ALTAHADY UNIVERSITY

FACULTY OF SCIENCE

DEPARTMENT OF ZOOLOGY

A STUDY IN THE EFFECT OF SMOKING ON SOME IMMUNOLOGICAL PARAMETERS

A thesis submitted in partial fulfillment of the requirement

for master degree of Science

By

INTISAR HAMAD ABDALLA

Under supervision of

Dr .Maher Naguib Abraheim **Dr. Ahmed Alsagheer daboob**

(2007-2008)

التاريخ : .....
الموافق : .....
الرقم الإشاري : .....

Faculty of Science
Department of Biology

Title of Thesis

A Study of The Effect of Smoking on some Immunological Parameters

By
Entesar Hamad Abdallah

Approved by:

Dr. Maher Naghib Ibraheim 1999
(Supervisor)

Dr. Ahmed A. Daboob .....
(Co-Supervisor)

Dr. Ali Mohamed Afan .....
(External examiner)

Dr. Fahima Hamed Haman .....
(Internal examiner)

Countersigned by:

Dr. Ahmed Farag Mhgoub
(Dean of faculty of science)

بسم الله الرحمن الرحيم

﴿ وما توفيقي إلا بالله عليه توكلت وإليه أنيب ﴾

الآية (88) من سورة هود

صدق الله العظيم

Dedication

My family and friends for their endless patience, and continuous support during the period of study ....., Especially to my brother Nasser.

Acknowledgements

Thank to Allah who helped and gave me the strength to complete my research project.

Frist, I would like to express my gratitude to supervisors Dr.Maher Naguib Ibraheim and Dr. Ahmed Alsagheer Daboob for their guidance and advice in criticizing, correcting my work from the first draft until it has taken its last from as the present thesis.

Second, I would like to thank the Dean of Sciences faculty, Head of Zoology department and Director of postgraduate office for offering me the opportunity to achieve this M. Sc. Thesis and to all my doctors and my colleagues at Biology Dept. for their kind cooperation and encouragement . My thanks also go to Al-Tahadi University, Sirte, for supplying all the facilities during the whole work.

I would like to thank the administration and employees of Ibn Sina Hospital and particularly the employees of the blood bank for their help and remarks they made on my study.

I would like also to offer my thanks and gratitude to the technical staff in the analysis department and the employees of the central library in the faculty of medicine for their help in providing me with references related to my research.

ABSTRACT

Smoking nowadays has become a potentially dangerous factor responsible for many health hazards. This study was carried out to investigate the effect of smoking on some immunological parameters. 299 subjects of either sex participated in this study.

None of the volunteers showed any past history of taking drugs, previous operation, other illness. Volunteers were devided in 3 categories. The first category included the control non smoking individual. The second group represented the passively smokers; who were exposed to smoke of cigarettes at least 5 years prior to the study. The last and third group of individuals, included the actively smoker ones, who were smoking at least 20 cigarettes per day for 5 years prior to the study. This study was carried out on 4 male groups (A, B, C and D male groups, according to age schedule) and another corresponding 4 female groups (E, F, G and H, also according to age). Each male or female group was subdivided into 3 subgroups; as mentioned before, control non smoking group, passively smoking group and actively smoking groups. None of the adult fertile females were pregnant or canplaing of any gynecological or abstetrical disturbance. Venous blood sample was taken from each volunteer for estimation of hemoglobin content, leukocytic count, and concentrations of IgA, IgM, IgG and C3. For adult fertile females, the boold sample was taken during mid luteal phase of menstrual and ovarian cycle.

Results of the present study, showed that smoking, either passive or active has a definite stimulatory effect on the immunological parameters estimated in this study; leukocytes count, IgA, IgM, and IgG. However, IgG showed the maximal significant elevation while IgM showed the minimal increases. Old age individuals of either sex in this study, have less reactive immune response,

than members of younger age. Moreover, females were more reactive to smoking than males. In this work, both males and females showed nearly an apparent similar reaction to smoking. It was also found that immune response was reacting in passive smoker, who were not actively smoking, in a nearly similar way, as in active smoker. So, it was advised for all peoples not smoke but also not to be exposed to smoke of cigarettes or any other alternatives.

Contents

CHAPTER IV	Pages
Discussion.....	67

	Pages
Recommendation	72
References.....	73
Appendix.....	85
Arabic summary.....	I

LIST OF TABLES

LIST OF FIGURES

List of abbreviations

TCR	T-Cell Receptor
Ig	Immunoglobulin
MBL	Mannan-Binding Lectin
MAC	Membrane Attack Complex
DAF	Decay Accelerating Factor
CD	Cluster of Differentiation
NK	Natural Killer
APC	Antigen Presenting Cells
MHC	Major Histocompatibilty Complex
HLA	Human Leukocyte-associated Antigens
T-cell	Thymus-derived lymphocytes
CMI	Cell-Mediated Immunity
Fab	Fragment Antigen Binding
Fc	Fragment Crystalline
MW	Molecular Weight
SLE	Systemic Lupus Erythematosus
HIV	Human Immunodeficiency Virus
IL-6	Interleukin-6
PMN	Polymorph nuclear
TNF	Tumor Necrosis Factor
FcR	Fragment Crystalline Receptor
COPD	Chronic Obstructive Pulmonary Disease
CSE	Cigarette Smoke Extract

ACTH	Adrenocorticotropic Hormone
AFC	Antibody Forming Cell
COHb	Carboxyhaemoglobin
ELISA	Enzyme Linked Immunosorbent Assay
EDTA	Ethylene Diamine Tetra Acetic acid
RID	Radio Immune Diffusion
PLC-δ1	Phospholipase C- δ1

INTRODUCTION

Smoking is one of the preventable cause of disease and death all over the world for instance, tobacco kills 400.000 Americans per / year by causing coronary heart disease, lung and other cancers, chronic obstructive lung disease including emphysema and bronchitis acute respiratory infections, and cerebral vascular accidents. Heart disease, cancer, stroke and chronic obstructive pulmonary disease are the top four leading cause of death in all countries, all possibly caused by smoking **(Geng *et al.*, 1996; Boyle and Waters, 2000).**

Tobacco smoke contains about 4.000 chemicals. However, nicotine is the major compound **(Goud *et al.*, 1994)**. Cigarette smoke affects a wide range of immunological functions in humans and animals including both cell-mediated and humoral immune response **(Finch *et al.*, 1999)**. It was found that chronic exposure to nicotine inhibits the antibody forming cell response, and this immune suppression might be related to the impairment of antigen-mediated signaling in T or B cell. Therefore, nicotine seems to affect both T and B cell functions of immune system, usually by suppressing some of their function. As proved that tobacco smoking is harmful not only to smokers but also to those who are living or working with them. Tobacco consumption is believed to be one of the world greatest preventable health problem. About 1.1 blillion people worldwide are addicted to nicotine with tobacco causing an estimated four millions premature deaths every year. At seems that disturbances in the immune system, might be one of the etiological factors leading to these deaths **(Basta, 2001).**

Toxic smoke components are immunosuppressant and may well play a part in complex immuno-pathogenesis intraction **(Medhat, 2006).**

Some investigators have demonstrated antigenic role of substances in cigarette smoking, resulting in the development of antigen-antibody complexes. These complexes are capable of causing pulmonary and peripheral changes in responses of the humoral and cell-mediated system **(Hersey *et al*., 1983).**

Both active and passive cigarette smoke exposure increase the risk of infections. Cigarette smoking is associated with a variety of alterations in cellular and humoral immune system function. These alterations include a decreased level of circulating immunoglobulins, a depression of antibody responses to certain antigens, a decrease in $CD4^+$ lymphocyte counts, an increase in $CD8^+$ lymphocyte counts, depressed phagocyte activity, and decreased release of proinflammatory cytokines **(Lidia *et al.*, 2004).**

Van Eeden and Hogg, (2000) found that polymorphonuclear leukocytes (PMNs) from smokers have phenotypic changes indicating bone marrow stimulation. They suggested that proinflammatory factors released from alveolar macrophages, such as tumor necrosis factor α, interleukin (IL) 1, IL-8, and granulocyte-macrophage colony-stimulating factor, are probably responsible for the stimulation of bone marrow by cigarette smoking.

