain for immunoglobulin (Ig) and different mechanisms for single transduction (Figure 1-5).

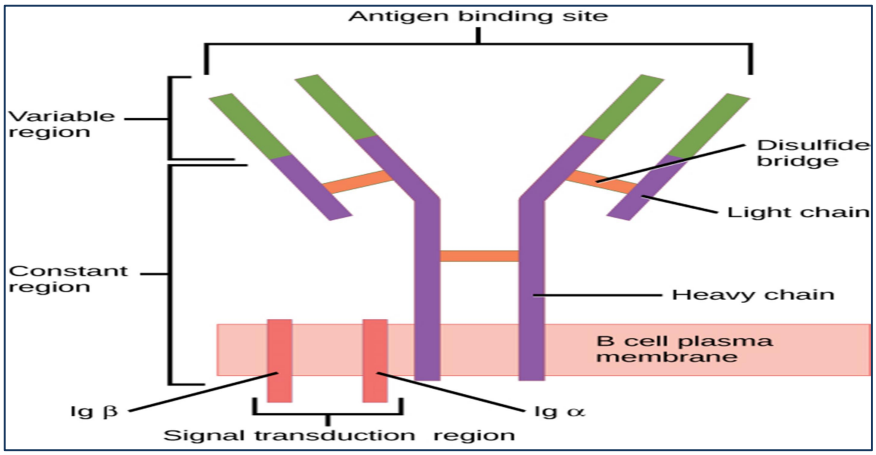

Figure (1-5): Structure of Immunoglobulin Receptor. (Wang *et. Al.*, 2009).

-Seven-transmembrane Spanning Family Receptors: which pass the membrane seven times and transduce the signal through G-protein pathway (Figure 1-6).

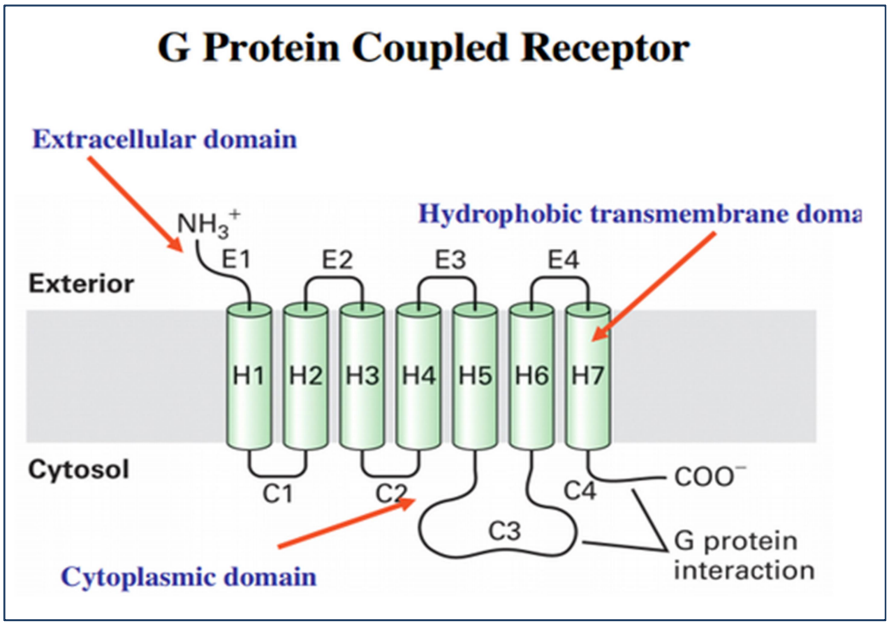

Figure (1-6): Structure of Seven-transmembrane Spanning Family Receptors. (Wang *et. Al.*, 2009).

1.2.5 Major histocompatibility complex MHC

The major histocompatibility complex (MHC) in the humans called human leukocyte antigen (HLA) has many different diverse immunological and non-immunological functions. The MHC is divided into two types of molecules encoded by the MHC classes: MHC class I and MHC class II. Both of them are recognized by different subsets of the T cells. The MHC class I molecules have the crucial task of the presentation of an antigenic protein to the T Cytotoxic lymphocytes (CD8+) while MHC class II presents an antigen to T Helper lymphocytes (CD4+)(Babatope, 2018).

Molecules of MHC class I are found on the surface of all nucleated cells of human body, while molecules of MHC class II are found on the surface of the Antigen Presenting Cell (APC). The MHC complex includes the most polymorphic genomes in the human genomes with many consequences related to the transplantation and the autoimmune disease. The adhesion molecules CD4+ and CD8+ are necessary to enhance the binding of the T cells and the APC. The expression of CD4+ and CD8+ is variable on the T cells. The normal ratio of CD4+ T cell to CD8+ T cells is approximately 2:1 in healthy population (Lisowska, 2012).

1.2.6 Overview of anesthetic agents

Anesthesia aims to allow surgery to be conducted in a safe manner, under the most favorable operating conditions and with the patient experiencing little pain, anxiety or other discomfort. Anesthesia can be achieved using regional anesthesia (including spinal and epidural anaesthesia, as well as peripheral nerve blocks) or general anesthesia; Anesthetists therefore use a combination of medicines including the volatile inhalations, intra-venous hypnotics and sedatives, muscle relaxants and opiates in order to achieve these goals. This is often referred to as ″balanced anaesthesia″ and aims to have synergism of desired effects without synergism of side effects (Robertson and Ridge, 2010).

The possible effects of anesthesia on the immune system have been discussed from the early 20th century. Graham in 1911 and Gaylord in 1916 respectively describe the influence of ether anesthesia on bacteriolysis and phagocytosis in human, and the effects of anesthetics on tumor growth in an animal model. Particularly in cancer patients,

immunosuppression after surgery accelerates the development of residual cancer cells and promotes the establishment of new metastases. Immunological effects affect the long-term outcomes of patients after surgery. Therefore, awareness of immunological properties in the surgical area is helpful for daily anesthetic management (Kurosawa and Kato, 2008). Figure (1-7), a schematic diagram illustrates the possible role of anesthesia on immune system, by direct and indirect effects through neuro-immune- endocrine interaction during surgical stress.

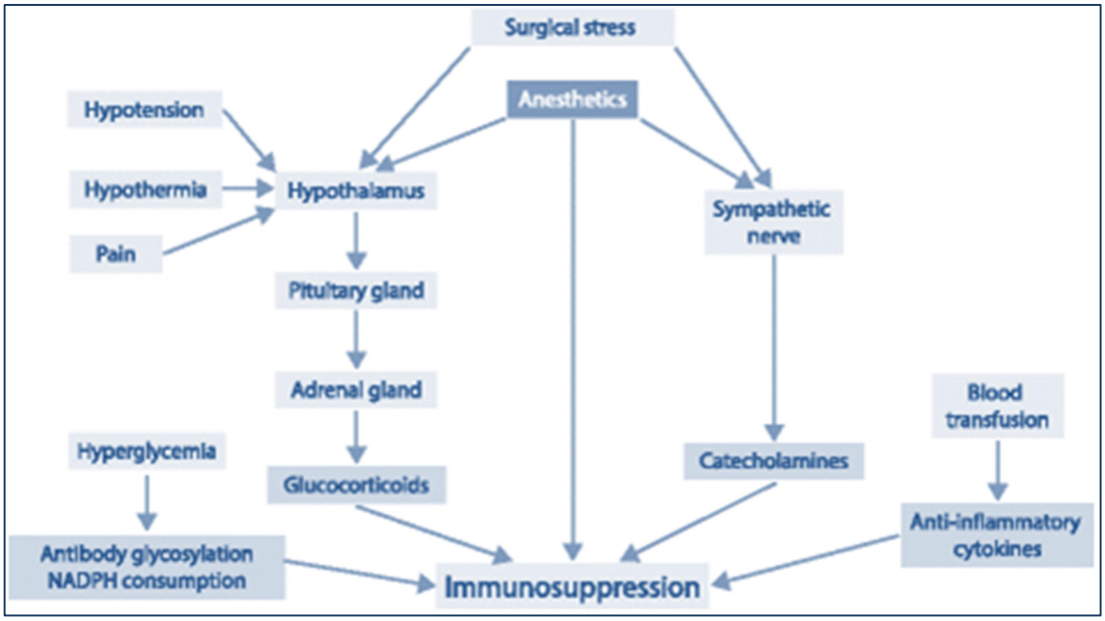

Figure (1-7): Schematic diagram of possible modulators of immune competence during anesthesia and surgery (Amari and Basnnawi, 2017).

1.2.6.1Classification of anesthetic agents

1.2.6.1.1 Intravenous anesthetics

The modern intravenous anesthesia began in the 1930s with the introduction of barbiturates into the clinical practice, such as thiopental. Benzodiazepines, like diazepam and midazolam, are gamma amino butyric acid (GABA) receptor agonists with sedative, hypnotic, anxiolytic, anticonvulsant, and muscle relaxant properties; they are commonly used in premedication or for sedation in minor procedures (Fahlenkamp, *et al*. 2011).

1. **Ketamine**:

Acts on N-Methyl-D-aspartate (NMDA) receptors, and differs from other anesthetics because of its strong analgesic effect with minimal respiratory depression. Ketamine acts at different levels of inflammation, interacting with inflammatory cell recruitment, cytokine production, and regulation of inflammatory mediators (Loix, De Kock *et al*., 2011). Etomidate is a GABA agonist used for induction of general anesthesia and sedation, and presents peculiar characteristics, such as adrenocortical suppression (Persson , 2013).

The anti-inflammatory effects of ketamine are still unclear but they could be connected with the suppression of macrophage production of TNF in the presence of bacteria (Chang, *et al.* 2005). ketamine inhibits production of proinflammatory cytokines by reduction of hepatic nuclear factor kappa B (NF-κB) activation in experimental model of sepsis (Song, *et al*. 2009).

The nuclear factor Kappa-B (NF-κB) proteins belong to a family of transcription factors that play a key role in the activation of many genes that regulate a large number of process such as synthesis of proinflammatory cytokines, cell proliferation and apoptosis, NF-κB is

also involved in the transcription of genes linked with sepsis. Thiopental and ketamine can inhibit the release of IL-1, IL-6, TNF -α and IL-8 triggered by endotoxin or lipopolysaccharide. Additionally, these drugs also increase the level of IL-10, an anti-inflammatory cytokine (Welters*, et al*. 2010).

Overproduction of the proinflammatory cytokines causes the enhanced systemic inflammatory response syndrome (SIRS). However, the predominance of anti-inflammatory cytokines may lead to higher risk of infections. The effect of ketamine and thiopental on the function of mast cells appears to be disadvantageous for patients with a high risk of infection. Mast cells have a protective role in host defense against pathogens due to their involvement in innate and acquired immune responses to infection. The inhibitory effects of thiopental and ketamine on mast cell function may induce a higher risk of infection (Fujimoto*, et al*. 2005).

2. Barbiturate anesthetics:

Thiopental is one of the most investigated anesthetic agents and is widely used for the induction of general anesthesia. Its inhibitory effects on the nonspecific immune system have been well documented in several studies. In clinically used concentrations, thiopental has been shown to inhibit the bactericidal functions of leukocytes; neutrophil polarization, chemotaxis, adherence, phagocytosis, and respiratory burst; and monocyte chemotaxis (Colucci, *et al*. 2013).

The ability of thiopental to inhibit monocyte chemotaxis and phagocytosis may also suppress the host response against pathogens and thereby enhance the risk of infection in immunocompromised patients. Chemotaxis plays a crucial role in the inflammatory response, because it

is involved in the migration of leukocytes through the walls of blood vessels to sites of infection (Wheeler*, et al*. 2011).

3. Propofol (2, 6-diisopropylphenol):

It is routinely used for short-term sedation, maintenance of anesthesia and for long-term sedation in critical patients. Propofol is characterized by perfect anesthetic effects and anti-inflammatory, antioxidant properties (Marik, 2005).

The drug owes these properties to its chemical resemblance to endogenous tocopherols. Studies have shown that after the use of Propofol patients exhibit a substantial increase in γ tocopherol, an agent causing inhibition of the inflammatory response. The anti-inflammatory properties of Propofol are also demonstrated by its ability to weaken the synthesis of prostaglandins (PGE) by lipopolysaccharide (LPS) activated monocytes. In addition, Propofol blocks the expression of intracellular adhesion molecule (ICAM-1) and vascular cell adhesion molecules (VCAM-1), which are important in the migration of leukocytes through the endothelium. Propofol reduced the concentration of cytokines (IL-1, TNF-α, IL-6) and stimulated the neutrophils to nitric oxide (NO) synthesis. The anti-inflammatory effect of Propofol has been confirmed by its inhibition of LPS stimulated macrophages, which can be regarded as a laboratory endotoxemic model (Hsing, *et al*. 2011).

4. Opioids:

Different opioids affect immune function differently depending on drug factors, host factors, and the duration of exposure (Cruz, *et al*. 2017). Opioids like morphine, fentanyl and pethidine, exert their effects either directly through receptors (μ, δ, κ) which are widely located on

both neural and immune cells or *via* the autonomic and central nervous systems. Although opioid effects are largely attributed to decreased central sympathetic nervous system outflow, opioids can also cause direct sympathetic nervous activation, which may suppress the proliferation and function of some immune cell populations and primary and secondary lymphoid tissues. Opioids cause the modulation of both innate and adaptive immunity, including cytokine and immunoglobulin synthesis, activation NK cells and phagocytosis (Ninković and Roy, 2013).

Morphine alone or with bupivacaine caused a significant increase of μ receptors mRNA in lymphocytes, which likely causes attenuation of cytokine synthesis. Additionally, at the same time a lowering of the percentage of NK cell was observed (Campana, *et al*. 2010).

Opioids have the ability to modulate the immune responses including cell proliferation and regulation of cytokine synthesis and secretion. Morphine induces the production of transforming growth factor beta (TGF-β) by stimulated lymphocytes, monocytes and macrophages, this concerns mainly long-term therapy which also causes a rise in IL-4 and IL-5 concentrations and a decrease in IL-2 and IFN-γ. Furthermore, opioids could modulate humoral immunity by decreasing both antibody secretion by normal B-lymphocytes and proliferation of multiple myeloma cells (Vassou, *et al*. 2008).

Studies investigating the influence of opioids on the production of pro-inflammatory monocyte/macrophage-derived cytokines have demonstrated a significant diminishing of TNFα, IL-1 and IL-6 concentrations following morphine treatment (Shavit, *et al*. 2005).

The pathways of morphine-induced inhibition of proinflammatory cytokine production via μ receptors are different but finally lead to the depression of NF-κB signaling. On one hand, the depression of NF-κB

signaling is caused by activation of the cyclic adenosine monophosphate (cAMP) response, and on the other, it follows the increase of nitric oxide activation after stimulation of the μ3 opiate receptor on monocytes. There is evidence that Toll-like receptors (TLR4, TLR2) are involved in the immunomodulatory effect of morphine. Stimulation of TLR signaling causes activation of intracellular pathways: the NF-κB and mitogen-activated protein kinase (MAPK) pathways play leading roles in the proinflammatory response, which is related more to processes of survival and apoptosis (Roy, *et al*. 2005).

Remifentanil, in comparison with fentanyl, causes a decrease in the IFN-γ/IL-10 ratio (a drop in IFN-γ), which mirrors the equilibrium of Th1/Th2. Concentrations of IL-6, TNF, IL-10 and IL-2 were not shown to change upon treatment with remifentanil or fentanyl(Dossow, *et al*. 2008).

5. Benzodiazepines:

Midazolam, a representative of benzodiazepines, is used mainly in premedication and sometimes intraoperative during anesthesia. Midazolam exerts its action via central and peripheral benzodiazepine receptors (CBR/PBR). The expression of PBR found on the surface of macrophages allows benzodiazepine to modulate pro-inflammatory and anti-bacterial functions of macrophages by blocking their ability to produce superoxide anions as well as IL-1, TNF and IL-6 cytokines (Song, *et al*. 2009).

Midazolam, a widely used benzodiazepine derivative, acts on GABA receptors by increasing neuronal permeability to chloride ions, leading to cell hyperpolarization. It is known to inhibit certain aspects of immune function. Midazolam binds to peripheral receptors on macrophages and

modulates their metabolic oxidative responsiveness in vitro. It has been suggested that clonazepam also binds to receptors on macrophages and inhibits their capacity to produce IL-1, IL-6, and TNF-α in a T-cell independent manner (Cruz, *et al.* 2017).

Both midazolam and Propofol modulate the transport and release of IL-8. Activation of leukocytes via LPS in the presence of Propofol and midazolam causes a decrease in the concentration of extracellular IL-8 while the intra-cellular concentration remains unchanged. The suppression of IL-8 release may increase the risk of infection in the post-operative period (Lisowska, *et al*. 2013).

1.2.6.1.2 Volatile anesthetics

Inhalational anesthetic agents have inhibitory effects on neutrophil function, decrease lymphocyte proliferation, and suppress cytokine release from peripheral blood mononuclear cells (Kurosawa and Kato 2008)(Kurosawa and Kato 2008). Halogenated anesthetics are known to suppress inflammatory cytokines in rat alveolar cells, In contrast, exposure to volatile anesthetics and mechanical ventilation has been shown to induce increased gene expression of pro-inflammatory cytokines (Cruz, ,. 2017).

Volatile anesthetics affect the expression of inducible nitric oxide synthase (iNOS) by reversible inhibition of voltage-dependent calcium channels and decreased intracellular calcium concentrations. Thus, in vitro effects of volatile anesthetics predominantly consist of inhibition of immune products, but these are generally transient, as well as dose- and time-dependent (Kurosawa and Kato,2008).

1-Isoflurane:

Isoflurane exposure leads to reduction in leukocyte counts and levels of systemic proinflammatory cytokines (TNF-α, IL-6 and IL-1β), as well as less macrophage activation and polarization toward the M2 phenotype. These effects were found to be protein kinase C dependent and also due to systemic inhibition of nuclear factor kappa-light chain-enhancer of activated B-cells (NF-κB). Pre-exposure to volatile anesthetics induces a systemic anti-inflammatory effect. On the other hand, exposure to isoflurane has been shown to lead to cognitive impairment and a small increase in IL-1β and activated caspase-3 levels in both young adult and elderly rats. These results suggest that isoflurane induces neuroinflammation, which then leads to cognitive impairment (Lee, *et al.* 2015).

1.2.6.1.3 Local Anesthesia

Local anesthesia is reversible loss of sensation in a defined area of the body, this temporary loss of sensation achieved by topical application or injection of agents blocking the sodium channels that facilitate nerve impulse in tissue. They can be administered peripherally (topical application, local infiltration and plexus block), or at level of spine(epidural and spinal)(Cook KA, 2017).

With regard to their anti-inflammatory properties, local anesthetics have been shown to affect PMNs directly, as well as macrophage and monocyte function. It decreases PMN adherence, migration, and accumulation at the site of inflammation. Since local anesthetics impair PMN presence and function, concerns have arisen that local anesthesia might increase susceptibility to infection, as local anesthetic-mediated depression of the PMN oxidative metabolic response may decrease the

host's ability to control bacterial proliferation (Hahnenkamp, *et al*. 2004). A leading theory for the beneficial effect of regional anesthesia on tumor progression is that regional block attenuates perioperative immunosuppression. During a major surgery, there is a measurable decrease of cytokines for cell-mediated immunity such as IL-2, IL-12, and IFN-γ. The number of circulating natural killer (NK) cells, cytotoxic T lymphocytes, and the ratio of T-helper 1 (Th1) to T-helper 2 (Th2) are also significantly reduced. Regional anesthesia blocks the afferent sensory transmission, efferent sympathetic activation, and the associated endocrine and metabolic responses. Intraoperative use of regional anesthesia lowers the plasma levels of cortisol and catechol amines (Sanders *et al*., 2010). Under spinal analgesia, the function of NK cells and the balance of Th1/Th2 are better preserved. In addition, most intravenous and volatile anesthetics are immunosuppresses; regional anesthesia decreases systemic opioid use and the amount of general anesthetics required. All these aspects of regional anesthesia help to maintain NK function and cell-mediated immunity, the first line and the most important defense against malignancy (Caza *et al*., 2008).

Local anesthetic agents categorized into two classes based on their structure:

1. **Para-amino benzoic acid(PABA):**

Also known as ester local anesthetic drugs and includes: benzocaine, cocaine, procaine, tetracaine, amylocaine, and chloroprocaine.

2. **Non-Para amino benzoic acid:**

It's called amide local anesthetic which includes: articaine, tonicaine, cinchocaine and lidocaine (lignocaine).

Each group is available in different formulations such as ointment, patches and injections. Lidocaine is the most common anesthetic agents used in surgical procedure since it's effective and non-toxic and free of sensitivity (Corbo, *et al*. 2016). Lidocaine decreased TNF-α-induced up-regulation of CD11b/CD18 surface expression on PMNs in vitro (Cassuto, *et al*. 2006).

In vitro lidocaine inhibits IL-1β and IL-8 release from epithelial cells, IL-1β secretion from mononuclear cell, and the neutrophil function, the phagocytosis, the migration of leukocytes, also it decrease the formation of reactive oxygen metabolite and attenuate the release of leukotriene and histamine (Kitamora, *et al*. 2015).

Lidocaine also inhibits interferon-inducible IL-10 secretion in intestinal epithelial cells, decrease IL-1β, IL-6, IL-8 on activated human umbilical vein endothelial cells; it also impairs the release of IL-2, TNF-α and IFN-γ. And in addition to the direct effect of local anesthesia on immune system , it also affect the immunity by blocking sympathetic nervous system and by decrease surgical stress (Kurosawa and Kato, 2008).

Chapter Two

Materials and Method

2. Materials and Methods

2.1 Materials

2.1.1 Instrument and Equipment

All instruments and equipment that have been used in study are listed in table (2-1).

Table (2-1): Instruments and Equipment with their Company.

No.	Equipment & instrument	Company/Country
1	Centrifuge	Hettich/Germany
2	Digital camera	Samsung/China
3	Distillator	Labteech/South Korea
4	EDTA tube	Laiwuyaohua/China
5	Eppendorf tube	Eppendorf/Germany
6	Exispin vortex centrifuge	Bioneer/S. Korea
7	Flow Cytometry Machine	BriCyte E6/India
8	High Speed Cold Centrifuge	Eppendorf/Germany
9	Glass tube (4 ml)	Laiwuyaohua/China
10	Incubator	Memmert/Germany
11	Micropipettes 5-50, 0.5-10, 100-1000µl	CYAN/Belgium
12	Microwave	Argose/Germany
13	Microcentrifuge tube	Biobasic/Canada
14	Plain tube	Laiwuyaohua/China
15	Refrigerator	Concord/Lebanon

16	Sensitive Balance	Sartorius/Germany
17	UV Trans illuminator	ATTA/S Korea
18	Vortex	CYAN/Belgium
19	Water Bath	Memmert/Germany

2.1.2 Flow cytometry Reagents

All Flow Cytometry assay kits that have been used in this study are listed in table (2-2).

Table (2-2): Reagents of Flow Cytometry with Company and their Origin.

Flowcytometry kit	Company	Origin
Monoclonal Ab conjugated PE-S dye for CD4 cells.	Thermo Fisher/ **Bioscience™**	USA
Monoclonal Ab conjugated PE-C dye for CD8 cells.	Thermo Fisher/ **Bioscience™**	USA
Monoclonal Ab conjugated FITC dye for CD16 cells.	Thermo Fisher/ **Bioscience™**	USA
Monoclonal Ab conjugated PI dye for MCP1protein.	Thermo Fisher/ **Bioscience™**	USA

2.1.2.1 Human CD4 Monoclonal antibody conjugated (SK3 (SK-3)), PE, eBioscience

Normal human peripheral blood cells have been stained with PE of Anti-Human CD4 monoclonal antibody (clone SK3 (SK-3)) that will react with human CD4, a 59-kDa cell surface receptor which expressed by majority of thymocytes and do not cross- block binding, so suggesting recognition of distinct epitopes. Cells in lymphocyte gate were used for

analysis to detect CD4 cells. Table (2-3) contains full information about this product that has been used in this study.

Table (2-3): Details of Flowcytometry Reagents of CD-4 cells.

Details	
Size/Dilution	100 tests/(5 µl (0.125 µg)/test)
Host/Isotope	Mouse/ IgG1, kappa
Class/ Type	Monoclonal/Antibody
Clone/ Conjugate	SK3 (SK-3)/ PE
Form /Concentration	Liquid/(5 µl/ Test)
Storage Buffer	PBS, pH=7.2, with 0.1%, gelatin 0.2%, BSA
Purification	Affinity chromatography
Contains/Storage condition	0.09% sodium azide/ 4°C. Store in dark

2.1.2.2 Human CD8a Monoclonal Antibody conjugated (SK1), PE-Cyanine7, eBioscience

This kit has been used for detection of CD8 cytotoxic T-cells, in which the peripheral blood of human, have been stained with PE-Cyanine7 of anti-human CD8a monoclonal antibody (clone SK1), which reacts with human CD8a monoclonal (32-34 kDa) cell surface receptor that expressed as either homodimers (CD8 alpha/alpha) or heterodimer (CD8 alpha/beta). Unstained cells represent the negative control profiles. The details of CD8 flow cytometry kit listed in table (2-4).

Table (2-4): Description of Flowcytometry Reagents of CD-8 cells.

Details	
Size/Dilution	100 tests/ 5 µl(0.06 µg)/test
Host/Isotope	Mouse/ IgG1, Kappa
Class/ Type	Monoclonal/Antibody
Clone/ Conjugate	SK1/ PE-Cyanine 7
Form /Concentration	Liquid/5 µl/ Test
Storage Buffer	PBS, pH 7.2,with 0.1% gelatin, 0.2% BSA
Purification/Immunogenic	Purified/ HumanCD8
Contains/Storage condition	0.9% sodium azide/ 4°C. Store in dark

2.1.2.3 Human CD16 monoclonal antibody conjugated (eBioCB16 (CB16) FITC

This kit has been used to recognize CD16 on natural killer cells (Fc gammaRIII), the low-affinity receptor for IgG with an apparent molecular weight of 50-80kDa, full details and information about this kit were listed below in table (2-5).

Table (2-5): Details of CD16 Monoclonal Antibody Conjugated FITC Flowcytometry Reagents.

Details	
Size/ Dilution	100 tests/(5 µl (0.125 µg)/test)
Host/Isotope	Mouse/ IgG1, kappa
Class/ Type	Monoclonal/Antibody
Clone/ Conjugate	EBioCB16 (CB16))/ FITC
Form /Concentration	Liquid/(5 µl/ Test)
Storage Buffer	PBS, pH=7.2, with 0.1%, gelatin 0.2%, BSA
Purification	Affinity chromatography
Contains/Storage condition	0.09% sodium azide/ 4°C. Store in dark

2.1.2.4 Human CCL2 (MCP1) Monoclonal Antibody Conjugated (5D3-F7), PE

The 5D3-F7 antibody has been used in this study for intracellular staining followed by flow cytometry analysis. This 5D3-F7 antibody would react with human monocyte chemoattractant protein-1(MCP-1). All the information of this kit has been listed below in table (2-6).

Table (2-6): MCP-1 Monoclonal Ab Flowcytometry Reagent's

Details	
Size/dilution	25µg/ (0.125 µg /test)
Host/Isotope	Mouse/ IgG1, kappa
Class/ Type	Monoclonal/Antibody
Clone/ Conjugate	5D3-F7/ PI
Form /Concentration	Liquid/0.2 mg/ml
Storage Buffer	PBS, pH=7.2, with 0.1%, gelatin
Purification	Affinity chromatography
Contains/Storage condition	0.09% sodium azide/ 4°C. Store in dark

2.1.2.5 Lysing and Fixation Reagents of Flowcytometry

Unique-Lyse® (USA), is the kit that used in this study which specially formulated and used for red blood cell lysis. The reagents provided with this kit listed in table (2-7).

Table (2-7): Lysis and Fixation Reagents with it's Origin.

Reagent Provided	Quantity Description	Company and origin
Erythrocyte lysing reagent **A**	50 ml sufficient for 500 test/not dilute	**Unique-Lyse /USA**
Erythrocyte lysing reagent **B**	500 ml sufficient for 500 test/not dilute	**Unique-Lyse /USA**

2.1.3 Kits of Enzyme linked immunosorbent assay

All enzyme linked immunosorbent assay kits that have been used in this study are listed in table (2-8).

Table (2-8): ELISA Kits with its Company and Origin.

ELISA kits	Origin	Company
Human IL-2 ELISA kits	**USA**	**Elabscience**
Human IL-10 ELISA Kit	**USA**	**Elabscience**
Human IFN-gamma ELISA kit	**USA**	**Elabscience**
Human TNF-α ELISA kit	**USA**	**Elabscinece**

2.1.3.1 Human IL-2 ELISA kit

This kit used for quantitative measurement of IL-2 concentration in patient's serum. The contents and volume of kit listed in table (2-9).

Table (2-9): Components of Human IL-2 ELISA Kit.

Reagents	Quantity
Micro ELISA coated Plate	(8 wells ×12 strips)x2
Reference Standard	2 vials
Reference Standard & Sample Diluent	1vial 20mL
Concentrated Biotinylated Detection Ab	1vial 120µL
Biotinylated Detection Ab diluent	1vial 10mL
Concentrated HRP Conjugate	1vial 120µL
HRP Conjugate Diluent	1vial 10mL
Concentrated Wash Buffer (25)	1vial 30mL
Substrate Reagent	1vial 10mL
Stop Solution	1vial 10mL
Plate sealer	5 pieces

2.1.3.2 Human IL-10 ELISA Kit

This kit was used for quantitative measurement of IL-10 serum concentration in patients, contents of kit is the same as in table (2-9).

2.1.3.3 Human Interferon-γ (IFN-γ) ELISA Kit

This ELISA kit is used for quantitative measurement of IFN-γ serum concentration, the content and quantity of this kit is the same as listed in (2-9).

2.1.3.4 Human TNF-α ELISA Kit

This kit was used for quantitative measurement of TNF-α serum concentration, and the contents and quantity of this kit as in table (2-9).

2.2 Methods

2.2.1 Subjects (Patients)

2.2.1.1 Study Design

This prospective study was conducted on 30 patients (16 males and 14 female) recruited from of Al-Diwaniaya Teaching Hospital in Al-Qadisiyah governorate, who were subjected to anesthetic drug during surgery procedure (details in appendix 2), age ranged was between 10-72 years old as illustrated in figure (2-1). The work was done during period from January to the end of April of 2018. Selection of patients based on type of surgery and type of anesthetic drug have been given to that patients. The study was in agreement with ethics of Al-Diwaniaya teaching hospital and verbal informed consent obtained from all patients participated in study before taking blood samples.

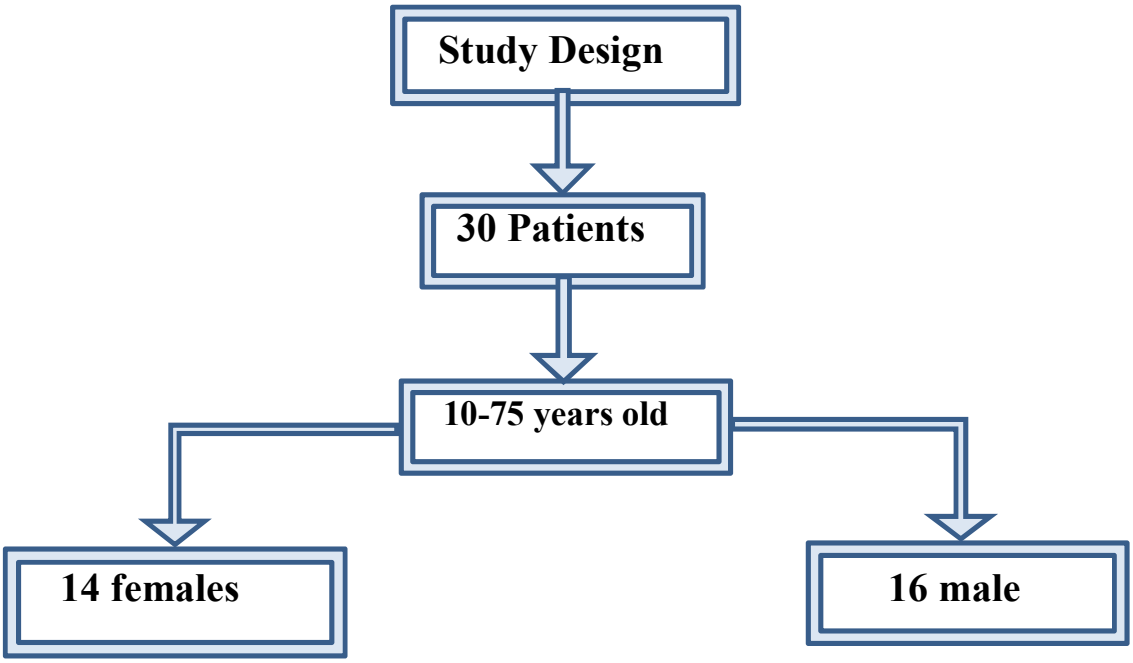

Figure (2-1): A Flow Chart Illustrating the Study Design.

The patients underwent to different types of orthopedic surgical operations and receiving different types of anesthesia (with different time of duration) including: general, local and regional anesthesia.

Table (2-10): Types of Surgery of Patients Participating in Study.

Patient	Type of surgery
1	Biopsy of Aneurysmal bone tumor
2	Bone fracture fixation
3	Bone Marrow Biopsy
4	Frozen Shoulder
5	Lateral Malleolar fracture
6	Bilateral Maxillofacial Biopsy
7	Radius fracture fixation
8	Carpal tunnel Syndrome
9	Left Knee Orthoscopy
10	Right Knee Orthoscopy
11	Tennis elbow
12	Scapula fracture
13	Hip Dislocation
14	Distal Radius fracture fixation
15	Ganglion cyst
16	Oblique mid-shaft Femur fracture
17	Metacarpal fracture
18	Frozen Shoulder
19	distal radius fracture repair
20	Carpal tunnel release
21	Shoulder dislocation
22	Metacarpal fracture
23	Right Knee Orthoscopy
24	Radius fracture repair
25	Ganglion cyst
26	External fixation removal
27	Carpal tunnel RELEASE
28	Right Knee Orthoscopy
29	Fixation under screen
30	Knee lipoma cyst

Table (2-10) including types of surgery have been conducted for patients. Further demographic characteristics of patients group including type of surgery, type and duration of anesthesia and other clinical disease, all are explained in appendix 2.

2.2.1.2 Inclusion Criteria of patients

-Both genders with different ages were included within study.

- Patients underwent certain types of surgery (only orthopedic surgery that listed in table 2-10).

2.2.1.3 Exclusive Criteria of patients

- Patients not consenting.

-Patient showing hypersensitivity reaction during administration of anesthesia has been excluded from study.

- Patient that passes away during surgery would excluded from study.

-Psychiatric patients are excluded.

-Patients who taking steroids or other immunosuppressive drugs are excluded from the study.

2.2.1.4 History and clinical assessment of patients

- Age and Gender.

- Clinical and medical history of patients.

- Type and duration of surgery.

-Type of anesthetic drug and rout of administration.

2.2.2 Blood Samples Collection

About 6ml of blood samples (90 samples), were drown aseptically from each patients with interval pre, perioperative and post-operative, 2 ml were collected in sterile test tube (plain tube) and allow to clot at room temperature for minutes to 1 hr., the sera were separated by centrifugation for 10 min. at 2500 r.p.m, (then serum would be divided into 4 Eppendorf tube, one tube for IL-2 ELISA assay , one for IFN-γ ELISA assay, one for IL-10 ELISA assay and one for TNF-α ELISA procedure), the separated sera were labeled and stored at -30◦C until in-vitro tests were performed. Another 2 ml of blood were collected in ethylinditetracitic acid (EDTA) tube for Flowcytometry analysis, one ml of blood sample used for WBCs profile analysis, and the last one ml of blood sample collected in gel tube for C-reactive protein analysis (CRP) procedure. Another 1ml for CRP test analysis, Figure (2-2). Blood sample drown from patients within 3 different period interval including pre, peri and post-operative interval.