Nicotine and other constituents have been associated with decreased numbers of Langerhans cells in the cervix in a cytologic examination **(Sedlacek, 1999).** The Langerhans cells are part of the antigen–T-lymphocyte cell-mediated immune response system and are responsible for recruiting $CD4^+$ lymphocytes, which are necessary for the local immune response **(Arany and Tyring, 1996).**

Nicotine stimulate catecholamine and corticosteroid release.These mediators might increase $CD8^+$ lymphocytes in the cellular-mediated system **(Miller, 1982).**

Smokers are exposed not only to nicotine, but also a vast array of chemicals that are known to be harmful, including nicotine and carbon monoxide, the two main toxic substances. Smoking throughout pregnancy, caffeine and alcohol consumption may increase the risk of spontaneous abortion **(Rasch, 2003 and Khoury *et al.*, 2004).**

Host defense against pathogenic microbes requires dramatically different responses, depending on the character of the pathogen and on the tissue under attack. Central to the immune system's ability to mobilize a response to an invading pathogen is its ability to distinguish self from nonself. The host has evolved both innate and adaptive mechanisms to respond to and eliminate pathogenic microbes **(David and Birmingham, 2003).**

1 - General features of innate and adaptive immunity:

Innate immune system includes barrier mechanisms, such as neutrophils, eosinophils, monocytes, and epithelial cell layers that express tight cell–cell contacts (tight junction, coadherine mediated cell) and the secreted mucus layer that overlays the epithelium in the respiratory, gastrointestinal, and genitourinary tracts, and the epithelial cilia that sweep away this mucus layer, permitting it to be constantly refreshed after it has been contaminated with inhaled or ingested particles. The innate response also includes soluble proteins and bioactive small molecules that are either constitutively present in biological fluids such as the complement proteins **(Yang *et al.*, 2002).**

All are released from cells as they are activated (including cytokines that regulate the function of other cells, chemokines that attract inflammatory leukocytes, lipid mediators of inflammation, and bioactive amines and enzymes that also contribute to tissue inflammation). Lastly the innate immune system includes cell surface receptors that bind molecular patterns expressed on the surfaces of invading microbes. Adaptive responses are based primarily on the antigen –specific receptors expressed on the surfaces of T and B-lymphocytes. Which assembled somatic rearrangement of germ line gene elements to form intact TCR and Ig (B-cell antigen receptor) genes. The assembly of antigen receptors from a collection of a few hundred germ line-encoded gene elements permits the formation of millions of different antigen receptors, each with potentially unique specificity for a different antigen. The innate and adaptive immune system are usually act together, with the innate response representing the first line of host defense and the adaptive response becoming prominent after several days. As antigen-specific cells amplify their responses by recruiting innate

effector mechanisms to bring about the complement control of invading microbes **(David and Birmingham, 2003).**

2- Discrimination of self from nonself

Tolerance mechanisms:

Immunological tolerance is an induced state of specific nonreactivity toward a substance that is ordinarily immunogenic. This type of specific tolerance depends on the interaction between antigen and immunologically competent cells and is characterized by the subsequent failure of these cells to participate in the immune response. Tolerance is restricted to the antigen eliciting its introduction and thereby differs from the nonspecific unresponsiveness induced by radiation antilymphocytes serum, or drugs in the absence of antigen. **(Allison, 1972; Peter *et al.*, 2000 a).**

Tolerance of T-cells induced in the thymus and of B-cells in the bone marrow is called central tolerance. But another mechanism to prevent autoimmunity is necessary, because most tissue–specific antigens are not present in the thymus or bone marrow but are present too small for the induction of tolerance **(Miller *et al.*, 1996 a; Miller and Basten, 1996 b; Peter *et al.*, 2000 a-b).**

3- Soluble mediators of innate immune system

The molecules collectively referred to as acute-phase proteins enhance resistance to infection and promote the repair of damaged tissue **(Gabay and Kushner, 1999).** Plasma levels of these proteins change rapidly in response to infection, inflammation, and tissue injury **(Peter *et al.*, 2000a).**

Cytokines constitute another group of soluble mediators. They act as messengers both within the immune system and between the immune system and other systems of the body, forming an integrated network that is highly involved in the regulation of immune responses (**Mire-Sluis and Thorpe, 1998**). The presence of a cytokine is sensed by a cell by means of specific cytokine receptors. There are soluble forms of cytokine receptors (**Heaney and Golde, 1998**). Some cytokines have a direct role in defense; for example, the interferons that are released by virally infected cells establish a state of viral resistance in the surrounding cells, cytokines, including interleukin-1, interleukin-6, and tumor necrosis factor, also provide costimulatory signals. However, not all signals from cytokines and cell-surface molecules are stimulatory. Interleukin-10 and transforming growth factor B often provide negative signals (**Keilholz *et al.*, 1998**).

Complement system

The complement system is one of soluble mediators of innate immunity. It is composed of more than 25 plasma proteins and cell surface proteins that include three activation pathways and soluble and membrane bound downmoulting regulatory pathways (**Liszewski *et al.*, 1996**).

Many of the proteins of the activation pathways are proteinases, and activation occurs in a cascade (**Fig.1**). The liver represents the primary source of circulating complement components .Other sources of certain complement components include monocytes, macrophages, fibroblasts, endothelial cells, mucosal epithelial cells, and adipocytes (**Qian *et al.*, 1999**). Three modes of complement activation have been described: 1) the