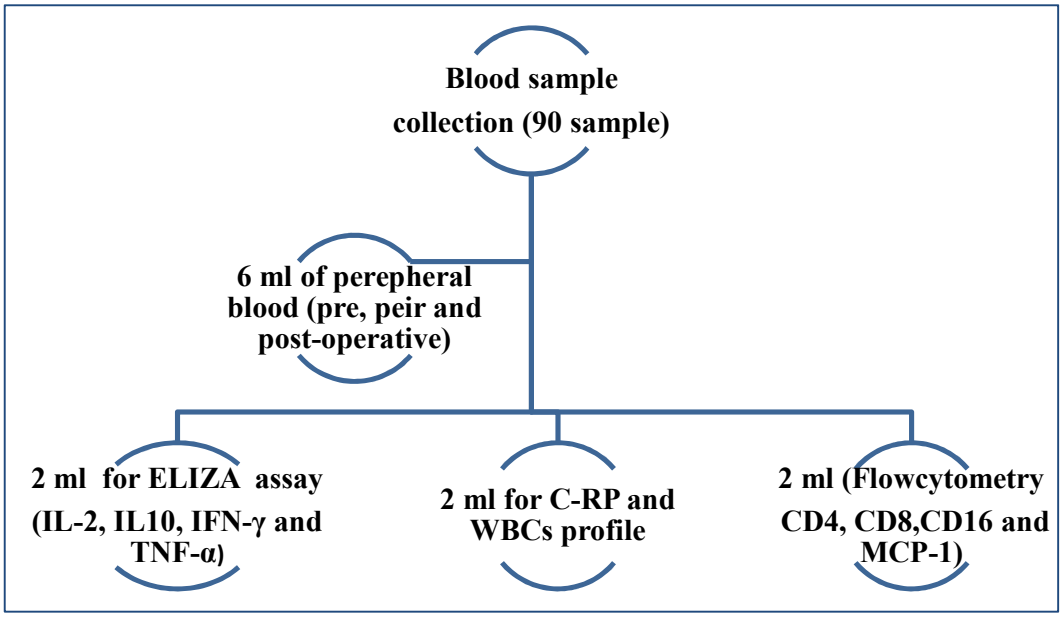

Figure (2-2): Flow Chart Illustrating Collection of Blood Sample.

2.2.3 Hematological Tests

2.2.3.1 C-Reactive Protein (CRP) test

In vitro test for quantitative immunological determination of CRP in human serum has been done by using automated machine (Cobas C311/Roche-Hitachi/Japan) system. 1 ml of blood sample collected in gel plain tube, then serum was extracted and transferred to special Hitachi tube specific for the machine. Software was designed to revealed the results immediately and give auto-reports within minutes.

Principle of test depends on that human C-reactive protein agglutinates with latex particles coated with monoclonal anti-CRP antibodies, then the precipitate is determined turbidimetrically at 552 nm (546nm on Cobas c501 and c311 analyzer).

2.2.3.2 White Blood Cells Profile (WBCs) Test

One (1ml) of blood sample have been collected aseptically in EDTA tubes for CBP test to estimate total white blood cells (WBC) count and absolute neutrophil count for patients pre and post operatively in order to study the effect of different cytokine levels on WBCs and neutrophil counts. This test done automatically by using (ABX Micros ES60/HORBA Medical-French) apparatus, which gave reports within minutes.

2.2.4 Immunological analysis (Flowcytometry Analysis)

Flowcytometry analysis is a laser based, biophysical technology employed in cell counting, sorting and biomarker detection, used in this study to detect CD4 helper T-cells, CD8 cytotoxic T-cells, CD16-NK cells and monocyte chemoattractant protein (MCP-1). The assay involves suspending cells in a stream of fluid and passing them by electronic detection apparatus, later each type of cell passes through beam of light. Fluorescents measurement taken at different wavelengths can provide quantitative and qualitative data about fluorochromes- labeled cell surface receptor or intracellular molecules such as cytokines. The specificity of test is achieved by optical filter which block certain wavelengths while transmitting others (Gondhalekar*, et al*. 2018).

Flowcytometry analysis was done by using one ml of EDTA treated blood which was freshly processed and analyzed by flowcytometry (BriCyte E6) machine within 24 hrs. according to kit instructions.

2.2.4.1 Principle of Flow Cytometry Assay

Polymer based dyes such as Invitrogen® Super Bright fluorochromes that have been used in this study, would exhibit non-specific interactions when using more than one polymer dye-conjugated

antibody together in the same Flowcytometry experiment. This nonspecific interaction result in mutually exclusive immune-stained populations that appear as under-compensated data. In order to correct this, Invitrogen® Super Bright staining buffer was included during staining each time when more than one Super Bright fluorochromes-conjugated antibody is used.

2.2.4.2 Procedure and Steps of Flowcytometry

1. One hundred microliter (100 µl) of reagent A were added to each sample and mix well by vortex and incubated for 10 min. in dark at room temperature.
2. One 1 ml of reagent B added to each sample and shakes well then incubated at room temperature in dark for about 20 min.
3. The mixture centrifuged at 300-600xg for about 5 min and the supernatant was discarded.
4. Cells were re-suspended in an appropriate volume (1 ml of PBS) flow-cytometry staining buffer.
5. Centrifuge at 300xg for 5 min. and the supernatant poured off and re-suspend in 300 ml of PBS (contain 1%paraformaldehyde for preservation of samples that would not be analyzed in the same day.
6. Fifty (50µL) of anti-coagulated whole blood were added to the bottom of 12x75mm polystyrene tube, along with the conjugated antibody, as directed by manufacturer's instruction. Mix well by vortex and then incubated at room temperature in the dark for about 10 min.
7. Samples were analyzed using Flow Cytometry (BriCyte E6/ India, Figure 2-1).

8. Lymphocyte and neutrophils phenotype carried out by gating according to forward scatter (FSC) histogram that depend on the size of cells, and side scatter (SSC) histogram depending on granularity of cells.
9. All results were expressed as percentage of cell positive for CD4,8,16 and MCP1 cells, considering the percentage of ≥ 20% as a +ve results, while the percentage ≤ 20% as a negative result according to (Bain, 2010)
10. Neutrophil selected electronically on the basis of their forward and side scatter characteristics and about 10,000 cells were being analyzed in each sample.

Figure (2-3): Flow Cytometer (BriCyte E6) Machine.

2.2.5 Immunological assay (ELISA assay)

2.2.5.1 Principles of Enzyme-linked immunosorbent test

Kits of ELISA have been 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 (IL-2, IL-10, IFN-γ and TNF-α). Samples or standard were then added to the appropriate micro-titer plate wells and combined with the specific antibody. Then a biotinylated detection antibody specific to (IL-2, IL-10, IFN-γ and TNF-α) and Avidin- Horseradish peroxidase (HRP) conjugate was added to each well and incubated, then free components were washed away.

After that substrate solution added to each well, only those wells that contain (IL-2, IL-10, IFN-γ and TNF-α), biotinylated detection antibody and Avidin- HRP conjugate appeared blue in color. Enzyme substrate reaction was terminated by addition of stop solution (sulphuric acid) and the color turned yellow. The optical density (OD) is measured immediately by spectrophoto-meter at 450 ± 2 nm wave length. The value of OD is proportional to concentration of serum marker (IL-2, IL-10, IFN-γ and TNF-α) and the concentration is in samples have been determined by comparing the optical density (OD) of the sample to the standard curve.

2.2.5.2 Reagents Preparation

• Washing solution had been diluted before used with DW (1:9).

• All reagents and samples were brought at room temperature and diluted as mentioned in kits instructions before using.

• All samples were centrifuged after thawing before assay.

•All the reagents have been mixed thoroughly by gently swirling before pipetting (avoid foaming).

2.2.5.3 Assay procedure

1. Adding Sample: One hundred 100µL of Standard, Blank, or Sample were added per micro ELISA plate well. The blank well is added with Reference Standard and Sample diluent. After that solutions mixed gently and cover the plate with sealer, and then incubated for 90 minutes at 37°C.

2. Biotinylated Detection Ab: liquid of each well were removed and immediately adding 100 µL of biotinylated detection Ab working solution to each well and incubate at 37°C for 1 hr.

3. Washing: All plate wells were aspirated and washed, and this process repeated three times. Wash done by filling each well with washing Buffer (approximately350µL) using squirt bottle.

4. HRP Conjugate: 100µL of HRP Conjugate working solution was added to each well and covered with the plate sealer, then incubated for 30 minutes at 37°C.

5. Washing: washing process was repeated 5 times as conducted in third step.

6. Substrate: 90µL of substrate Solution was added to each well and covered with a new Plate sealer, then incubated at 37°C for about 15 minutes.

7. Stop reaction: 50µLof Stop Solution was added to each well. Then, the color turns to yellow immediately (Figure 2-4).

8-Optical Density (OD) Measurement: OD for each well is calculated at once by using a micro-plate reader spectrophotometer at wave length 450nm.

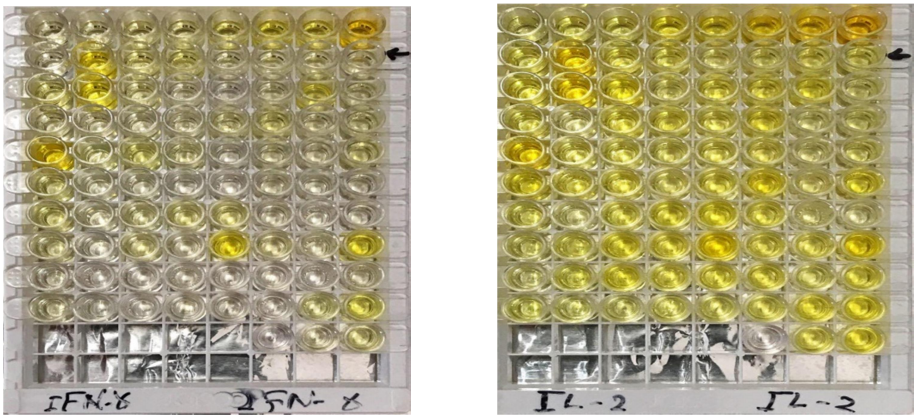

Figure (2-4): Final Color of ELISA Assay Products.

2.2.5.4 Calculating of optical density and standard curve

Enzyme linked-immune sorbent assay results were calculated depending on average of the duplicate reading for each standard and samples optical density, the standard curve was plotted by mean OD value for each standard on y-axis against the concentration on the x-axis and drew a best fit curve through the points on the graph.

1-Calculation of OD and SC of Human IL-2

Figure (2-5) represents the standard curve of human IL-2, which calculated depending on O.D on y-axis versus concentration on x-axis as shown below.

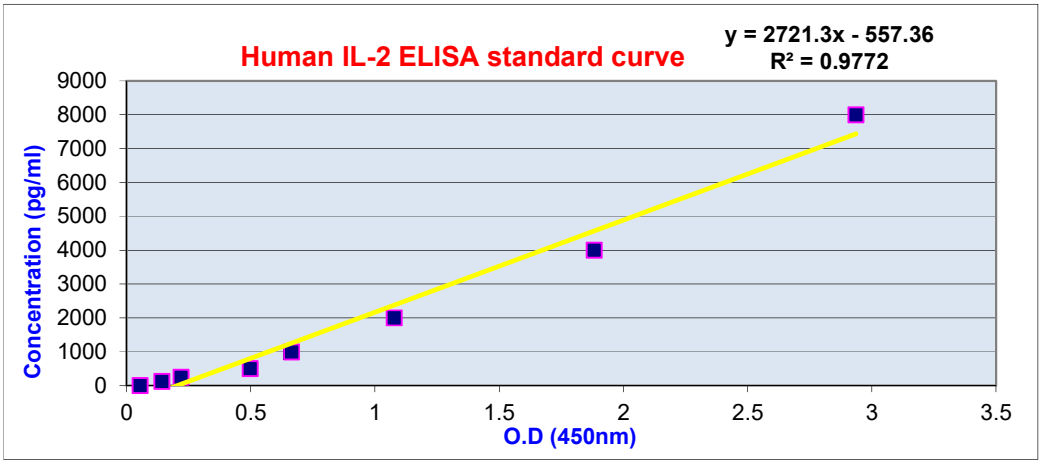

Figure (2-5): Standard Curve for human IL-2.

2- Calculation of OD and Standard Curve of Human IL-10

Results of human IL-10 ELISA kit were calculated depending on OD reading for each standards and samples. SC was plotted by means of OD value for ach standard on y-axis against concentration on x-axis as below in figure (2-6).

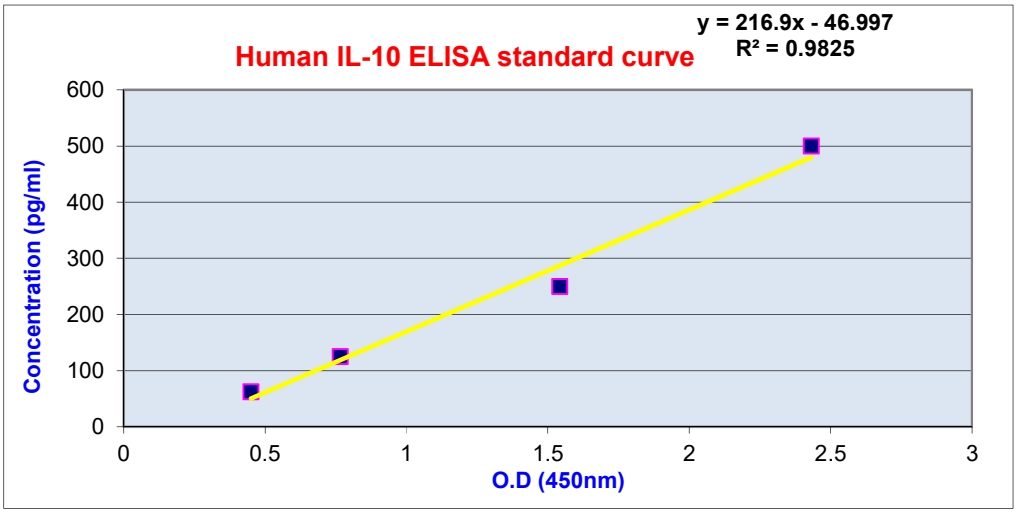

Figure (2-6): Standard Curve of Human IL-10.

3- Calculation of OD and SC of human IFN-γ

Enzyme linked-immune sorbent assay results were calculated by depending on reading of optical density for each standard and sample, and the SC was plotted by means of OD value for each standards and samples on y-axis against the concentrations on x-axis and drew a best fit curve throughout the points on the graph as shown in figure (2-7).

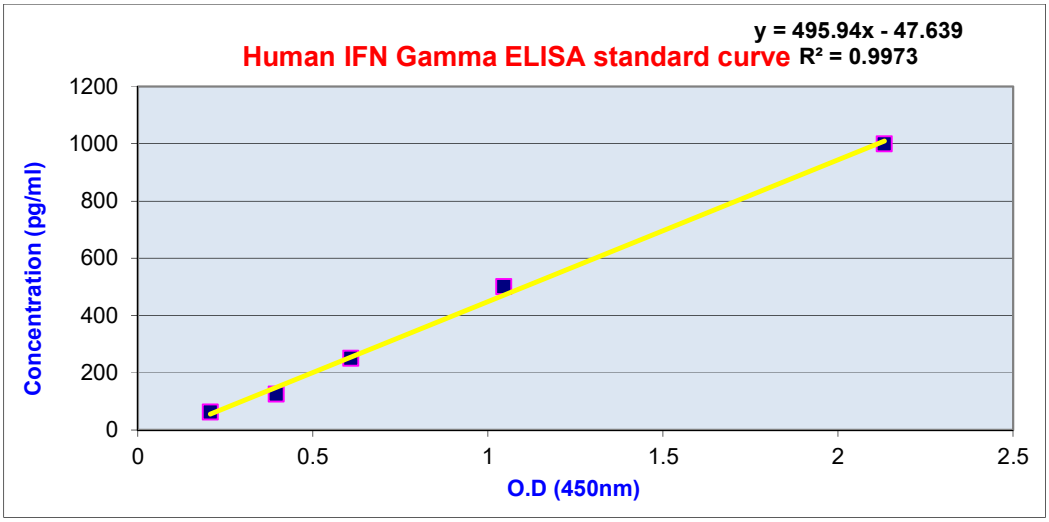

Figure (2-7): Standard Curve of human IFN-γ.

4-Calculation of optical density and SC of human TNF- α

Figure (2-8) below represent the standard curve (SC) of human TNF-α cytokine, which plotted by means of OD value for standard on y-axis versus concentration on x-axis as shown below.

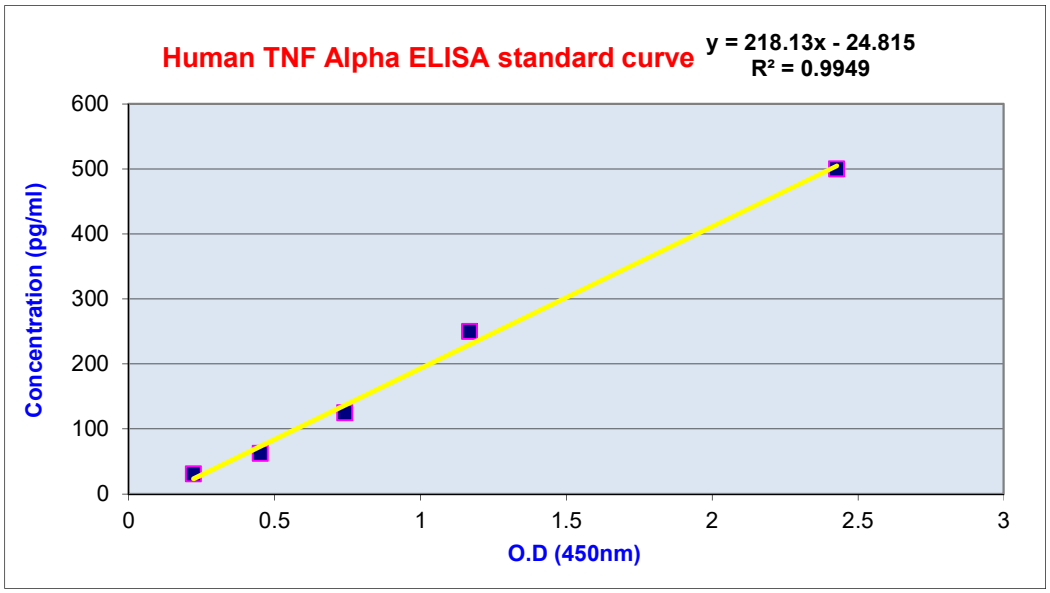

Figure (2-8): Standard Curve of human TNF-α.

2.2.6 Statistical Analysis

An expert statistical advice was sought for, the data were translated into computerized data base structure, which was examined for errors using rang and biological data cleaning method, and any inconsistencies were treated. All data were analyzed by using Statistical Package for Social Sciences (SPSS) software version 20 in association with Microsoft Excel 2016.

Compliance of quantitative random variables were normally distributed (Gaussian curve), and were thus analyzed by using Kolmogorov-Smirnov test. All variables were non-normally distributed, such variables that could be described by means of interquartile rang and median. Non-parametric test used to assess differences in median between two groups using Mann-Whitney equation, while differences among three groups assessed by using Kruskal-Wallis test.

Statistical significance, direction and the strength of linear correlation between two quantitative variables, one is being distributed non-normally, this correlation was measured by Spearman's rank linear correlation coefficient (Sorlie, 1995).

Significant associations of measured odd ratio (OR) and 95% of confidence interval (CI) were assessed using Chi-squared formula, and all statistics were inspected using bilateral probability, and such estimate considered significant if its P value ≤ 0.05 (Weng, *et al.* 2006).

Chapter Three

Results

3. Results

3.1 Demographic characteristics of the patients group

All results included in this chapter based on analysis of data belong to 30 patients, mean age 35.67 ±17.53 years with wide range of age variation from 10 to 72 years. This study included 16 males and 14 females with proportions of 53.3% and 46.7%, respectively; the male to female ratio was 1.14:1. According to type of anesthesia, this study enrolled 10 patients with general anesthesia, 10 patients with regional anesthesia and 10 patients with local anesthesia.

Table (3-1): General characteristics of patients.

Characteristic	Value
Number of cases	30
Age	
Mean ±*SD* (years)	35.67 ±17.53
Range (Min.-Max.) years	62 (10-72)
Gender	
Male, *no* (%)	16 (53.3)
Female, *no* (%)	14(46.7)
M:F ratio	1.14:1
Type of anesthesia	
General, *no* (%)	10 (33.3%)
Local, *no* (%)	10 (33.3%)
Regional, *no* (%)	10 (33.3%)
Duration of anesthesia	
Mean ±SD (Minute)	44.33 ±19.85
Range (Min.-Max.) minutes	75 (15-90)
Chronic illness	
Hypertension, *no* (%)	3 (10%)
Diabetes mellitus, *n* (%)	3 (10%)
IHD, *no* (%)	1 (3.3%)
Asthma, *no* (%)	1 (3.3%)
Agranulocytosis, *no* (%)	1 (3.3%)

SD: Standard deviation; no: number of cases; IHD: ischemic heart disease.

The mean duration of anesthesia was 44.33 ±19.85 minutes and it ranged from 15-90 minutes. Hypertension was seen in 3 patients (10%), diabetes was seen also in 3patients (10%), ischemic heart disease was seen in a single patient (3.3%), a single patient (3.3%) suffered from asthma and agranulocytosis was seen in a single patient (3.3%),as shown above in table (3-1).

3.2 Hematological (Blood) tests Results

3.2.1C-Reactive Protein Analysis

A high level of CRP in blood is a marker of inflammation, that caused by a wide variety of conditions. CRP measured in milligrams of CRP per liter of blood (mg/L). The American heart association and U.S centers for disease control and prevention have defined risk groups as follows: low risk less than 1.0 mg/L, average risk is 1.0- 3.0 mg/L, and the higher risk is above 3.0 mg/L (Prtecia *et al*., 2018).

3.2.1.1 Blood Levels of C-reactive protein (CRP) in Patients Enrolled in This Study

CRP was measured before operation as a baseline reading and then another estimation of CRP was conducted for each participating patient after completion of surgery. There was highly significant rise in CRP blood level post-operatively when compared with pre-operative blood level, 2.65 (4.99) and 3.99 (5.64) respectively (P <0.001), as seen in figure (3-1).

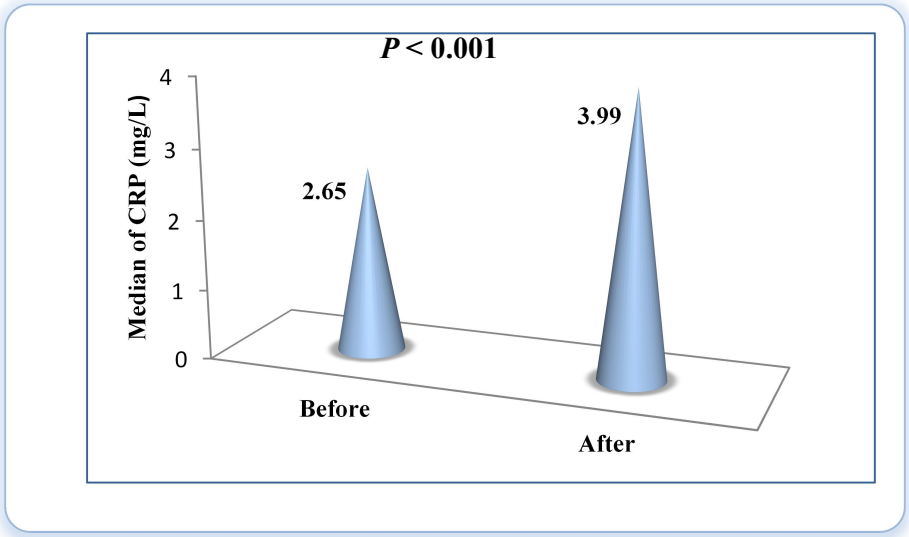

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Figure (3-1):Half-life of CRP level before and after surgery

3.2.1.2 Correlation of Patient's Age and Gender with CRP Levels

The study showed no significant correlation between CRP level and age of patients (p= 0.164, r = 0.261), as seen in figure (3-2).

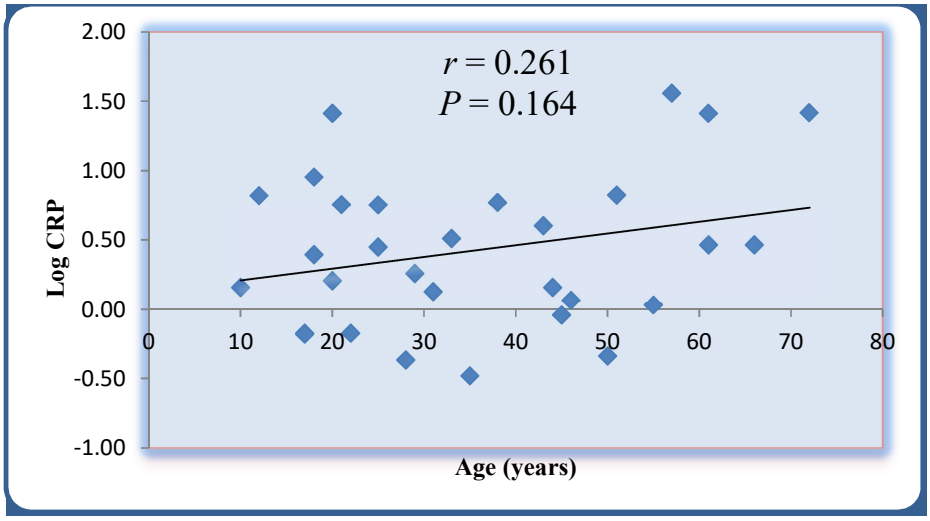


Figure (3-2): Correlation between Log CRP and Age.

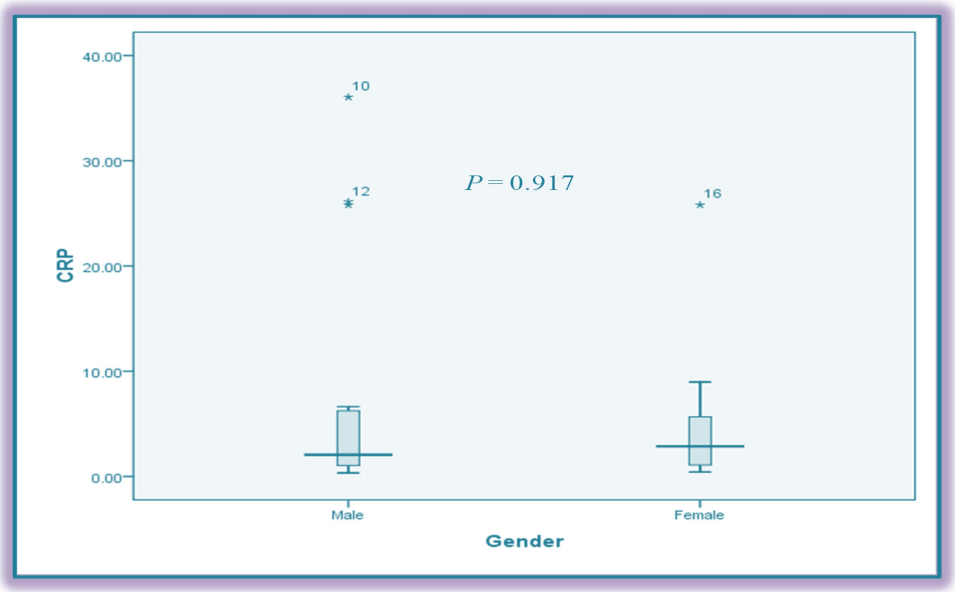


Figure (3-3): Comparison of CRP level between male and female patients

When compared CRP blood levels between male and female, the results showed that a higher CRP level were seen in female compared with male, but generally there is no significant difference in base line level of CRP between male and female, 2.06mg/l (5.46) versus 2.86mg/l (4.92), respectively (p=0.917) as in figure (3-3).

3.2.1.3 Comparison of CRP levels with kinds and time of duration of anesthesia

Higher level of CRP could be seen in patients group that receiving general anesthesia (5.76mg/L), while the lower CRP levels were seen in patients receiving local anesthesia (1.31mg/L), but generally there is no significant differences in post-operative CRP level and different types of anesthesia (p=0.104) as in figure (3-4). While figure (3-5) show the effect of time duration of anesthesia on the CRP level, that reveal there is no significant

association between CRP level and duration of anesthesia(p=0.197,r=0.24).

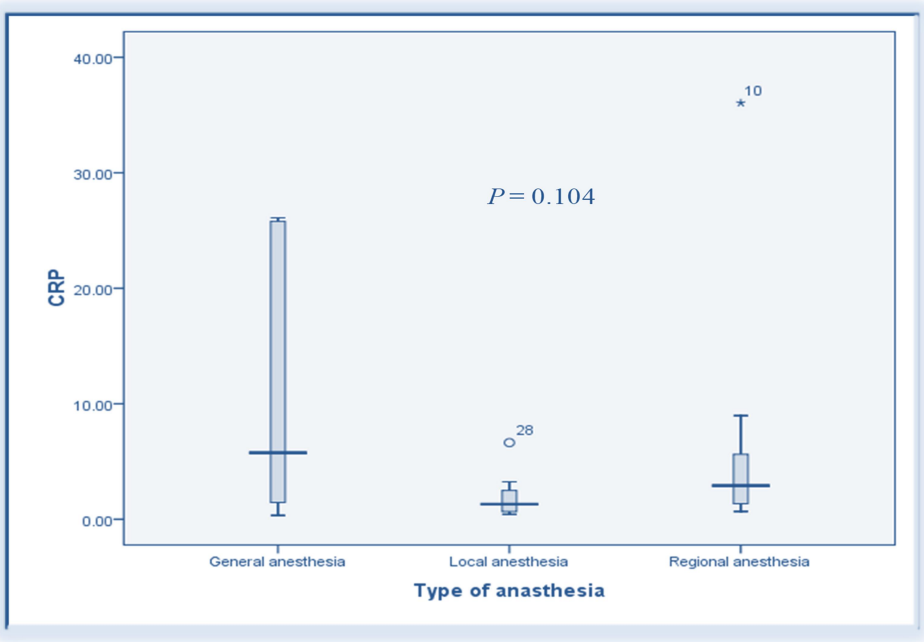


Figure (3-4): Comparison of CRP level among Types of Anesthesia.

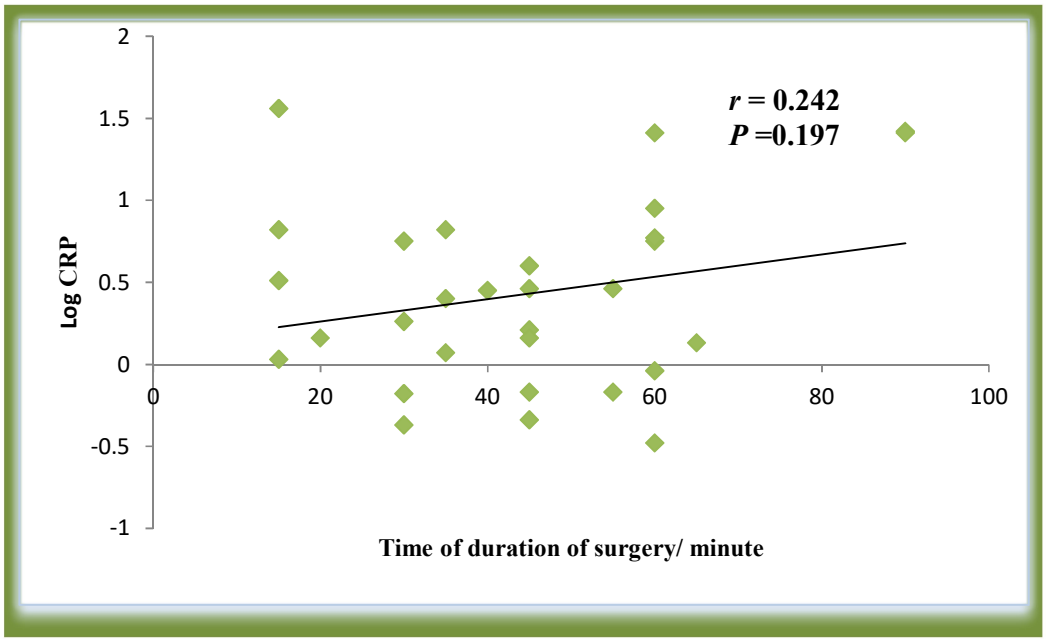


Figure (3-5): Correlation Between Duration of Anesthesia and Post-Operation CRP Level.

3.2.2 White Blood Cells Count (WBCs) assay results

The importance of this test is to investigate the leukocyte differential count (LDC) that gives the counts and percentage of each WBC type, like: neutrophil, lymphocytes, monocytes, eosinophil and basophil.

3.2.2.1 Counts of WBCs in relation to anesthesia time baseline

The results revealed a significant rise in neutrophil count after operation in comparison with its baseline level before operation, 6.74 ±2.89 versus 5.62 ±2.75 respectively ($P < 0.001$), while the level of lymphocytes showed significant decline following operation, 2.85 ±1.13 versus 3.03 ±1.15 respectively, ($P <0.001$). The level of monocyte and eosinophil also got significantly reduced after operation ($P < 0.001$), whereas, the level of basophil showed no significant alteration after operation (P = 0.687), as shown in table (3-2).

Table (3-2): WBCs Count Before and After Anesthesia.

WBC	Before	After	*P*
Neutrophil, Mean ±SD	5.62 ±2.75	6.74 ±2.89	<0.001
lymphocyte, Mean ±SD	3.03 ±1.15	2.85 ±1.13	<0.001
Monocyte, Mean ±SD	0.65 ±0.25	0.63 ±0.24	<0.001
Eosinophil, Mean ±SD	0.31 ±0.40	0.30 ±0.40	<0.001
Basophil, Mean ±SD	0.03 ±0.03	0.03 ±0.04	0.687

WBC: white blood cells; SD: standard deviation

3.2.2.2 Correlation between WBCs Count /Percentage with Gender and Age

No significant correlation was found between WBCs counts and percentage with gender of patients ($P > 0.05$), as demonstrated in table (3-3). Regarding the correlation between WBC count / percentage and age of patients, the results also showed no obvious association ($P > 0.05$), as listed in table (3-4).

Table (3-3): Correlation of WBCs ount and percentage with Gender.

WBC	Total *No* = 30 Mean	SD	Male *No* = 16 Mean	SD	Female *No* = 14 Mean	SD	*P*†
Neutrophil	5.62	2.75	5.66	2.70	5.57	2.91	0.927
% Neu	47.09	23.09	46.44	24.53	47.84	22.22	0.872
Lymphocyte	3.03	1.15	2.96	1.21	3.11	1.12	0.728
% lymph	34.69	16.63	34.14	17.25	35.32	16.52	0.850
Monocyte	0.65	0.25	0.67	0.23	0.63	0.27	0.713
% Mon	6.98	2.16	7.18	2.06	6.74	2.31	0.586
Eosinophil	0.31	0.40	0.19	0.14	0.44	0.55	0.083
% Eos	3.16	3.45	2.12	1.46	4.36	4.61	0.076
Basophil	0.03	0.03	0.03	0.03	0.04	0.03	0.312
% Baso	0.38	0.30	0.34	0.29	0.43	0.31	0.385

*significant only at $p \leq 0.05$. SD: standard deviation. † Independent samples t-test; *No*: cases number

Table (3-4): Correlation of WBC Count and % with Age of Patients.

WBC	Age	
	r	***P***
Neutrophil	-0.059	0.757
% Neu	0.157	0.407
Lymphocyte	0.143	0.452
% lymph	0.066	0.729
Monocyte	-0.008	0.968
% Mon	-0.030	0.876
Eosinophil	-0.013	0.945
% Eos	-0.010	0.960
Basophil	0.335	0.071
% Baso	0.306	0.100

r: correlation coefficient

3.2.2.3 Correlation of WBCs Counts/Percentage with Types and Duration of Anesthesia

No significant correlation was found between type of anesthesia and WBC counts (P > 0.05), as showed in table (3-5); also no significant correlation was found between duration of anesthesia and WBC counts (P > 0.05), as in table (3-6).

Table (3-5): Correlation of WBCs count/ % with Type of Anesthesia.