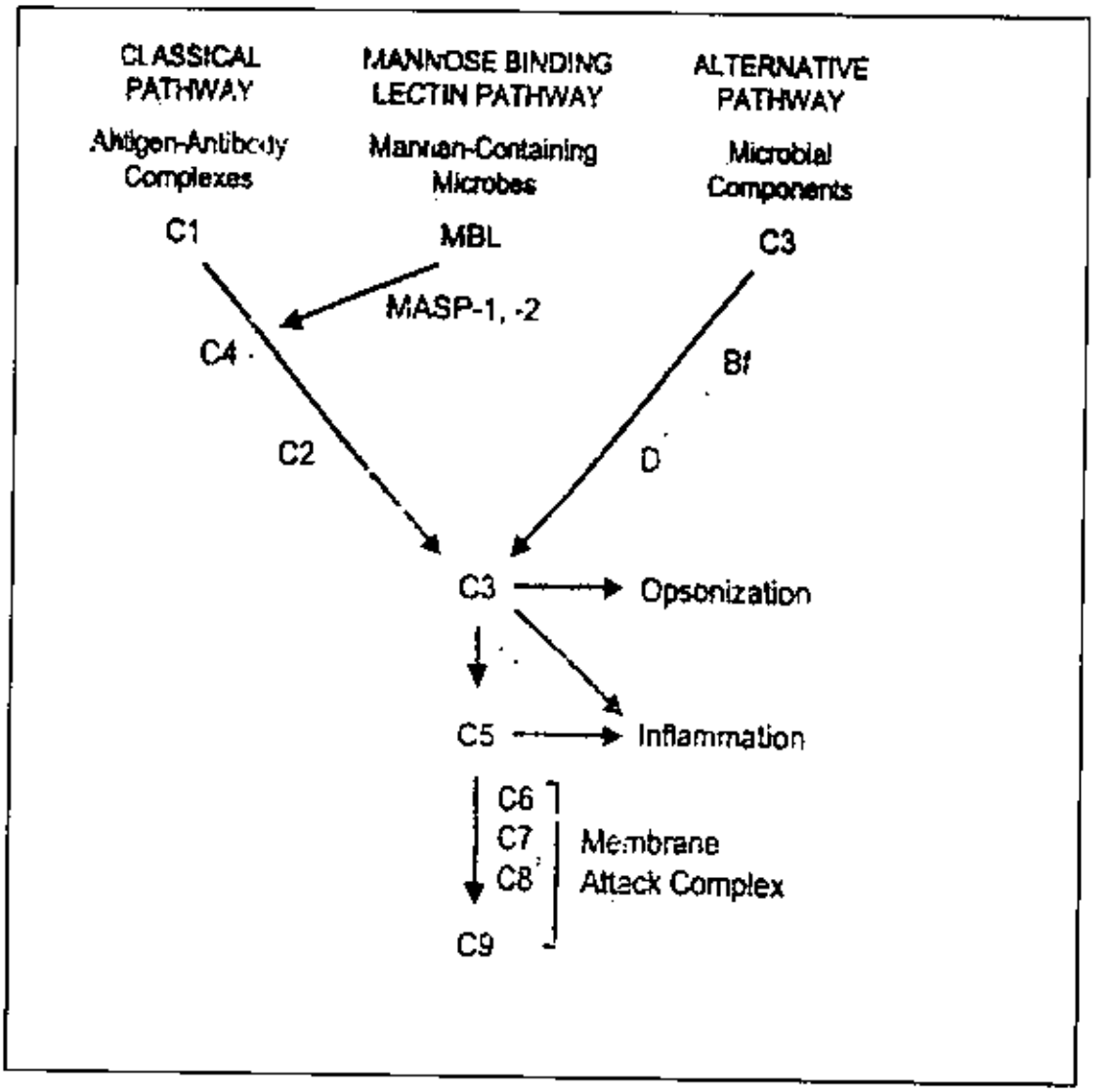

Fig.(1) Pathway of complement activation (David and Birmingham, 2003)

classical, 2) alternative pathway, 3) lectin pathway. **The classical pathways**, the first to be described, is initiated by IgM or IgG antibody bound to its cognate antigen. The cascade starts with activation of the C1 complex. Antibody, or sometimes other substances, binds to C1q and induced its conformational change leading to subsequent activation of the two enzymes, C1r and C1s; the latter then sequentially cleaves C4 and C2. **Alternative pathway** components are called factors followed by a specific latter. During activation some components are initially cleaved into fragments. The smaller fragment, designated "a" is released, the larger "b" fragment is usually deposited on the surface of the activating cell **(Walport, 2001).**

The third activation pathway is triggered by microbial cell wall components containing mannans and is called the lectin pathway of complement activation **(Gal and Ambrus, 2001).**

The complement pathways are triggered by different factors (Kamradt and Mitchison, 2001):

1) Classical pathway by antigen–antibody immune complexes, apoptotic cells.C- reactive protein bound to ligand, certain viruses and bacteria.

2) Alternative pathway by bacterial endotoxin, fungal cell walls, viruses and tumour cells.

3) Mannan-binding lectin (MBL) pathway is activated by microbes with terminal mannose groups. MBL has a similar structure to C1q and activates through the classical pathway with out the requirement of antibody.

The pathways converge in the activation of C3 (by the formation of either classical or alternative C3 convertase) leads into a final common pathway with the assembly of components C5–C9 to form the membrane attack complex (MAC) which assembles into a doughnut-like transmembrane. Channel leading to cell lysis by osmotic shock. Complement activation is focused at-cell membrane host cells are protected from complement–mediated lysis by inhibitory surface molecules, for example (DAF). Most organisms lack any protective molecules and are therefore susceptible to complement **(Kamradt and Mitchison, 2001).**

Functions of complement system (Walport, 2001)

a- Anti- infective function:

- Opsonization by C3b and C4b.
- Chemotaxis–attraction of phagocytes by chemoattractant activation products.
- Activation of leukocytes by anaphylactions (C5a, C3a, and C4a); via receptors on leukocytes .
- Lysis of bacteria.

b- Interplay between innate and adaptive immune system :

Immunomodulation of B-cell responses to specific antigen through binding of complement receptors on B-cell surface, thus augmenting antibody responses and immunological memory.

4-Pattern-recognition receptors of the innate immune system:

The receptors of the innate immune system are expressed on many effector cell of the innate immune system, most importantly on macrophages, dentritic cells. Functionally, they can be divided into three classes: Secreted, signaling and endocytic. Secreted pattern-recognition molecules function as opsonins by binding to microbial cell walls and flagging them for recognition by the complement system and phacocytes. Signaling receptors recogenize pathogen-associated molecular patterns and activate signal-transduction pathways that induce the expression of a variety of immune-response genes, including inflammatory cytokine **(Ruslan and Charles, 1997; 2000).** Another endocytic pattern-recognition receptor, the macrophage scavenger receptor, binds to bacterial cell walls and is an essential part of the clearance of bacteria from the circulation **(Thomas, 2000).**

5- Cellular elements of the immune response

An intact immune response includes subsets of leukocytes which can be discriminated, morphologically through the use of conventional histological stains and by surface phenotype as defined by monoclonal antibody binding to registered differentiation antigens. These differentiation antigen are assigned (CD) numbers. There currently more than 260 defined CD antigen. Mature, circulating leukocytes differentiate from pluripotent hematopoietic stem cells which differentiate first into lymphoid and myeloid stem cells. Lymphoid stem cells differentiate further into the three major population of mature lymphocytes cell, B-cell, and NK cell. These lymphocyte subsets can be discriminated by surface phenotype. T-cells are defined by their cell surface expression of the TCR, a transmembrane heterodimeric protein that

binds processed antigen displayed by APC. T-cells exist in several subtypes and subsets of those types. B-cells are phenotypically defined by their binds processed antigen displayed by APC. T-cells exist in several subtypes and subsets of those types. B-cells are phenotypically defined by their expression of the B-cell receptor for antigen membrane anchored Ig. Cells of the granulocyte lineage that play prominent immune roles include neutrophils, monocytes eosinophils, basophils, and mast cells **(David and Birmingham, 2003).**