WBC	General *No* = 10 Mean	SD	Local *No* = 10 Mean	SD	Regional *No*= 10 Mean	SD	*P*†
Neutrophil	5.93	3.04	5.27	2.35	5.65	3.07	0.875
% Neu	52.74	21.01	41.63	24.44	46.90	24.70	0.577
lymphocyte	3.05	1.07	3.05	1.12	2.98	1.35	0.988
% lymph	33.22	12.80	36.17	18.09	34.68	19.89	0.929
Monocyte	0.73	0.28	0.65	0.27	0.57	0.18	0.399
% Mon	7.41	2.03	7.15	2.18	6.37	2.33	0.550
Eosinophil	0.16	0.13	0.31	0.41	0.45	0.54	0.276
% Eos	1.78	1.40	3.22	3.49	4.49	4.49	0.220
Basophil	0.03	0.02	0.02	0.01	0.05	0.04	0.088
% Baso	0.28	0.20	0.32	0.22	0.55	0.39	0.090

SD; standard deviation. *No:* number of the cases; † Kruskal Wallis H test

Table (3-6): Correlation of WBC counts/percentage and duration time of anesthesia.

WBC	Duration of surgery	
	r	*P*
Neutrophil	0.219	0.245
% Neu	0.215	0.253
Lymphocyte	0.014	0.940
% lymph	-0.108	0.568
Monocyte	0.217	0.250
% Mon	0.043	0.820
Eosinophil	-0.188	0.320
% Eos	-0.230	0.222
Basophil	-0.136	0.473
% Baso	-0.222	0.238

r: correlation coefficient

3.2 .2.4 Correlation of WBC counts/ percentage with CRP Levels and Inflammatory and Immune Markers

Results revealed no significant association between leukocytes counts and blood level of CRP ($P > 0.05$), as listed in table (3-7), while the correlation between leukocytes counts and immune marker showed that there is only a significant correlation between Log CD8 preoperatively with eosinophil counts and percentage (p=0.016,0.017) respectively like seen in table (3-8).

Table (3-7): Correlation of WBC counts/percentage and CRP levels.

WBC	CRP	
	r	*P*
Neutrophil	0.118	0.536
% Neu	0.004	0.984
Lymphocyte	-0.002	0.991
% lymph	-0.128	0.501
Monocyte	0.109	0.565
% Mon	-0.039	0.839
Eosinophil	-0.064	0.735
% Eos	-0.082	0.668
Basophil	0.318	0.087
% Baso	0.214	0.256

r: correlation coefficient.

In table (3-9) the results showed a significant positive association between IL-2 pre with monocyte count (p= 0.009) and percentage (p=0.007). Also there was obvious positive association between IL-10 pre with monocyte count and % (p=0.037 and 0.05) and negative association with basophile, IL-10 postoperative also showed a negative association with eosinophil. TNF-α peri significantly associated with eosinophil (p=0.029 and 0.011), also there is a significant positive association between IFN-γ preoperative and monocyte (p=0.05, 0.018), and IFN-γ post with eosinophil (p=0.008, 0.01) as seen below in the table.

Table (3-8): Correlations of WBC counts/percentage with immune markers.

Parameter		neutrophil	% Neu	lymphocyte	% lymph	Monocyte	% Mon	Eosinophil	% Eos	Basophil	% Baso
Log CRP	*r*	0.066	0.056	0.009	-0.166	0.081	-0.075	0.059	0.034	0.271	0.138
	P	0.728	0.768	0.963	0.380	0.669	0.692	0.755	0.859	0.148	0.466
Log CD4Pr	*r*	-0.178	-0.116	0.029	0.115	-0.180	-0.038	0.196	0.217	0.166	0.227
	P	0.348	0.543	0.881	0.545	0.341	0.843	0.299	0.249	0.381	0.228
Log CD4Pe	*r*	-0.303	-0.172	-0.134	0.209	-0.253	0.115	-0.274	-0.216	0.105	0.223
	P	0.104	0.364	0.481	0.268	0.177	0.543	0.143	0.251	0.581	0.237
Log CD4Po	*r*	-0.266	-0.104	-0.012	0.097	-0.226	-0.009	-0.010	0.008	0.170	0.152
	P	0.155	0.583	0.950	0.611	0.230	0.964	0.956	0.967	0.370	0.421
Log CD8Pr	*r*	-0.083	0.066	0.284	0.105	-0.082	-0.153	0.437	0.432	0.230	0.236
	P	0.663	0.728	0.128	0.580	0.668	0.421	0.016	0.017	0.222	0.210
Log CD8Pe	*r*	-0.037	0.077	0.013	0.010	-0.288	-0.282	-0.035	-0.028	0.173	0.154
	P	0.846	0.687	0.947	0.957	0.123	0.131	0.854	0.883	0.361	0.417
Log CD8Po	*r*	-0.116	-0.203	-0.118	-0.030	-0.072	0.069	-0.169	-0.152	0.194	0.175
	P	0.542	0.281	0.534	0.874	0.707	0.718	0.371	0.421	0.303	0.355
Log CD16Pr	*r*	-0.087	-0.291	-0.164	0.078	-0.273	-0.087	0.103	0.147	-0.026	0.163
	P	0.647	0.118	0.386	0.682	0.145	0.646	0.586	0.437	0.890	0.389
Log CD16Pe	*r*	-0.349	-0.335	0.042	0.281	-0.097	0.220	-0.062	-0.006	-0.055	0.133
	P	0.059	0.071	0.826	0.132	0.609	0.243	0.744	0.976	0.773	0.483
Log CD16Po	*r*	-0.147	-0.017	-0.240	0.123	-0.218	0.080	-0.063	-0.014	-0.108	0.056
	P	0.439	0.929	0.201	0.518	0.248	0.675	0.739	0.941	0.571	0.767
Log MCP-1Pr	*r*	-0.062	-0.223	-0.118	0.048	-0.092	0.032	0.072	0.107	0.008	0.115
	P	0.743	0.236	0.535	0.801	0.627	0.866	0.704	0.573	0.968	0.544
Log MCP-1Pe	*r*	-0.030	-0.217	-0.109	0.020	-0.060	0.034	-0.100	-0.094	-0.203	-0.141
	P	0.873	0.250	0.566	0.918	0.752	0.860	0.598	0.622	0.282	0.456
Log MCP-1Po	*r*	-0.045	-0.091	-0.098	0.058	-0.114	-0.006	0.074	0.100	0.002	0.098
	P	0.813	0.634	0.606	0.760	0.547	0.975	0.696	0.599	0.993	0.608

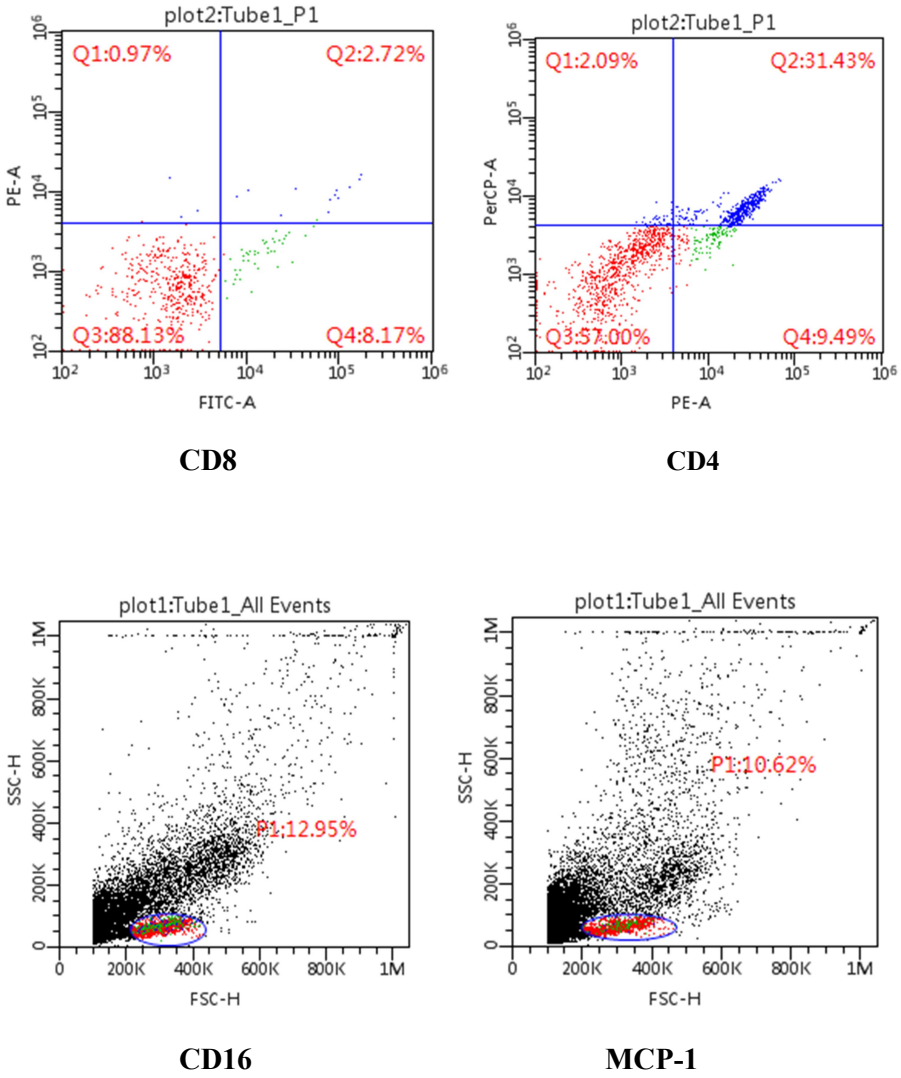
Table (3-9): Correlations of WBC counts and percentage with inflammatory markers.

Parameter		neutrophil	% Neu	lymphocyte	% lymph	Monocyte	% Mon	Eosinophil	% Eos	Basophil	% Baso
LogIL-2pre	*r*	0.044	-0.362	-0.209	-0.259	0.469	0.483	-0.331	-0.376	-0.340	-0.385
	P	0.819	0.049	0.268	0.167	0.009	0.007	0.074	0.041	0.066	0.035
LogIL-2peri	*r*	-0.158	-0.323	0.084	0.271	-0.048	0.139	0.084	0.129	-0.176	0.034
	P	0.404	0.082	0.659	0.147	0.801	0.464	0.659	0.495	0.353	0.859
LogIL-2post	*r*	-0.079	-0.137	0.062	0.074	-0.243	-0.204	0.352	0.356	-0.022	0.011
	P	0.679	0.470	0.745	0.699	0.196	0.280	0.056	0.053	0.910	0.953
LogIL-10pre	*r*	0.185	-0.359	-0.231	-0.222	0.383	0.361	-0.291	-0.337	-0.405	-0.401
	P	0.329	0.051	0.219	0.238	0.037	0.050	0.118	0.069	0.026	0.028
LogIL-10peri	*r*	-0.417	-0.328	0.011	0.528	-0.335	0.183	0.153	0.264	-0.086	0.259
	P	0.022	0.077	0.952	0.003	0.071	0.332	0.419	0.159	0.653	0.167
LogIL-10post	*r*	-0.173	-0.128	0.016	0.033	-0.268	-0.174	0.432	0.437	0.087	0.067
	P	0.361	0.499	0.933	0.863	0.152	0.358	0.017	0.016	0.649	0.725
Log TNF-αpre	*r*	0.104	0.102	-0.164	-0.134	0.210	0.201	-0.312	-0.326	-0.040	-0.063
	P	0.586	0.593	0.387	0.481	0.266	0.286	0.094	0.079	0.833	0.741
Log TNF αperi	*r*	-0.174	-0.151	0.046	0.279	-0.309	-0.108	0.399	0.458	0.033	0.241
	P	0.357	0.426	0.809	0.135	0.097	0.571	0.029	0.011	0.862	0.199
Log TNF-αpost	*r*	0.156	0.092	-0.292	-0.123	-0.310	-0.277	0.180	0.195	0.114	0.109
	P	0.411	0.630	0.117	0.516	0.095	0.139	0.342	0.303	0.548	0.567
Log IFN-γ pre	*r*	0.076	-0.428	-0.313	-0.262	0.354	0.427	-0.309	-0.346	-0.347	-0.375
	P	0.691	0.018	0.092	0.161	0.055	0.018	0.097	0.061	0.060	0.041
Log IFN-γ peri	*r*	-0.311	-0.290	-0.096	0.405	-0.327	0.133	-0.060	0.035	-0.077	0.213
	P	0.095	0.120	0.613	0.026	0.078	0.485	0.752	0.853	0.684	0.258
Log IFN-γ post	*r*	-0.051	0.069	0.068	-0.053	-0.236	-0.316	0.478	0.466	0.225	0.148
	P	0.788	0.715	0.721	0.779	0.208	0.089	0.008	0.010	0.232	0.436

3.3 Subject Immunological Analysis Results

3.3.1 Results of Flow Cytometry Analysis of Cells' Immune Markers

Flowcytometry results were expressed as a percentage of positive cells, considering the percentage of (R2+R4) ≥ 20% as a positive results while the percentage of (R2+R4) < 20% as a negative results, as seen below in figure(3-5), further figures for flowcytometry results listed in appendix chapter (Appendix 3).



CD8 **CD4**

CD16 **MCP-1**

Figure (3-6): Results of Flowcytometry Analysis for CD4, CD8, CD16 and MCP-1 Detection on Lymphocytes.

3.3.1.1 Correlation Between Median Levels of Immune Markers and Operation Time-line

Observation of CD4 lymphocytes counts reveled significant rise during time of anesthesia (42.23mg/L), however it showed significant reduction post-operatively (37.12 mg/L), but the reduction did not reach baseline count. In addition, the observation of CD8 lymphocytes counts reveled significant rise during time of anesthesia (28.21 mg/L), however it showed significant reduction post-operatively (22.91 mg/L), but the reduction did not reach baseline count. Moreover, the results of CD16 NK cells count reveled significant rise during time of anesthesia (21.05 mg/L) however, it showed significant decrease post-operatively (13.350 mg/L), but the reduction did not reach baseline count, it has been noticed also that the level of MCP-1 got raised significantly in association with anesthesia induction (13.24 mg/L), however it returned back to its baseline level following surgery, as outlined in figure (3-7) and table (3-10).

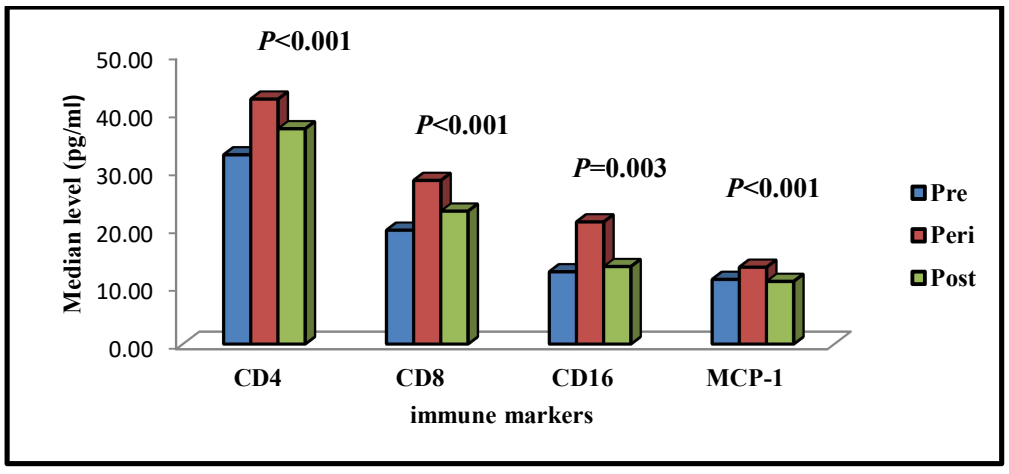


Figure (3-7): Median level of Immune Markers in Relation to Operation Timeline.

Table (3-10): Median level of immune markers in relation to operation timeline.

Marker	Pre-operatively	Peri-operatively	Post-operatively	*P*†
CD4, median (IQR)	32.62 (7.80)	42.23 (17.08)	37.12 (15.11)	<0.001
CD8, median (IQR)	19.62 (7.67)	28.21 (12.18)	22.91 (12.40)	0.003
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

† Friedman test; CD: Cluster of designation; IQR: inter-quartile range.

3.3.1.2 Correlation of Immune Markers With Gender and Age

Table (3-11) showed that the count of CD4 lymphocytes, CD8 and CD16 lymphocytes before, perioperative and post-operatively, had no significant association with gender (p-value > 0.05). It was also obvious, that the level of the chemotactic chemokine MCP-1 remains insignificantly altered before, within and after operation (P > 0.05), as listed below.

Table (3-11): Lymphocytes Immune Markers in Relation to Gender.

Marker	Total n = 30	SD	Male n = 16	SD	Female n = 14	SD	*P*†
	Mean		Mean		Mean		
CD4Pr	32.62	7.80	35.84	6.79	32.45	12.90	0.252
CD4Pe	42.23	17.08	46.10	17.49	35.68	16.26	0.070
CD4Po	37.12	15.11	37.12	16.09	37.37	17.94	0.454
CD8Pr	19.62	7.67	20.87	6.54	18.12	11.57	0.589
CD8Pe	28.21	12.18	29.48	7.75	26.99	15.49	0.236
CD8Po	22.91	12.40	25.93	14.92	19.14	10.53	0.328
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	13.35	5.79	11.18	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

*Significant at P< 0.05. SD: standard deviation. Values were expressed as median (Inter-quartile range); n: number of the cases; † Mann Whitney U test.

Log transformation was carried out in order to make the distribution of variables related to CD4, CD 8 lymphocytes, CD 16 natural killer cells and the level of MCP-1, normal. Neither immune cells, lymphocytes and natural killer cells, nor MCP-1 showed significant correlation with age of patients ($P > 0.05$), as demonstrated in table (3-12).

Table (3-12): Correlation of Immune Markers with Age.

Marker	r	*P*
Log CD4Pr	0.339	0.067
Log CD4Pe	0.324	0.081
Log CD4Po	0.337	0.068
Log CD8Pr	0.186	0.325
Log CD8Pe	0.203	0.282
Log CD8Po	0.231	0.219
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	0.969

*Significant at $P< 0.05$ *r*: correlation coefficient; CD: cluster of designation.