5.1) T-lymphocytes

The major class of T-cells is defined by its suffice expression of the **α, β** T-cell receptor. This receptor has evolved primarily to recognize peptide antigens presented in a complex with class I or class II MHC proteins. T-cells differentiate into several different subsets, some of which $CD8^+$ T cells act primarily to kill cells infected with intracellular microbes **(Mescher, 1995).**

a)- T-cell subpopulations

In the blood and secondary lymphoid organs, 60% to 70% of T cells are $CD4^+$ $CD8^-$ {$CD4^+$}and 30% to 40% are $CD4^-$ $CD8^+$ {$CD8^+$}.$CD4^+$ cells are generally designated (helper cells) and work to activate both humoral immune responses (B-cell help) and cellular responses (delayed-type hypersensitivity responses) **(David and Birmingham, 2003).**

$CD8^+$ cells show a major cytotoxic activity against cells infected with intracellular microbes and against tumor cells, but also contain regulatory cells that down regulate immune responses suppressor cells **(Shevach, 2002).**

b)- Antigen recognition by T-lymphocytes

A major role of the T-cell arm is to identify and destroy infected cells. T-cells can also recognize peptide fragments of antigens that have been taken up by APC by phagocytosis or pinocytosis. The way that the immune system has evolved to permit T-cells to recognize infected host cells is to require that the T-cells recognize both a self-component and a microbal structure. This is done through family of MHC molecule as shown in (**Fig.2**). MHC molecules also called the "HLA" are cell surface glycoproteins that bind peptide fragments of proteins that either have been synthesized within the cell class I MHC molecules or that have been ingested by the cell and proteolyrically processed (class II MHC molecules) **(David and Birmingham, 2003).**

Class I MHC molecules: There are three major HLA class I molecules, designated HLA–A, B and C each encoded by a distinct gene.

Class II MHC molecules: Like the class I molecules, the class II HLA molecules consist of two polypeptide chains but in this case both are MHC encoded transmembrane proteins and are designated α and β **(Bjorkman, 1997; Kamradt, and Mitchison, 2001).**

The class I molecule remains anchored in the plasma membrane by the tranemembrane region of chain. The class II proteins are expressed constitutively on B-cells, dendritic cells, monocytes, macrophages and all cells that present antigens to $CD4^+$ T-cells. Expression of MHC class II proteins can also be inducced on many additional cell types, including epithelial and endothelial cells **(Skoskiewicz *et al.*, 1985; klien, 2000).**

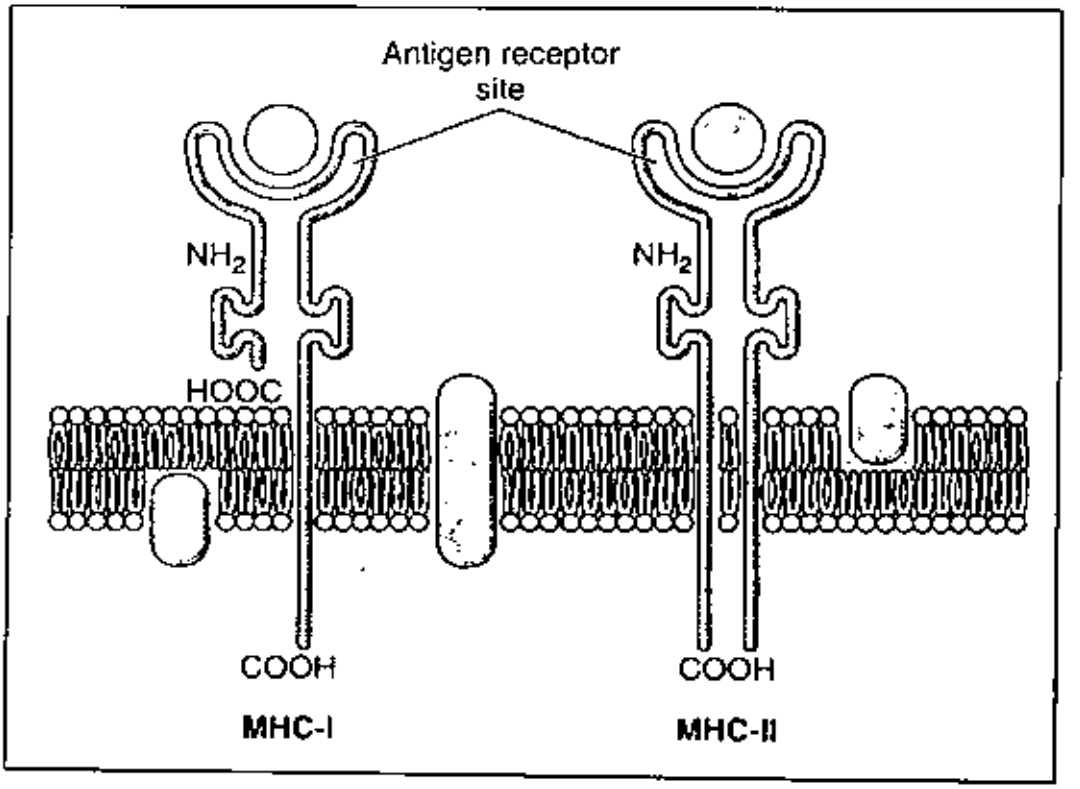

Fig.(2) Structure of HLA Class I and Class II Molecules (Tough DF, Sprent J, 1995).

c)- T-cell development

Each individual T-cell bears antigen recptors of a single specificifty. Selection of cells carrying functional TCR genes occurs in the thymus, a complex lymphoid organ located in the anterior mediastinum at the base of the neck. The subcapsular zone of thymus gland, is where bone marrow-derived prothymocytes, begin to differentiate, proliferate, and rearrange their TCR β chains.The cells then move to the thymic cortex where the chain gene elements rearrange, potentially forming a functional, mature α, β TCR. In the cortex, cells test whether their receptors have sufficient affinity for self MHC molecules to permit them ultimately to recognize antigen–MHC complexes. This involves interactions between the developing lymphocyte and the specialized cortical epithelium **(Van Ewijk, 1991; Miller, 2002).**

d)- Cell –mediated immunity

Cell–mediated immune phenomena are these in which lymphocytes and macrophages predominate. Characteristically such responses can be transferred passively from a sensitized individual to a non-sensitized one by cells, but not by circulating antibody. T-cell play a central role in the induction and execution of CMI. Although effector cells other than lymphocytes may participate in the response as shown in **(Fig.3)**, most reactions of cellular immunity develop more slowly than those mediated by antibody **(Paul and Benacerraf, 1977; Von Andrian and Mackay, 2000).**

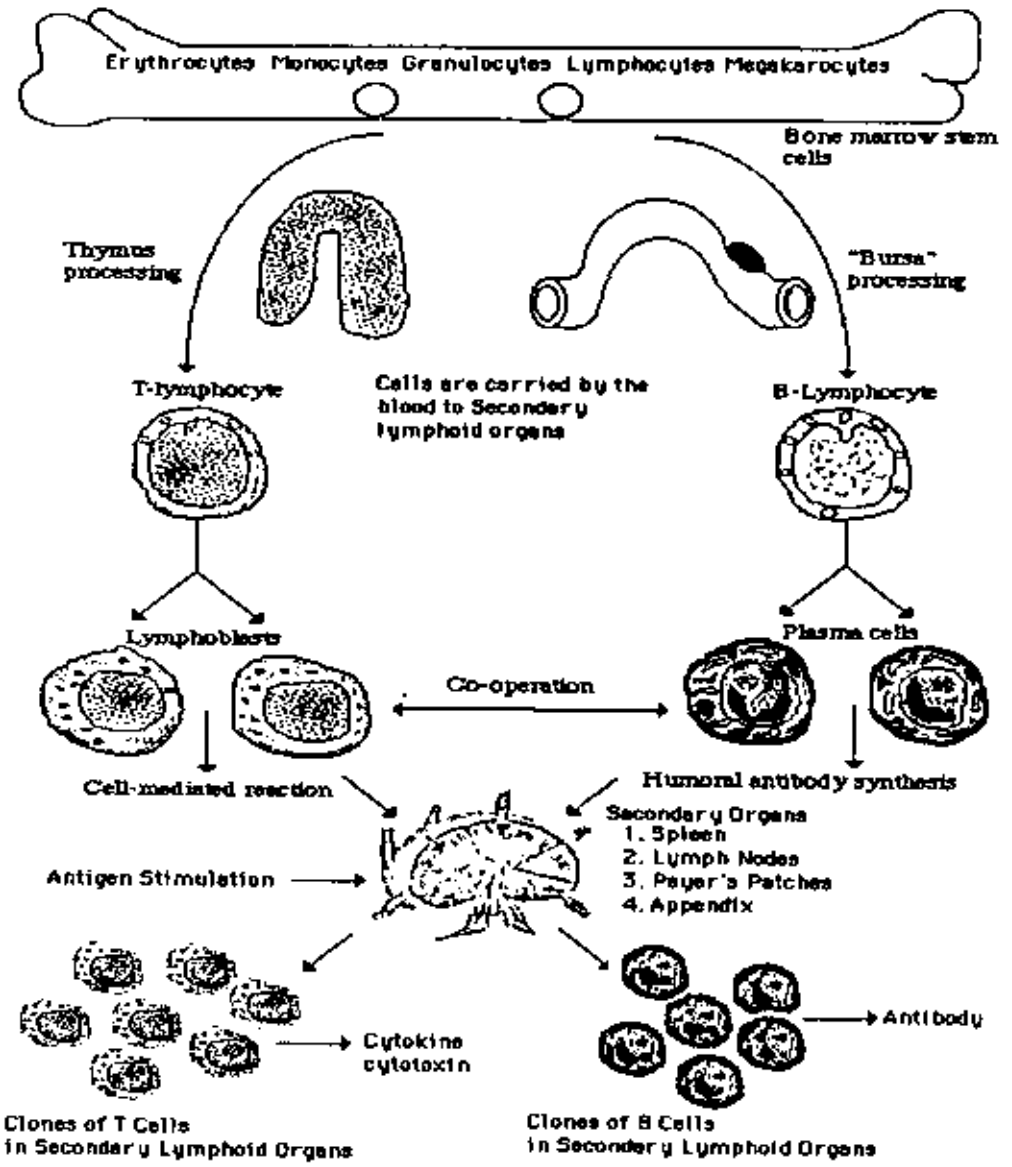

Fig.(3) Acquired immunity (Von Andrian and Mackay, 2000)

5.2) B- lymphocytes

B-cells constitute approximately 15% of peripheral blood leukocytes. They are defined by their production of Igs **(David and Birmingham, 2003).** B-cells differentiate from hematopoictic stem cells in the bone marrow **(Bassing *et al.*, 2002).**

a)- Major populations of B cells

The B-cells that develop earliest during ontogeny are referred to as B1 cells. Most B1 cells express CD5, an adhesion and signaling cell-surface molecule. They are the source of the so–called natural antibodies. In most cases, natural antibodies have a relatively low affinity **(Hayakawa *et al.*, 1999).**

Most B cells lack the CD5 molecule, and because they develop slightly later in ontogeny, they are referred to as B2 cells. Before they encounter antigen, mature B2 cells coexpress IgM and IgD antibodies on their cell surface, but by the time they become memory cells, they have usually switched to the use of IgG, IgA, or IgE as their antigen receptors **(Peter, 2000a).**

b)- **Immunoglobulins**

Immunoglobulins (Igs) molecules are the effector products of B cells and although they all have a broadly similar structure, they show minor differences within the main immunological classes IgG, IgM, IgA, IgD and IgE. The main biological features of the human antibodies are summarized in table **(1) (Janeway and Travers, 1994).**

Antibodies are glycoproteins. They consist of two heavy chains and two light chains as show in **(Fig.4)**, the heavy chain determines the antibody isotype or class, i.e IgG, IgA, IgM, IgD or IgE. The major regions of immunoglobulin are as follows: **Variable V domains:** have variations in the amino acid sequence between immunoglobulins, with short segments of hypervariable regions. Antigen binding occurs in the area where the loops bearing the hypervariable regions of the light and heavy chains come together, called the **fab** region. The shape of the binding site determines the "goodness of fit" or affinity / avidity of any particular antibody for an antigen. **Idiotypes** are markers found in the hypervariable region and are associated with the antigen-binding site. The idiotype is antigenic, idiotypes and anti-idiotypes are thought to make a network regulating the production of antibody. **The Fc** region is formed from the constant domains in which the amino acid sequences are relatively conserved. This is the part that binds to cell-surface immunoglobulin receptors or causes complement fixation, hence it controls the effects of the antibody molecule after it has bound its antigen **(Kamradt and Mitchison, 2001).**

Immunoglobulin isotypes and their functions (Scofied, 2004)

1- Elimination of infective organisms by :

- Binding to prevent adhesion and invasion of organisms (e.g. preventing the entry of poliovirus and other enteroviruses).
- Opsonization of particles for phagocytosis.
- Lysis (in combination with complement).

2- Antitoxin activity (e.g. in prevention of tetanus)

3- Immune regulation, acting as antigen receptor on B cells and presenting the antigen to helper T-cells.