3.3.1.3 Relation of Immune Marker with Type and Duration Time of Anesthesia

Immune marker have been analyzed in relation to type of anaesthesia and the results showed that the count of CD 4 lymphocyte, CD8 and 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 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 (3-13).

Table (3-13): Immune Markers in-relation to Type of Anesthesia.

Marker	General		Local		Regional		*P*
CD4Pr	32.79%	16.53%	33.42%	5.15%	32.33%	13.05%	0.810
CD4Pe	40.23%	17.68%	40.26%	17.48%	42.23%	17.725	0.830
CD4Po	36.70%	19.63%	38.56%	12.03%	34.46%	20.84%	0.940
CD8Pr	18.74%	9.82%	21.26%	7.67%	21.76%	14.79%	0.369
CD8Pe	29.34%	14.70%	27.13%	6.47%	29.52%	19.08%	0.844
CD8Po	22.08%	17.69%	25.98%	11.5%	18.17%	15.44%	0.368
CD16Pr	13.24%	5.51%	15.09%	6.47%	14.33%	7.77%	0.145
CD16Pe	20.09%	17.68%	22.98%	15.83%	21.16%	11.99%	0.557
CD16Po	12.85%	7.06%	13.35%	7.70%	13.28%	5.88%	0.866
MCP-1Pr	2.32%	11.725	11.33%	6.23%	11.70%	13.62%	0.084
MCP-1Pe	13.98%	15.19%	15.00%	5.77%	12.46%	6.50%	0.673
MCP-1Po	5.01%	10.41%	11.44%	8.77%	10.97%	9.82%	0.093

*Significant at P< 0.05 Values were expressed as median (Inter-quartile range); *n*: number of the cases; † Kruskal Wallis H test.

Regarding the correlation of immune marker with time duration of anesthesia, the results showed that neither immune cells, lymphocytes and natural killer cells, nor MCP-1 showed significant correlation with duration of anesthesia ($P > 0.05$), as seen in table (3-14).

Table (3-14): Correlation of Immune Markers with Duration of Anesthesia.

Marker	r	*P*
Log CD4Peri	0.186	0.325
Log CD4Post	0.093	0.627
Log CD8Peri	-0.276	0.139
Log CD8Post	-0.358	0.052
Log CD16Peri	-0.508	0.174
Log CD16Post	0.049	0.799
Log MCP-1Peri	0.069	0.719
Log MCP-1Post	0.095	0.618

*Significant at P< 0.05 *r*: correlation coefficient; CD: cluster of designation

3.3.1.4 Correlation between immunological markers and pre-operative CRP levels

Neither immune cells, lymphocytes and natural killer cells, nor MCP-1 showed significant correlation with CRP ($P > 0.05$), as demonstrated in table (3-15).

Table (3-15): Correlation of Pre-Operative CRP with Immunologic Markers.

Marker	r	*P*
Log CD4Pre	-0.022	0.908
Log CD4Peri	-0.047	0.803
Log CD4Post	-0.159	0.402
Log CD8Pre	-0.227	0.228
Log CD8Peri	-0.212	0.261
Log CD8Post	-0.309	0.097
Log CD16Pre	-0.075	0.694
Log CD16Peri	-0.168	0.374
Log CD16Post	-0.151	0.427
Log MCP-1Pre	0.079	0.680
Log MCP-1Peri	0.022	0.909
Log MCP-1Post	-0.052	0.783

*Significant only at p≤0.05. *r*: correlation coefficient; CD: cluster of designation

3.3.2 Cytokines ELISA Assay Results

Cytokine ELISA assay results showed that higher level of cytokine was IL-2 cytokine in all time of operation whether pre, peri and post-operative, also IL-2 was the higher level recorded in all types of anesthesia, but mainly was in general anesthesia. The lowest level of cytokine was IL-10 in all anesthesia time line and higher level was seen with general anesthesia, as listed estimated below in details.

3.3.2.1Correlation of Cytokines Levels with Operation Timeline

Higher level of cytokine was IL-2 mainly post-operative median level (1257.7pg/ml), and lower level was seen with IL-10 mainly pre-operative median level (36.08). Although the level of interleukin-2 (IL-2 p=0.393), interleukin-10 (IL-10 p=0.131), interferon -gamma (IFN-γ p=0.740) and tumor necrosis factor-alpha (TNF-α p=0.741), all showed no significant change in relation to time of anesthesia whether pre, peri and post-operative ($P > 0.05$), as seen in figures (3-8) and (3-9).

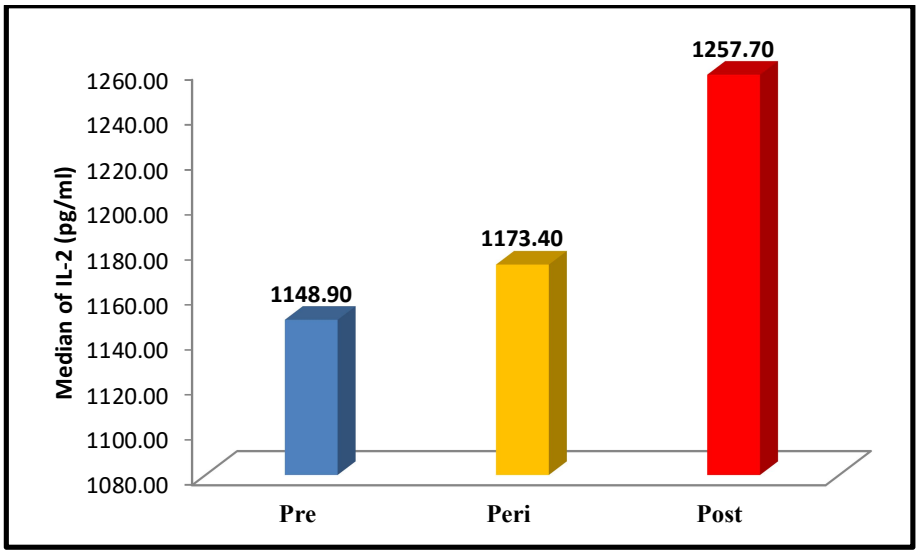


Figure (3-8): Level of IL-2 in relation to duration time of anesthesia.

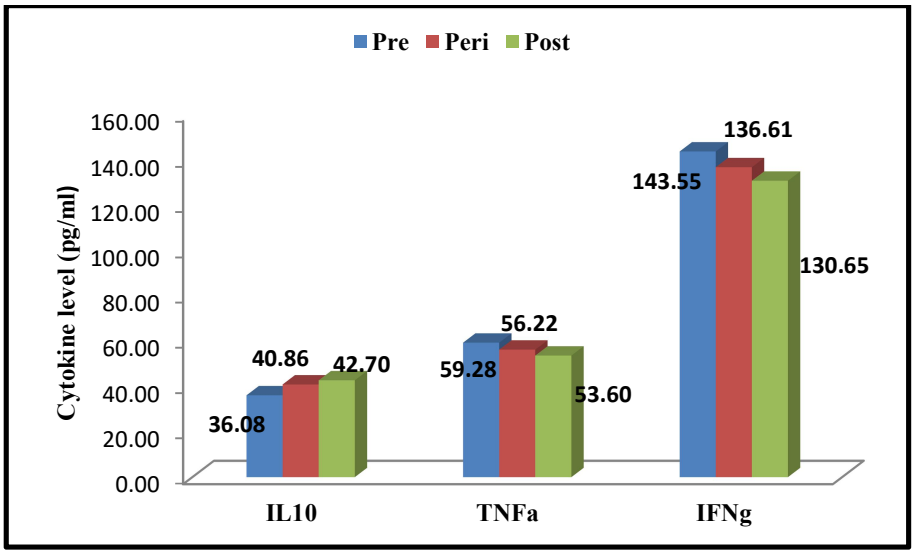


Figure (3-9): Level of IL-10, TNF-α and IFN-γ in relation to anesthesia duration.

3.3.2.2 Association of Cytokines level with Gender and Age:

The result listed in table (3-16) and (3-17), which associate between cytokines serum level (pre, pier and post-operatively) with gender and age, showed no significant association between male and female as well as no significant correlation with age of patients (p>0.05) for all cytokines including IL-2, IL-10, IFN-γ and TNF-α, as shown below.

Table (3-16): Association between Cytokine level and Gender.

Cytokine	Male (n=16)		Female (n=14)		*P*
	Median	IQR	Median	IQR	
IL-2 pre	1142.10	891.23	1148.90	586.44	0.803
IL-2 peri	1225.10	413.64	973.38	889.19	0.967
IL-2 post	1274.10	300.02	1110.80	764.68	0.677
IL-10 pre	36.08	38.07	36.41	25.11	0.603
IL-10 peri	37.06	22.89	45.19	30.04	0.244
IL-10 post	48.56	32.75	39.55	27.77	0.467
TNF-α pre	58.08	46.84	61.02	28.41	0.835
TNF-α peri	53.50	31.62	56.55	13.14	0.560
TNF-α post	55.02	14.23	51.97	26.13	0.519
IFN-γ pre	140.82	106.51	147.52	64.60	0.835
IFN-γ peri	130.40	71.91	137.35	29.88	0.560
IFN-γ post	133.88	32.35	126.93	59.39	0.519

*Significant only at p≤0.05. IL: Interleukin; TNF: tumor necrosis factor; IFN: interferon IQR: interquartile rang.

Table (3-17): Correlation between Age and Cytokine Levels.

Cytokine	r	*P*
LogIL-2 pre	-0.124	0.515
LogIL-2 peri	0.128	0.501
LogIL-2 post	0.149	0.434
LogIL-10pre	-0.082	0.666
LogIL-10 peri	0.140	0.461
LogIL-10 post	0.051	0.788
Log TNF-α pre	-0.109	0.565
Log TNF-α peri	0.091	0.632
Log TNF-α post	-0.052	0.784
Log IFN-γ post	-0.109	0.568
Log IFN-γ peri	0.087	0.649
Log IFN-γ post	-0.047	0.803

*Significant only at p≤0.05. *r*: correlation coefficient; IL: Interleukin; TNF: tumor necrosis factor; IFN: interferon

3.3.2.3 Correlation of Cytokines with types and duration of anesthesia

Analysis of data to correlate the cytokines level (IL-2, IL-10, IFN-γ and TNF-α) with types of anesthetic drugs (general, local, and regional anesthesia) showed no significant association between these cytokine level and type of anesthesia ($P > 0.05$), as described in table (3-18).

Table (3-18): Correlation Between Cytokine Levels and Type of Anesthesia.

Cytokine	General anesthesia		Local anesthesia		Regional anesthesia		*P*
	Median	IQR	Median	IQR	Median	IQR	
IL-2pre	1064.50	998.72	1448.20	491.88	1125.80	412.28	0.136
IL-2peri	905.34	597.33	1291.80	797.34	1147.50	830.00	0.227
IL-2post	1226.50	392.55	1406.10	408.87	1113.50	459.21	0.199
IL-10pre	35.00	34.81	36.41	33.40	37.49	21.80	0.885
IL-10pri	32.07	15.68	42.92	14.43	56.91	29.93	0.139
IL-10post	35.98	46.69	38.79	20.44	54.30	29.18	0.110
TNF-α pre	62.11	35.12	63.64	41.34	45.86	14.01	0.115
TNF-α peri	51.53	19.63	58.51	42.70	56.22	12.54	0.558
TNF-α post	50.88	22.31	54.58	13.69	55.02	28.85	0.686
IFN-γ pre	150.00	79.85	153.46	93.99	113.04	31.87	0.115
IFN-γ peri	125.94	44.63	141.81	97.07	136.61	28.52	0.558
IFN-γ post	124.46	50.71	132.88	31.12	133.88	65.59	0.686

*Significant at p≤0.05, *n*: number of the cases; † Kruskal Wallis H test.

Considering the time of duration of anesthesia, in table (3-19) the result revealed that there is no significant association between cytokines level and duration of anesthesia as in below table.

Table (3-19): Correlation Between Cytokine Levels and Duration of Anesthesia.

Cytokine	r	*P*
Log IL-2 pre	-0.223	0.236
Log IL-2 peri	-0.078	0.681
Log IL-2 post	0.105	0.581
Log IL-10 pre	0.142	0.454
Log IL-10 peri	-0.079	0.678
Log IL-10 post	0.046	0.809
Log TNF-α pre	-0.190	0.315
Log TNF-α peri	-0.155	0.412
Log TNF-α post	-0.105	0.580
Log IFN-γ pre	-0.189	0.317
Log IFN-γ peri	-0.159	0.401
Log IFN-γ post	-0.112	0.557

*Significant only at p≤0.05.

3.3.2.4 Correlation between Cytokines level and CRP

Results of this statistical analysis, that correlate the association between CRP blood level and cytokines serum concentration, showed no significant association as seen in table (3-20).

Table (3-20): Correlation Between Cytokine Levels and CRP Level.

Cytokine	r	*P*
Log IL-2 pre	0.003	0.988
Log IL-2 peri	-0.005	0.978
Log IL-2 post	-0.016	0.932
Log IL-10 pre	0.023	0.905
Log IL-10 peri	-0.098	0.607
Log IL-10post	-0.051	0.787
Log TNF-α pre	-0.042	0.826
Log TNF-α peri	0.152	0.421
Log TNF-α post	0.045	0.812
Log IFN-γ pre	-0.033	0.862
Log IFN-γ peri	0.143	0.451
Log IFN-γ Post	0.049	0.799

*Significant only at $p \leq 0.05$. *r*: correlation coefficient; IL: Interleukin; TNF: tumor necrosis factor; IFN: interferon.

Chapter Four

Discussion

4. Discussions

4.1 Overview and Justification for the Selection of Type of Surgical Specialty

Although any patient undergoing anesthesia, whether general, regional or local, is going to undergo physiological stress attributed to neural and humoral responses of the body, it was intended in this study to make sure that all patients will experience the, as much as possible, same environmental factors related to surgical operation, and therefore all patients were selected from orthopedic unit. Variation in type of surgical operation was proved by several authors to be associated with different types of responses to same anesthesia techniques (Eyelade, *et al*. 2016, Liang*, et al.* 2017).

4.2 Variation in WBCs Count Before, During and After Operation

In the current study, there is significant rise in neutrophil count after anesthesia in comparison with baseline level, this results agree with Deirmengian, *et al*. (2011) and with Sayit and Terzi, (2017). The proposed mechanism of rising neutrophil count is most probably due surgical trauma and associated stress with neurohumoral effect (Deirmengian, *et al*. 2011). In this current study, there is significant decline in lymphocyte count after anesthesia in comparison with baseline level and this results consistent with Dąbrowska and Słotwiński, (2014). The proposed mechanism for the reduction in lymphocyte count is the disturbance in apoptosis of lymphocyte through bcl2 dependent mechanism, by dysregulation of anti-apoptosis and pro-apoptosis signals equilibrium.

The results of study showed 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. This finding agree with (Sayit and Terzi 2017).

In addition, in the current study, there is 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, this results agree with results of Sayit and Terzi, (2017) study.

Moreover, the study revealed that there is no significant change in basophil count after anesthesia in comparison with baseline level, this results agree with Sayit and Terzi, (2017), the reason is also due to changes in immune mediators as a response to tissue injury and stress accompanying surgery This is in

The study showed that post-operative WBCs count and differential counts were not significantly correlated to age of the patients. This finding disagrees with Chen, *et al*., (2016).

The concept of aging of 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, the source of all cells involved in immune system (Ventura, *et al*. 2017); however some proposed a role for low estrogen in post-menopausal women since the observation that reduced estrogen is associated with higher rate of apoptosis among WBC and especially neutrophils (Chen, *et al*. 2016).

The finding of present study showed that post-operative WBCs count and differential were not significantly correlated to gender of the patients. These results consistent with the results of Valiathan *et al*., (2016). However; Chen *et al*., in (2016) suggested that lymphocyte count is significantly higher in women than man and that neutrophils are significantly lower in women than men and they explained these findings due to role of estrogen and concept of aging of bone marrow stem cells.

In addition, the present study showed that post-operative WBCs count and differential were not significantly correlated to duration of anesthesia. This finding is agree with (Kurosawa and Kato 2008; Costa, *et al*. 2013; Sayit and Terzi 2017). The study showed that the post-operative WBCs count and differential were not significantly correlated to type of anesthesia, which agree with results of (Kurosawa and Kato 2008; Costa, *et al*. 2013; Cho, *et al*. 2017).

It appears that, the changes in WBCs count happened as a response to the stress accompanying surgical operation that is the mirror of humeral and neural stimulation and that trauma and tissue damage associated with surgical incision is the main stimulant factor behind these stress responses (Lisowska, 2012; Yuki and Eckenhoff, 2016). Accordingly, there will be no significant correlation with the count of WBCs and the type and duration of anesthesia. In addition, the present study showed that post-operative WBCs count and differential were not significantly correlated to the level of C-reactive protein and this finding is in accordance with Godoy, *et al*. (2010) and Boersema, *et al.* (2018).

The CRP is an acute phase reactant and has been shown to rise significantly in this study, a finding that is similar to Godoy *et al*. (2010) and Boersema, *et al*. (2018). The explanation for the rise of CRP is most likely to inflammation that accompanies tissue injury at time of surgery

with increase in hepatic production of this acute phase reactant ((Boersema, *et al*. 2018).

4.3 Variation in Cytokine Markers' Level Before, During and After Operation

Results of study showed that the level of cytokines (IL-2 and IL-10) became significantly higher during operation, whatever the type and duration of anesthesia, in comparison with their levels before operation and that their level continued to rise insignificantly after operation, however, it did not return back to the same level before operation. These results are in agreement with (Volk, *et al*. 2004; Tureci, *et al*. 2011; Cheng, *et al.* 2013; Basnawi 2017).

The proposed mechanism for these findings in our study, may be attributed to surgical stress that enhances a number pro-inflammatory mediators such as IL-2, produced by inflammatory cells which then activate hypothalamic-pituitary-adrenal axis, thereby pro- and anti-inflammatory cytokines and neuro-hormonal system and additively potentiate their abortive effect on the immune system; the immune-abortive effect that is predominant before operation due to active neuro-hormonal and high level of cytokines may reversely affect clinical outcome of individuals following surgery. This opinion has been adopted by several other authors (Volk, *et al.* 2004; Tureci, *et al.* 2011; Cheng, *et al.* 2013; Basnawi 2017).