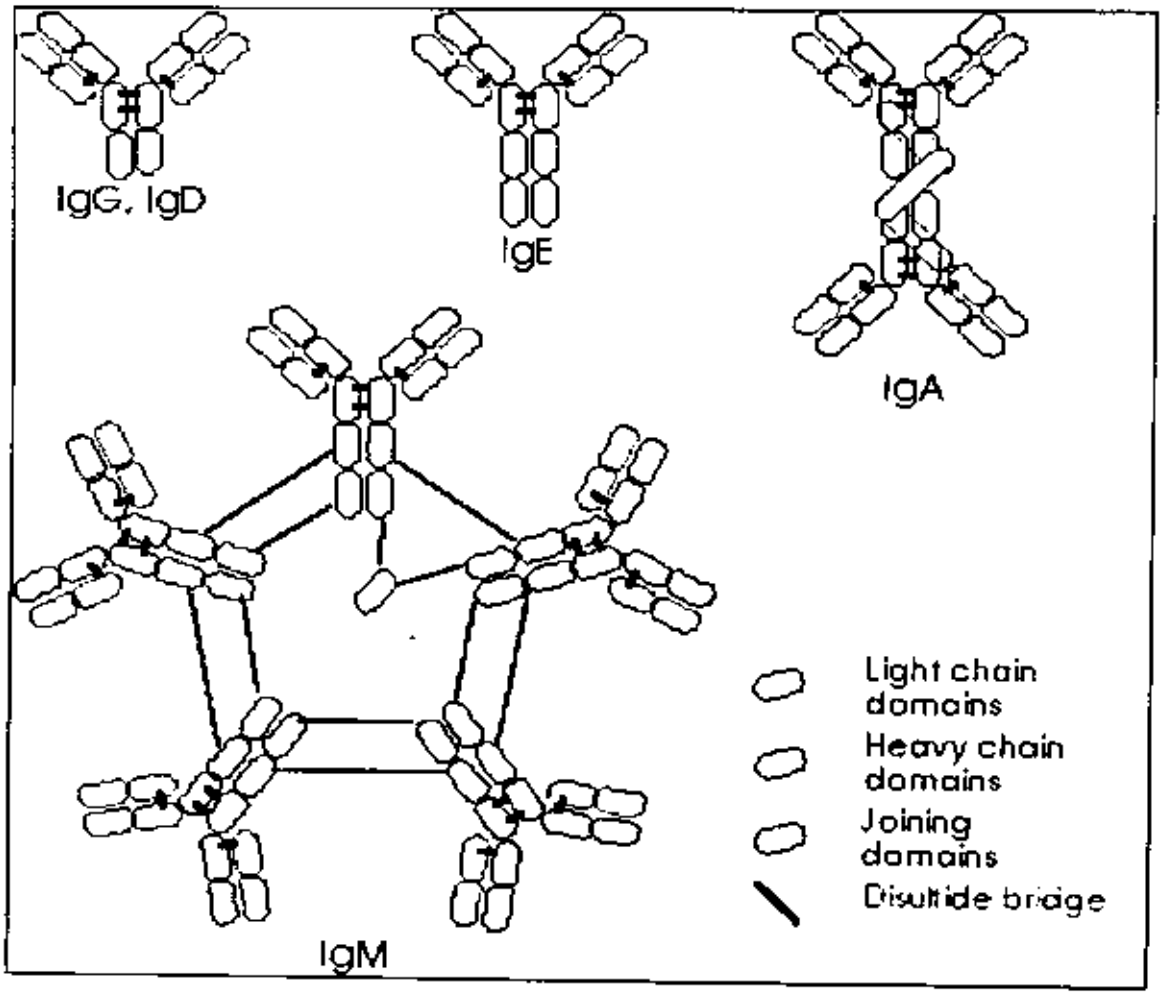

Fig.(4) Structure of immunoglobulin classes (Kamradt and Mitchison, 2001)

IgM: The antibody is confined mainly to the intravascular pool. It is the main immunoglobulin produced early in the primary immune response. It is a large pentameric molecule, bound together by the joining (J) chain as shwon in (**Fig.4**). It does not cross the placenta and is not normally produced in the child until after birth, antigen-specific IgM is a good marker for intrauterine infection. It is usually a sign of a cute infections. IgM is present on the surface of virtually all uncommitted B cells, it is composed of five **H2L2** units (each similar to one IgG unit) and one molecule of **J** chain. It is the most efficient immunoglobulin in agglutination, complement fixation, and other antigen-antibody reactions and is important also in defense agains bacteria and viruses, (**klein, 1993; Kamradt and Mitchison, 2001).**

IgG: This is the most abundant immunoglobulin in serum, present as a monomer. IgG is the antibody of secondary response, and has high antigen affinity. It is the only antibody to crosse the placenta in significant quantities. There are four subclasses:IgG**1**, IgG**2**, IgG3 and IgG**4**. IgG**1** and IgG3 are produced mainly in reponse to protein antigens, such as viruses. These subclasses are good opsonins, binding Fc receptors on neutrophils and activating complement. IgG**2** and IgG**4** are produced in response to polysaccharide antigen (e.g. the capsule of bacteria) and are the major opsonins for such organisms. Each IgG molecule consists of two **L** chains and two **H** chains linked by disulfide bonds (molecular formula **H2L2**) as shown in (**Fig.4**). Because it has two identical antigen-binding sites, it is said to be divalent.**(Roitt, 1994; Kamradt, and Mitchison, 2001).**

Table (1): Comparison between different types of immunoglobulin (Janeway and Travers, 1994).

Characteristics of immunoglobulin	IgG	IgA	IgM	IgE	IgD
Heavy chain symbol	Γ	α	μ	E	δ
Molecular weight×(1000)	150	170 or 400	900	190	150
Serum concentration(mg/ml)	0.5-10	0.5-3	1.5	0.003	0.03
Serum half-life(days)	23	6	5	1-5	2-8
Fixes complement	+	-	+	-	-
Concentration of total immnoglo-bulin serum	80	13	6	<1	<1

IgA: This is mainly the antibody of secretion such as milk, saliva, and tears, being present in the respiratory, gastrointestinal and urinary tracts.There are two subclasses, IgA1 and IgA2, their functions appear to be similar. IgA is mainly monomeric in the serum, but dimeric in secretions, as the molecules being complexed by a joining (*J*) chain. IgA in serum binds to a poly-Fc receptor for IgA and IgM on the basal surface of enterocytes and hepatocytes **(Kamradt and Mitchison, 2001).** It protects mucous membrances from attack by bacteria and viruses. Each secretory IgA molecule (MW 400,000) consists of two **H2L2** units, some IgA exists in serum as a monomer **H2L2** (MW 170.000) there are at least two subclasses, IgA1 and IgA2 **(Roitt, 1994).**

IgD: Serum levels are very low and its function uncertian. IgD is present on the surface of B-lymphocytes, and may have an immunoregulatory role. Levels are high in condition with B-cell activation such as SLE, HIV infection and Hodgkin's disease. It also occurs on cells of some lymphatic leukemia **(Abbas *et al.*, 1994; Kamradt and Mitchison, 2001).**

IgE: IgE is a monomer that is normally present in very low level in serum, as most is membrane-bound to the high-affinity receptors on mast cells and basophils. Its main physiological role is its anti-nematode activity, but its most common clinical relevance is in the pathogenesis of type 1 hypersensitivity (a topic or allergic) disease **(Roitt, 1994; Scofied, 2004).**

c)- Immune protection by antibodies

When B cells undergo terminal differentiation into plasma cells, they acquire the ability to produce and secret high level of antibodies. These antibodies can be directly protective if they inhibit the binding of a microorganism or toxin to the corresponding cellular receptor (**Burton *et al.*, 1998**). Antibodies are synthesized in various lymphoid organs, depending to large extent on the antigens portal entry. In regional lymph nodes following intradermal or subcutaneous stimulation, in the spleen and sometimes in the bone marrow and the lung following intravenous injection and in the subepithelial lymphoid tissues when antigen penetrates the gastrointestinal or respiratory mucosa (**Uhr and Finkelestein, 1967; Peter *et al.*, 2000a**).

Adults produce 3 to 4 g of secretory IgA per day . This form of IgA occurs selectively in saliva, colostrum , and other fluids . It is synthesized by plasma cells underlying mucosal surfaces and then transported across the epithelium by the polyimmunoglobulin Fc receptor (**Krajci *et al.*, 1995**). On the luminal side, the released antibodies prevent the adhesion of microbes to the surface of host cells. A second type of epithelial Fc receptor, FcR, has a number of functions,including transferring maternal IgG across the placenta. This mechanism offers important protection to the fetus before the full development of its own immune system. FcR also specifically transfers IgG from breast milk (which also contains IgA and IgM) across the intestinal epithelium of the neonate (**Ghetie and ward, 1997; Peter *et al.*, 2000b**).

d)- Cluster of differentiation (CD) classification

The CD nomenclature classifies over 300 molecules both within and outside the immune system, using monoclonal antibodies. As the surface molecule has many different epitopes (antibody–binding sites) as shown in **(Fig.5),** several different antibodies may react (a cluster). As initially most of the surface molecules defined the stage of development of the cell, they were termed "differentiation" molecules. The term CD therefore refers to a specific target molecule on a cell that is recognized by one or more antibodies and defines a particular cell type or function **(Scofied, 2004).**

5.3) Natural killer cells

NK cells are thought to represent a third lineage of lymphoid cells. When activated, they have the morphology of a large granular lymphocyte. They develop in the bone marrow under the influence of IL-2, IL-15, and bone marrow stromal cells **(David and Birmingham, 2003; Roitt, 1988).**

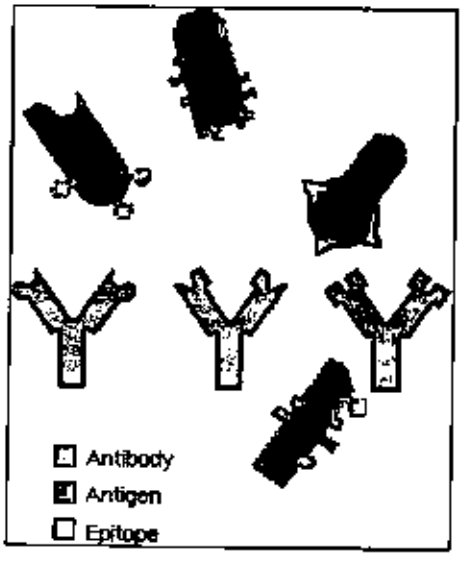

Fig.(5) antibody–binding sites (Scofied, 2004).

They represent only a small fraction of peripheral blood cells and a small fraction of lymphoid cells in the spleen and other secondary lymphoid tissue. NK cells have no antigen- specific receptors although NK cells are not target cell–specific. They exhibit target cell selectivity by mechanisms independent of antibody and antibody–dependent cellular cytotoxicity **(Brown and Borutaite, 2001).**

5.4) Phagocytic cells

The major phagocytic cells are neutrophils, macrophages, and monocytes as shown in **(Fig.6).** These cells engulf pathogenic microbes and use intracellular vacuoles to focus toxic effector such as nitric oxide, superoxide and degradative enzymes, in an effort to destroy the organism **(David and Birmingham, 2003).**

a)- Neutrophils

Like most of the cells involved in immune responses, are not static within a particular tissue. Neutrophils travel within the blood, either flowing freely as part of the circulating pool or rolling a long the vascular endothelium as the marginating pool. The neutrophil (PMN cell) is a specialized microbicidal (microbe–killing) phagocyte. The human body cotains over 10^{11} polymorpho-nuclear leucocytes/Kg, most of which are in the bone marrow **(Kamradt and Mitchison, 2001).**

Neutrophils produce large quantities of reactive oxygen species that are cytotoxic to bacterial pathogens and enzymes that appear to participate in tissue remodeling and repair after injury. Neutrophils have been recognized

to produce substantial amounts of the cytokines; TNF and IL-12, as well as certain chemokines (**Rossetti *et al.*, 2002**).

b)- Monocytes and macrophages

Mononuclear phagocytes consist mainly of macrophages and monocytes. Monocytes are more differentiated and have more endocytic activity than do macrophages. Macrophages are found in virtually all tissues, especially surrounding blood vessels and near epithelial cells. They generally have what is called a stellar morphology. Once they are differentiated, monocytes and macrophages do not divide under normal circumstances. Macrophages in different tissues have different morphologies and different function. For example, liver macrophages (**Kupffer cells**) are located in the sinusoids. They are the major cellular system responsible for the clearance of particulate material or microbes from the circulation. Kupffer cells play a central role in the acute-phase response. Alveolar macrophages efficiently remove particulate materials from the alveolar spaces. They also secrete proteases and bactericidal molecules. Macrophages in bone (osteoclasts) are specialized multinucleated giant cells that are involved in bone resorption and turnover. Phagocytic cells of the monocyte –macrophage lineage also play key roles in the adaptive immune response by taking up microbial antigens. Processing them by proteolysis to peptide fragments and presenting them in to forms that can activate T-cell responses (**Randolph, 1999; David and Birmingham, 2003**).

5.5) Eosinophils

Eosinophils have cytoplasmic granules that contain toxic molecules as shown in **(Fig.6)** and enzymes that are active against helminthes and other parasites **(Iacy and Moqbel, 2001)**. Eosinophils have receptors for IgE which is the major antiparasite antibody, particularly against nematodes. Eosinophils bind IgE via the FcεR, and toxic metabolites are released from the eosinophil granules directly into the parasite surface **(Kamradt and Mitchison, 2001)**.

5.6) Mast cells and basophils

Mast cells consist of two populations, which are distinguished by their enzyme content. The mast cells contain both trypsin and chemotrypsin and are called connective tissue mast cells. Basophils are found in very small numbers in the blood ,basophils and mast cells are morphologically similar cells, have cell surface expression of high-affinity receptors for IgE. They are key initiators of immediate hypersensitivity responses and the host response to helminthic parasites, releasing histamine other performed mediators from their granules and producing important quantities of lipid mediators that stimulate tissue inflammation edema, and smooth muscle contraction. Mast cells play prominent roles in the host response to bacterial infection as well **(Abraham and Malaviya, 1997; Kay, 2001)**.