Some authors suggested that opioid use may cause rise in IL-10 through receptor (μ receptors) on the cell surface of macrophages and lymphocytes; these receptors when activated lead ultimately to increase secretion of anti-inflammatory mediator (IL-10) by certain intracellular

mechanism that till now is unclear (Volk, *et al*. 2004; Kurosawa and Kato 2008; Liang, *et al*. 2016).

The present study showed that the level of cytokines (IFN-gamma and TNF-alpha) 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 (Volk, *et al*. (2004) and Cheng, *et al*.(2013). The explanation for the fall in the level of these cytokines is most probably due to the anti-inflammatory effect subjected by IL-10, which is an anti-inflammatory cytokine that acts by autocrine and paracrine mechanisms that causes suppression of secretion of pro-inflammatory cytokines such as (IFN-gamma and TNF-alpha) by the same cell secreting IL-1o and other nearby cells, an effect that is named as shifting from T-helper 1 into T-helper 2 predominance (Volk, *et al*. 2004; Kurosawa and Kato 2008; Liang, *et al*. 2016).

The study also showed no significant correlation between any of the cytokines and gender of the patients. This finding is in consistent with (Cheng, *et al*. 2013; Berger, *et al*. 2016; Liang, *et al.* 2016; Gołąbek-Dropiewska, *et al*. 2018). This means that gender of patients whether male or females have nothing to do with the level of inflammatory cytokines. 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 (Cheng, *et al.* 2013; Berger, *et al*. 2016; Liu, *et al*. 2016).

The results showed no significant correlation between any of the cytokines and age of the patients, that agree with (Cheng, *et al*. 2013; Liu, *et al.* 2016; Gołąbek-Dropiewska, *et al*. 2018). The explanation for the lack of significant correlation between these cytokines and the age of the patient is most likely due a relatively small sample size; however, substantial amount of published literature document the negative correlation between age and immune markers due the concept of aging of the immune system (De Toda, *et al*. 2016).

Study also showed no significant correlation between any of the cytokines and duration of anesthesia, in agreement with (Cheng, *et al.* 2013; Berger, *et al*. 2016; Gołąbek-Dropiewska, *et al.* 2018). That's to say, the duration of anesthesia has nothing to do with the level of inflammatory cytokines; 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 supervene the level of these markers get changed with disregard to the duration of anesthesia (Frieri, *et al.* 2017).

Another results of study showed no significant correlation between any of the cytokines and type of the anesthesia, that consistent with (Lisowska, 2012; Cheng, *et al*. 2013; Berger, *et al.* 2016). The same previous explanation is proposed to explain the later finding; once tissue injury supervene the level of these markers get changed with disregard to the duration of anesthesia (Basnawi 2017; Cruz, *et al.* 2017, Frieri; *et al*. 2017). Surgical trauma results in a metabolic, hemodynamic, endocrine, and immune reaction that continue for a minimum of several days (Basnawi 2017, Cruz, *et al*. 2017).

4.4 Variation in Immune Markers Level Before, During and After Operation

The present study showed that the level of immune markers, CD4, CD8, 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).

These results agrees with Heimlich, *et al*. (1999) which revealed that CD4 and CD8 lymphocyte counts become significantly higher than baseline reading during and after surgical operation, and that the CD4/CD8 ratio decreases slightly during and after operation; however, the change is not significant. In addition, the results of the present study agree with Volk, *et al.* 2004), that showed the CD4 and CD8 lymphocyte counts become significantly higher than baseline reading during and after surgical operation; nevertheless, it disagrees with Dąbrowska and Słotwiński, (2014).

Moreover, the finding of the present study is similar to the finding of Berger, *et al*. (2016) which illustrated that the level of the chemoattractant marker (MCP-1) becomes significantly higher during and after operation when compared to its baseline level before operation. Added to that, the results also agree with(Boland, *et al*. 2014; Paul and Lal, 2017) in which the count of CD16 natural killer cells becomes significantly increased at and after operation in comparison with pre-operative baseline levels.

The increase in the level of CD4 and CD8 lymphocytes is attributed to increase in the level of cytokines, pro-inflammatory mediators and

acute phase reactants (such as MCP-1), as a response to the stress accompanying surgical operation that is the mirror of humeral and neural stimulation. The rise in cytokines certainly will cause an increment in the number of CD4 and CD8 lymphocyte counts (Lisowska, 2012). Trauma and tissue damage associated with surgical incision is the main stimulant factor behind these stress responses (Lisowska, *et al*. 2013) Natural killer cell is an important player of the innate immunity and its count is expected to rise during physiologic stress (Yuki and Eckenhoff., 2016).

In this study, there was no significant correlation between gender and immune markers (MCP-1, IL-10, CD4, and CD8) and these results are in agreement with (Landgraeber, *et al*. 2014; Berger, *et al*. 2016; Karadeniz, *et al*. 2017). This means that gender of patients whether male or females has nothing to do with the count of lymphocytes, helper and cytotoxic cells, and also with the count natural cells and the immune marker MCP-1. 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 immune cells, lymphocytes and natural killer cells as well as and on the level of the chemotactic factor MCP-1(Everett, *et al*. 2013).

Moreover, in the present study, there was no significant correlation between age and immune markers (MCP-1, IL-10, CD4, and CD8) and these results are in agreement with Karadeniz *et al*. (2017) and disagree with De Toda *et al*. (2016). The explanation for the lack of significant correlation between these immune markers and the age of the patient is most likely due a relatively small sample size; however, substantial amount of published literature document the negative correlation between age and immune markers due the concept of aging of the immune system (De Toda, *et al*. 2016). Also among the results of study, there was no

significant correlation between duration of anesthesia and immune markers (MCP-1, IL-10, CD4, and CD8) and these results consistent with (Karadeniz, *et al*. 2017; Song *et al*. 2017). That's to say, the duration of anesthesia has nothing to do with counts of CD4, CD8 lymphocytes and natural killer cells and the level of the chemokine MCP-1; 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 supervene the level of these cells and markers get rise with disregard to the duration of anesthesia (Frieri, *et al.* 2017).

In addition, other finding of this study showed that there was no significant correlation between type anesthesia and immune markers (MCP-1, IL-10, CD4, and CD8) which agree with (Berger, *et al*. 2016; Karadeniz, *et al*. 2017). The same previous explanation is proposed to explain the later finding: once tissue injury supervene the level of these cells and markers get rise with disregard to the duration of anesthesia (Basnawi 2017; Cruz *et al*. 2017; Frieri *et al*. 2017). Surgical trauma results in a metabolic, hemodynamic, endocrine, and immune reaction that continue for a minimum of several days (Basnawi 2017; Cruz, *et al*. 2017).

4.5 Correlations among Immune Markers and Cells Involved in Innate and Adaptive Immune Response

In the present study there was negative significant correlation between monocyte count and IL-2 (before anesthesia) which means that patients with low level of IL-2 will have less monocyte count than patients with patients with high IL-2; in addition to that we found significant negative correlation between IL-2 (before anesthesia) and

basophil and eosinophil counts; this seems to be merely a statistical association and following thorough search in the available published articles we failed to find a scientific explanation in term of cellular cytokine interaction.

Moreover, the results showed significant positive correlation between the count of monocyte and the level of IL-10 (before anesthesia). (Prasse, *et al.* 2007; Xiu, *et al.* 2015) observed that IL-10 is associated with increase in the count of monocyte count, a finding that is similar to the finding of the present study. The mechanism of this correlation is till now unclear according (Prasse, *et al*. 2007; Xiu, *et al*. 2015).

Added to that, this study revealed a negative significant correlation between IL-10 (before anesthesia) and peripheral basophil count. Larson et al., in 2012 found that the response of basophils is down regulated by a mechanism that is dependent on IL-10; however, no published article, after thorough search in net, raised the issue of a correlation between the count of peripheral blood basophils and the level of IL-10. In addition to that, we found in this study, that the peripheral eosinophil count was significantly positively correlated with both TNF-alpha and IFN-gamma and simultaneously significantly negatively correlated with IL-10. This finding is in accordance with Choi *et al*. (2015).

Likewise, the relative concentrations of cytokines stored within eosinophils have not been studied. Here, we demonstrate that human blood eosinophils are not singularly outfitted with Th2-associated cytokines but rather, constitutively store a cache of cytokines with nominal Th1, Th2, and regulatory capacities, including IL-4, IL-13, IL-6, IL-10, IL-12, IFN-γ, and TNF-α. We demonstrate further rapid and differential release of each cytokine in response to specific stimuli. As

agonists, strong Th1 and inflammatory cytokines elicited release of Th2-promoting IL-4 but not Th1-inducing IL-12. Moreover, a large quantity of IFN-γ was secreted in response to Th1, Th2, and inflammatory stimuli. Delineations of the multifarious nature of preformed eosinophil cytokines and the varied stimulus-dependent profiles of rapid cytokine secretion provide insights into the functions of human eosinophils in mediating inflammation and initiation of specific immunity (Spencer *et al*. 2009).

Conclusions

And

Recommendations

Conclusions

1. There is no significant effect for anesthesia on immune response in patients undergoing orthopedic operations.
2. Changes in cells, immune markers and cytokines were mainly attributable to tissue trauma during operation that is mediated by neuro-humeral response.
3. Age and gender of patients has no significant effect on immune response during operation.
4. Type and duration of anesthesia showed no significant impact of immune response.
5. CRP has no significant correlation with clinical characteristic of the study group.

Recommendations

1. Larger sample study, multicentric and for a longer duration is to be planned and conducted in order to validate the finding of the present study.
2. Study of the effect of type and duration of anesthesia on other modalities of surgical operations, such as neurosurgery, general surgery, thoracic surgery …etc.
3. Study of the correlation between type and duration of anesthesia and other immune mediators and cellular response.
4. Study the risk of infection in relation to type and duration of both surgical operation and anesthesia.

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Appendix

Appendix

Appendix 1: Patient's Questionnaire Form:

Patient no.	Age	gender	Type of surgery	Duration of surgery	Clinical history

Appendix 2: Demographic characteristic of patient's group and anesthesia type.

Patient no.	Age	Gender	Type of Surgery	Type of Anesthesia	Duration of Surgery	Other Disease
1.	10 y	F	BIOPSY OF Aneurysmal bone tumor	GA Ketamine pentothal propofol Midazolam atracure	20 min.	no
2.	20 y	M	Bone fracture fixation	GA Ketamine neostigmine propofol midazolam	45 min.	no
3.	25 y	M	Bone Marrow Biopsy	Spinal Bupivacaine Midazolam I.V	30 min.	No
4.	50 y	F	Frozen Shoulder	Local and Spinal lidocaine Bupivacaine	45 min	Diabetic Hypertensive

				Midazolam I.V		
5.	25 y	F	Lateral Malleolar fracture	Spinal Bupivacaine Midazolam I.V	40 min	no
6.	12 y	F	Bilateral Maxillofacial Biopsy	GA Ketamine pentothal propofol atracure	15 min	History of Agranulocytosis (Sarcoma)
7.	20 y	M	Radius fracture fixation	GA Ketamine pentothal propofol Midazolam atracure	60 min	Asthmatic
8.	33 y	F	Carpal tunnel Syndrome	Local Lidocaine Midazolam i.v	15 min	no
9.	35 y	M	Left Knee Orthoscopy	GA Ketamine	60 min	no

				pentothal propofol Midazolam atracure		
10.	57 y	M	Right Knee Orthoscopy	Spinal Bupivacaine Midazolam I.V	15 min	diabetic
11.	55 y	F	Tennis elbow	Local lidocaine	15 min	diabetic
12.	72 y	M	Scapula fracture	GA Ketamine pentothal propofol Midazolam atracure	90 min	hypertensive
13.	45 y	M	Hip Dislocation Car accident	GA Ketamine pentothal propofol atracure	60 min	no
14.	21 y	F	Distal Radius fracture	GA	60 min	no

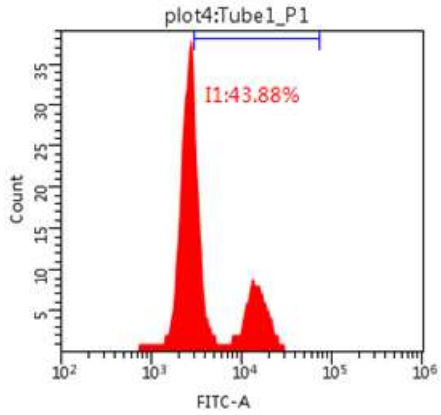
			fixation	Ketamine pentothal propofol Midazolam atracure		
15.	18 y	M	Ganglion cyst	Local and Spinal lidocaine Bupivacaine Midazolam I.V	35 min	no
16.	61 y	F	Oblique midshaft Femur fracture	GA Ketamine pentothal propofol Midazolam atracure	90 min	Hypertensive and Heart disease
17.	38 y	M	Metacarpal fracture	GA Ketamine pentothal propofol Midazolam	60 min	no

18.	46 y	M	Frozen Shoulder	Local and Spinal lidocaine Bupivacaine Midazolam I.V	35 min	no
19.	31 y	M	distal radius fracture repair	Spinal Bupivacaine Midazolam I.V	65 min	no
20.	43 y	F	Carpal tunnel release	Local and Spinal lidocaine Bupivacaine Midazolam I.V	45 min	no
21.	22 y	M	Shoulder dislocation	Local and Spinal lidocaine Bupivacaine Midazolam I.V	45 min.	no

22.	18 y	F	Metacarpal fracture	Local and Spinal lidocaine Bupivacaine Midazolam I.V	60 min	No
23.	66 y	F	Right Knee Orthoscopy	Local and Spinal lidocaine Bupivacaine Midazolam I.V	55 min	no
24.	17 y	M	Radius fracture repair	Local and Spinal lidocaine Bupivacaine Midazolam I.V	55 min	No
25.	28 y	F	Ganglion cyst	Local lidocaine and midazolam i.v	30 min	No
26.	44 y	M	External fixation removal	Local lidocaine and midazolam i.v	45 min	No

27.	29 y	F	Carpal tunnel release	Local lidocaine and midazolam i.v	30 Min	No
28.	51 y	M	Right Knee Orthoscopy	Local and Spinal lidocaine Bupivacaine Midazolam I.V	35 Min	No
29.	17 y	F	fixation under screen	Local and Spinal lidocaine Bupivacaine Midazolam I.V	30 min	No
30.	61 y	M	Knee lipoma cyst	Local and Spinal lidocaine Bupivacaine Midazolam I.V	45 min	no

Appendix 3(A): Histogram of Flowcytometry products for preoperative sample.



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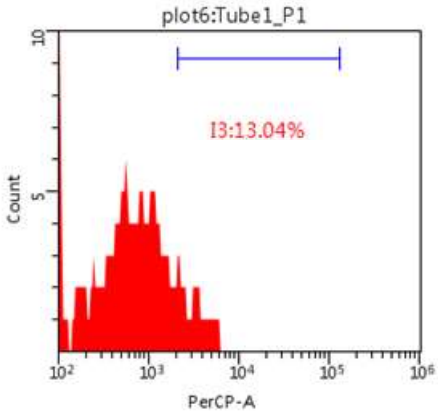

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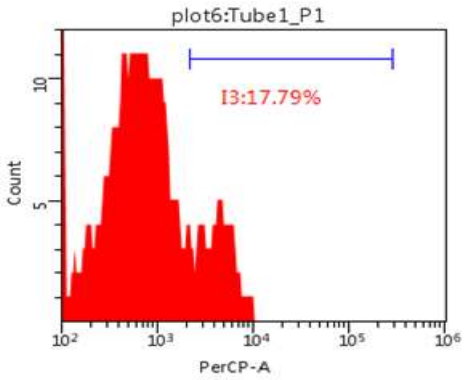
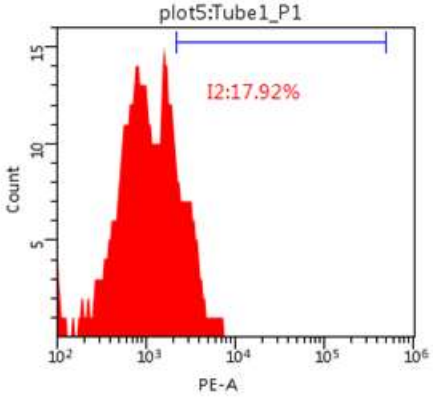
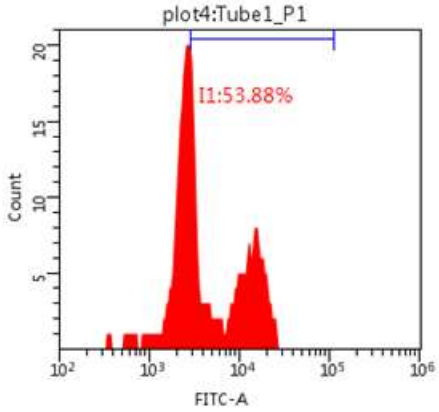


MCP-1



CD4

Appendix 3(B): Histograms of flowcytometry products for peri-operative sample.



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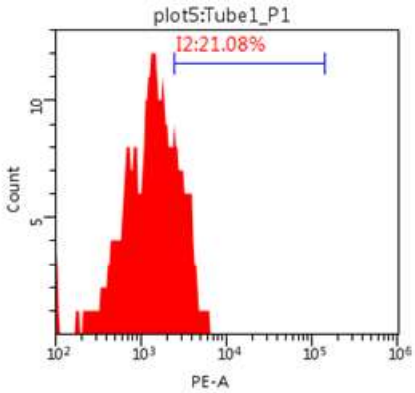

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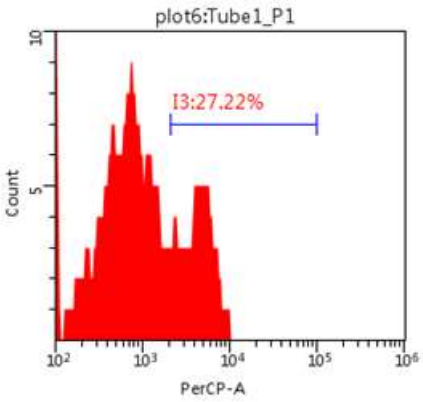

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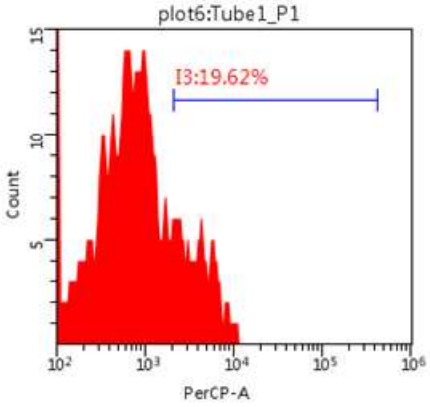

MPC-1

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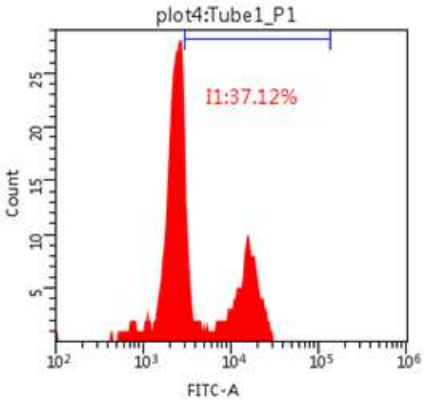
Appendix3(C): Figures of flowcytometry products for post-operative samples.



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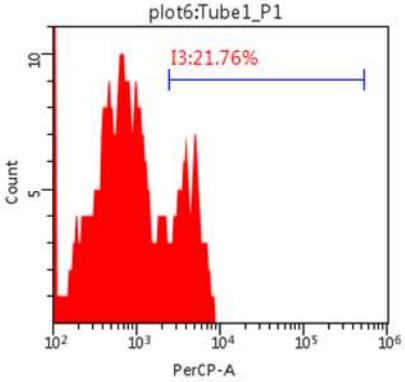

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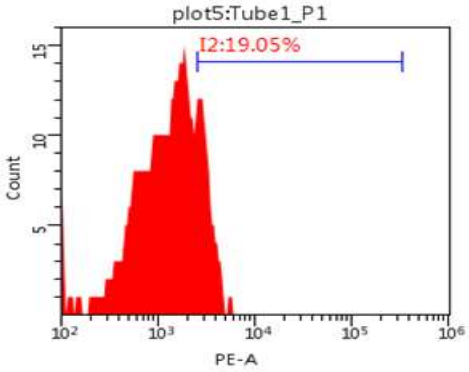

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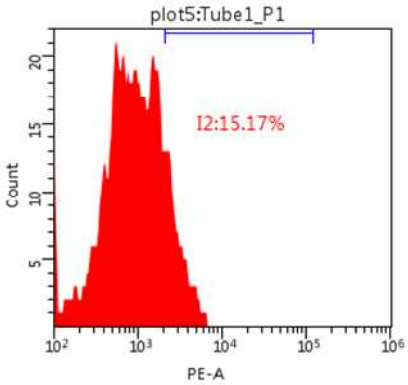

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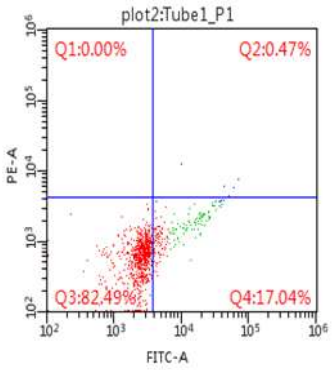

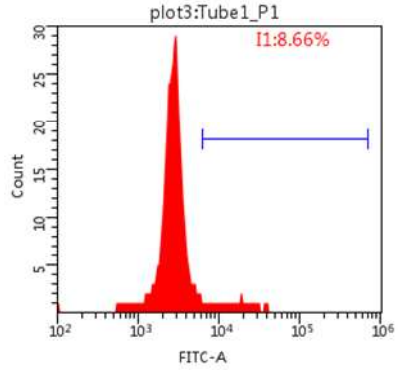

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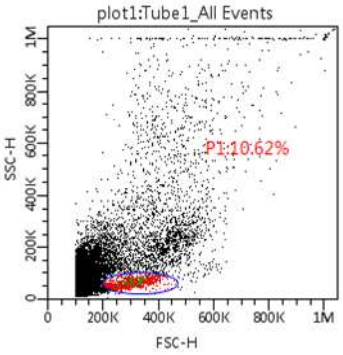

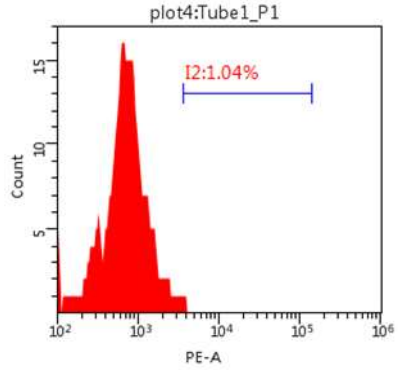

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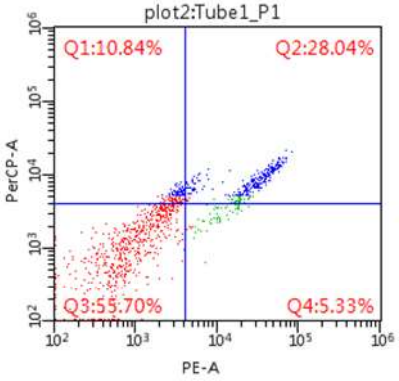

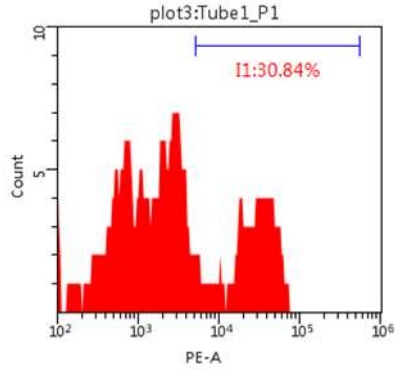

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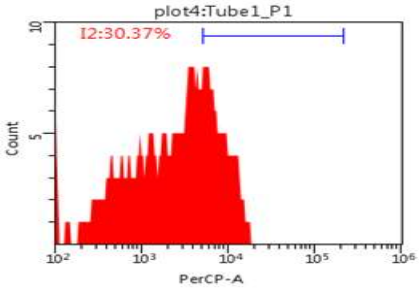

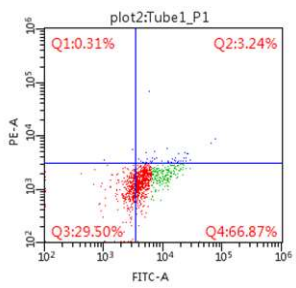

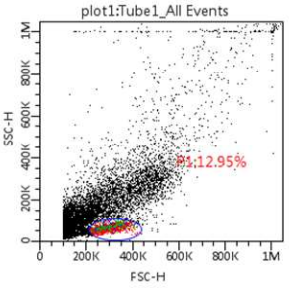

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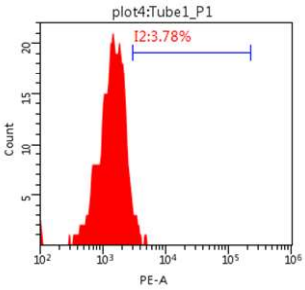

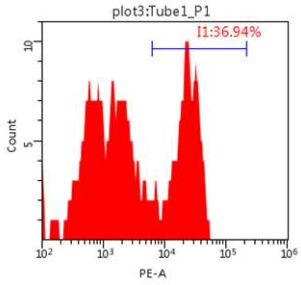

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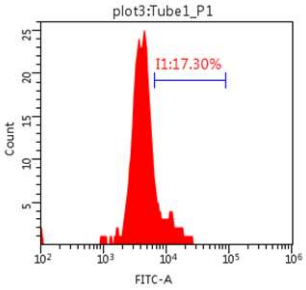

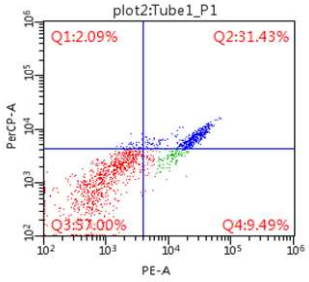

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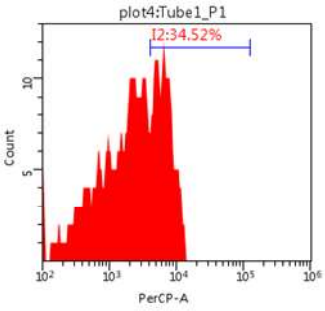
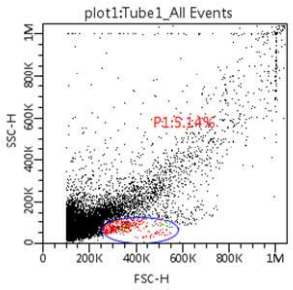


CD8



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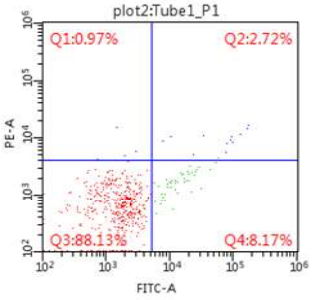

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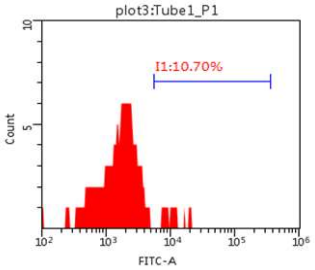
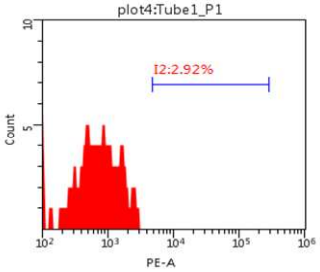
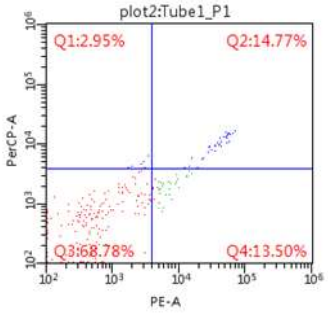
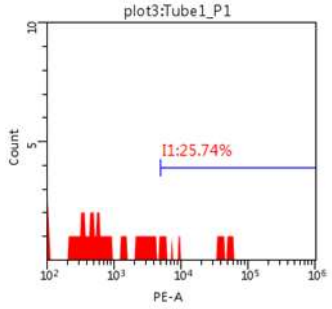


CD8

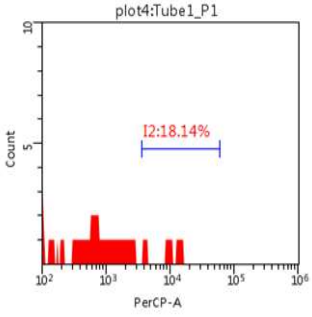


CD4





CD4



CD8

الخلاصة

الخلاصة:

يعتبر التخديرهو العامل الرئيسي الذي يؤثر على النتائج في ما بعد اجراءالجراحة للمريض. تعتبرعوامل التخدير ، الإجهاد الجراحي والصدمة لها تاثير على الاستجابة المناعية التي يمكن أن تسبب زيادة أو نقصان الوسائط المناعية إما عن طريق التأثير المباشر أو عن طريق تحفيز نظام الغدد الصم والجهاز العصبي.ان آلية التعديل المناعي هي عن طريق تغيير التوازن بين الوسائط المؤيدة للالتهابات والمضاد للالتهابات وغيرها من الوسائط.

الهدف من هذه الدراسة هو تقييم الدور المحتمل للتخدير والجراحة على المناعة الفطرية والخلوية عن طريق قياس مستويات IL-2 و TNF-α و IFN-γ التي هي مؤيدة للالتهابات و IL-10 مضاد للالتهابات من قبل ELISA. تم استخدام قياس التدفق الخلوي لتحديد CD4 و CD8 و CD16 و MCP-1. تم قياس البروتين التفاعلي C (CRP) وخلايا الدم البيضاء قبل وبعد الجراحة.

أجريت هذه الدراسة على 30 مريضًا من ردهةجراحة العظام وتنظير المفاصل 16 ذكورًا (53.3٪) و 14 من الاناث (64.7٪) من مختلف الفئات العمرية 10-72 سنةحيث تم تعيينهم من قبل قسم العظام والروماتيزم في مستشفى الديوانية التعليمي خلال الفترة من يناير من عام 2018 حتى نهاية أبريل من عام 2018. تم استخدام ثلاثة أنواع من التخدير ، 10 من المرضى مع التخدير العام ، و 10 مرضى تخدير مناطقي و 10 من المرضى التخدير الموضعي مع فترة التخدير تتراوح بين 75 دقيقة (15-90).

تم جمع عينات الدم في ثلاث فترات زمنية مختلفة لكل مريض ، الأولى هي قبل الجراحة 24 ساعة قبل الجراحة ، وكانت العينة الثانية أثناء الجراحة والعينة الثالثة 12 ساعة بعد انتهاء الجراحة.

في هذه الدراسة ، كان هناك زيادة كبيرة في مستوى البروتين التفاعلي C بعد الجراحة مقارنة مع مستوى المصل قبل الجراحة 2.65 (4.99) و 3.99 (5.64) على التوالي (P <0.001).

ايضا تم ملاحظة في هذه الدراسة هناك زيادة كبيرة في مستوى البروتين التفاعلي C بعد الجراحة مقارنة مع مستوى المصل قبل الجراحة 2.65 (4.99) و 3.99 (5.64) على التوالي (P <0.001).

في الخلايا اللمفاوية الذي شهد ارتفاعًا ملحوظًا خلال فترة التخدير CD4 تم معاينة مستوى (42.23) ، إلا أنها أظهرت انخفاضًا كبيرًا بعد الجراحة (37.12) ، ولكن هذا الانخفاض لم في الخلايا الليمفاوية CD8 يصل إلى المستوى قبل العملية. بالإضافة إلى ذلك ، فإن مستوى شهد ارتفاعًا ملحوظًا خلال فترة التخدير (28.21) ، ومع ذلك فقد أظهر انخفاضًا كبيرًا بعد الجراحة (22.91) ، ولكن الانخفاض لم يصل إلى مستوى قبل العملية .

وعلاوة على ذلك ، أظهرت نتائج خلايا CD16 NK ان المستوى قد شهد ارتفاعًا ملحوظًا خلال فترة التخدير (21.05) ، إلا أنها أظهرت انخفاضًا كبيرًا بعد الجراحة (13.350)، ولكن لم يصل الانخفاض إلى المستوى قبل العملية ، وقد لوحظ أيضًا أن مستوى 1- MCP تم ارتفاعه بشكل كبير بالترافق مع بدأ التخدير (13.24) ، إلا أنها عادت إلى مستوى قبل العملية بعد الجراحة.

اضافة لذلك ، أظهرت نتائج خلايا CD16 NK ارتفاعًا ملحوظًا خلال فترة التخدير (21.05) ، إلا أنها أظهرت انخفاضًا كبيرًا بعد الجراحة (13.350)، ولكن لم يصل الخفض إلى خط الأساس ، وقد لوحظ أيضًا أن مستوى 1- MCP قد ارتفع بشكل كبير بالتزامن مع بدأ التخدير (13.24) ، إلا أنها عادت إلى مستوى قبل العملية بعد الجراحة.

وقد تم تحليل عوامل المناعة فيما يتعلق بانواع التخدير وأظهرت النتائج أن عدد خلايا CD4 اللمفاوية CD8 و CD16 NK لم تختلف اختلافا تحليليا احصائيا كبيرا فيما يتعلق بنوع التخدير ، سواء التخدير العام والموضعي والمناطقي ، في جميع الحالات سواء قبل ، في وقت أو بعد العملية ($P> 0.05$).فيما يتعلق بالعلاقة بين عوامل المناعة ومدة التخدير ، أظهرت النتائج أنه لا الخلايا المناعية والخلايا اللمفاوية والخلايا القاتلة الطبيعية ، ولا 1-MCP أظهرت ارتباطًا كبيرًا مع مدة التخدير ($P> 0.05$) ،

كان مستوى 2-IL أعلى من اي سايتوكين اخر بعد العمليات الجراحية حيث ان المتوسط الاحصائي كان (1257.7pg/L) ،بينما اقل تركيز لوحظ بالنسبة الى 10-IL قبل اجراء العملية حيث كان المتوسط الاحصائي (36.08 pg/L). على الرغم من أن مستوى interleukin-2(IL-2 $p=0.393$)، interleukin-10 (IL-10 $p = 0.131$)، interferon -gamma ((IFN-γ $p = 0.740$)) وعامل تنخر الورTNF-α ($p = 0.741$) ، كل ذلك لم يطرأ عليه أي تغير معنوي احصائي فيما يتعلق بفترة التخدير سواء قبل أو بعد أو بعد العملية ($P> 0.05$).

بينت النتائج التي تربطت بين مستوى تركيز المصل السايتوكيني (ما قبل ، اثناء وما بعد الجراحة) مع الجنس والعمر ، عدم وجود أي ارتباط معنوي احصائي بين الذكور والإناث وعدم وجود اي ارتباط أحصائي مع عمر المرضى ($p> 0.05$) لجميع السيتوكينات بما في ذلك IL -2 و IL-10و IFN-γ و TNF-α. .

تم تحليل البيانات ل مستوى السايتوكينات IL-2 ، IL-10 ، IFN-γو TNF-α مع أنواع من أدوية التخدير (عام ، التخدير المناطقي ، والموضعي) تبين عدم وجود ارتباط كبير بين مستوى السيتوكين ونوع التخدير($P> 0.05$) .

وزارة التعليم العالي و البحث العلمي
جامعة القادسية
كلية الطب
فرع الأحياء المجهرية

التأثير المناعي المحتمل لأدوية التخدير على الأستجابة المناعية للمرضى الذين يخضعون لجراحة العظام

اطروحة مقدمة الى
مجلس كلية الطب – جامعة القادسية
كجزء من متطلبات نيل درجة فلسفة الدكتوراه
في علم الاحياء المجهرية الطبية

مقدمة من قبل
خالد لهمود ياسين
ماجستير احياء مجهرية طبية/ جامعة القادسية-2013
بكالوريوس صيدلة / جامعة بغداد – 1994

بإشراف

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