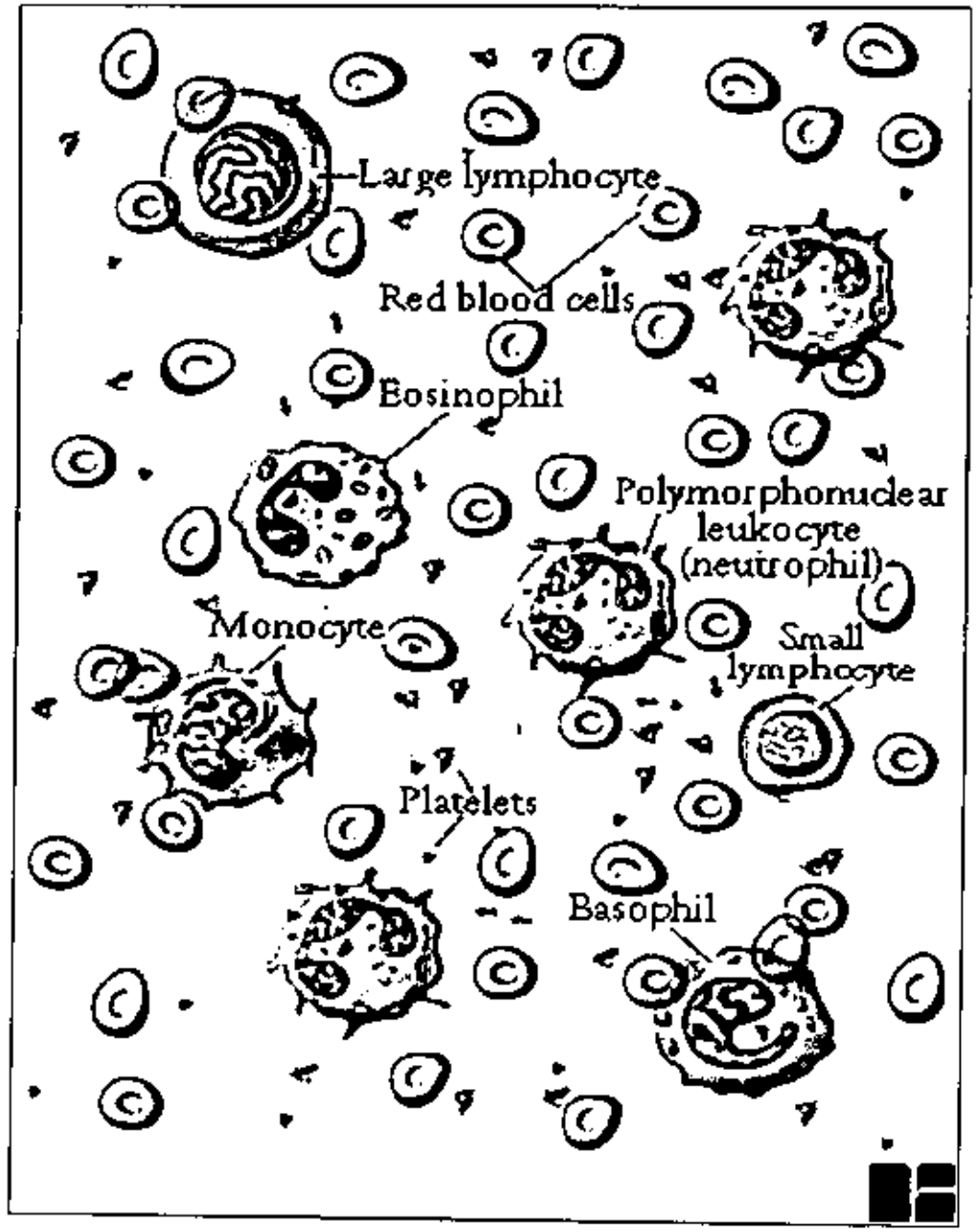

Fig.(6) Cells of immune system (Kay,2001)

6- Smoking

Smoking will be discussed in the following sequence:

6.1- History of smoking

6.2- Types of smoking

6.3- Zonal structure of the cigarette

6.1- History of smoking

Columbus discovery of the Americans introduced Europeans to tobacco and presaged its modern use. Cigarette smoking was less frequent than other forms of tobacco using until the late 1880, but by 1945, cigarette smoking has largely replaced chewing, snuffing and cigar smoking. Currently cigar smoking is once again on the rise **(Fishman *et al.*, 1998).** Smoking habit may be indirectly promoted among teens through television, cinema and tobacco advertisement, as viewing movie, depictions of tobacco use is associated with higher receptivity to smoking prior to trying the smoking behavior itself **(Sargent *et al.*, 2002).**

6.2- Types of smoking

Smoking patterns are not confined to cigarettes, cigar and pipe only; several traditional forms of tobacco smoking and use are known, for example:

1- Bidi and hooka in Bangladesh, India and Indonesia.

2- Chutta in India and Latin America.

3- Chillum and Sulpa in India and Nepal.

4- Cheroots and Kreteks in Indonesia.

5- Narguila, Goza and Shisha in Middle East including Egypt and Libya **(Omar and Sadek, 1995).**

The tobacco contained in Shisha is called " tombak " while that in goza is called " Meassel " which is molassed tobacco (Tobacco:Meassel=1:3). Each consumed unit of tobacco in goza is called " Korsi " and is equivalent in weight to one cigarette. The duration of the puff in goza is double that in cigarette **(Salem, 1979).** The sublimate of the cigarette with water filter is less than that of the cigarette with cellulose acetate filter (1.07 % and 2.81 % respectively). The nicotine content of the condensate is 0.35 % and 0.106 % respectively. The nicotine content in the sublimate of goza is 0.068 % and that in cigarette is 0.106 % respectively **(Salem, 1973).**

6.3- Zonal structure of the cigarette

Keith (1982) stated that there are four functional components forming the modem machine rolled cigarette as shown in (Fig.7) which are :-

a) Combustion zone:

It is the red glowering tip of the cigarette. In this zone burning is relatively complete, the tobacco being converted to simple products such as carbon monoxide, carbon dioxide and water with extraction of most of the oxygen. The temperature is the highest in the central portion of this zone and the yield of polycyclic hydrocarbons is the greatest **(Wynde and Haffman, 1987).** The cone temperature can be reduced by the use of additives such as sulphur, magnesium carbonate or vanadium pentoxide. Theses substances reduce the yield of polycyclic hydrocarbons **(Miller *et al.*, 1988).**

b) Pyrolysis zone:

The pyrolysis zone lies immediately behind the glowering tip where combustion of high temperature 600-1050 C (**Baker, 1984**) and a reducing gas rich in hydrogen encourage both conversion of carbon dioxide into carbon monoxide and pyrolysis of the tobacco to a variety of noxious hydrocarbons which condense on very small nuclei emitted by the combustion zone yielding an extremely dense smoke cloud (10^8-10^{10}/ ml) of spherical particles with a relatively uniform in size "range 0.2-1 micron, median 0.15-0.20 micron on a number average 0.5-0.6 micron on a mass average" **(Keith, 1982).**

c) Distillation Zone:

Where temperature of the smoke drops quite rapidly to about 40°C as it passes among the cigarette. Cooling favors the condensation of smoke constituents with a high boiling point. There is also a technology for coagulation of the smoke particles. Nevertheless, little mechanical filtration occurs and in the region immediately adjacent to the pyrolysis zone the temperature remains high enough for 20-30% of nicotine additives such as menthol to distill the smoke without alteration of the chemical composition. In this segment, smoke can diffuse through the wrapper into the room, while the maternal inhaled by the smoke can be diluted by room air. It is further possible to change the characteristics of the smoke in this zone by pre-treatment of the tobacco or replacement of some of the tobacco by less toxic filler **(Kiefer, 1982).**

d) Terminal filter:

Majority of modern cigarettes incorporate a filter, which traps components of the gas phase. Efficiency of various categories of filters ranges from 25-75% depending upon the puff resistance the smoke is prepared to accept. Incorporating an air vent into the filter, which dilutes the smoke, can diminish this pressure drop and slow its passage through the filter-encouraging particle trapping, depending on the routing of the air stream previously trapped and deposited. Volatile constituents may also be reincorporated into the smoke. The filtration of smoke particles, nicotine and tar normally proceeds roughly in parallel. Unfortunately such compounds give flavor to the smoke and for this reason an effective filter may be unacceptable to the dedicated smokers by use of:

1. Carbon filter for example one can achieve a ten fold selectively in the removal of irritants such as acrolein.

2. Cellulose acetate filters in contrast give a selective extraction of phenol (2-3folds) and other polar compounds.

3. Hydrocarbon polymers containing filters are particularly effective in removing non- polar compounds **(Keith, 1982).**

7- The aim of the study:

The aim of this study is to assess the effects of smoking on some immunoglobulin parameters.

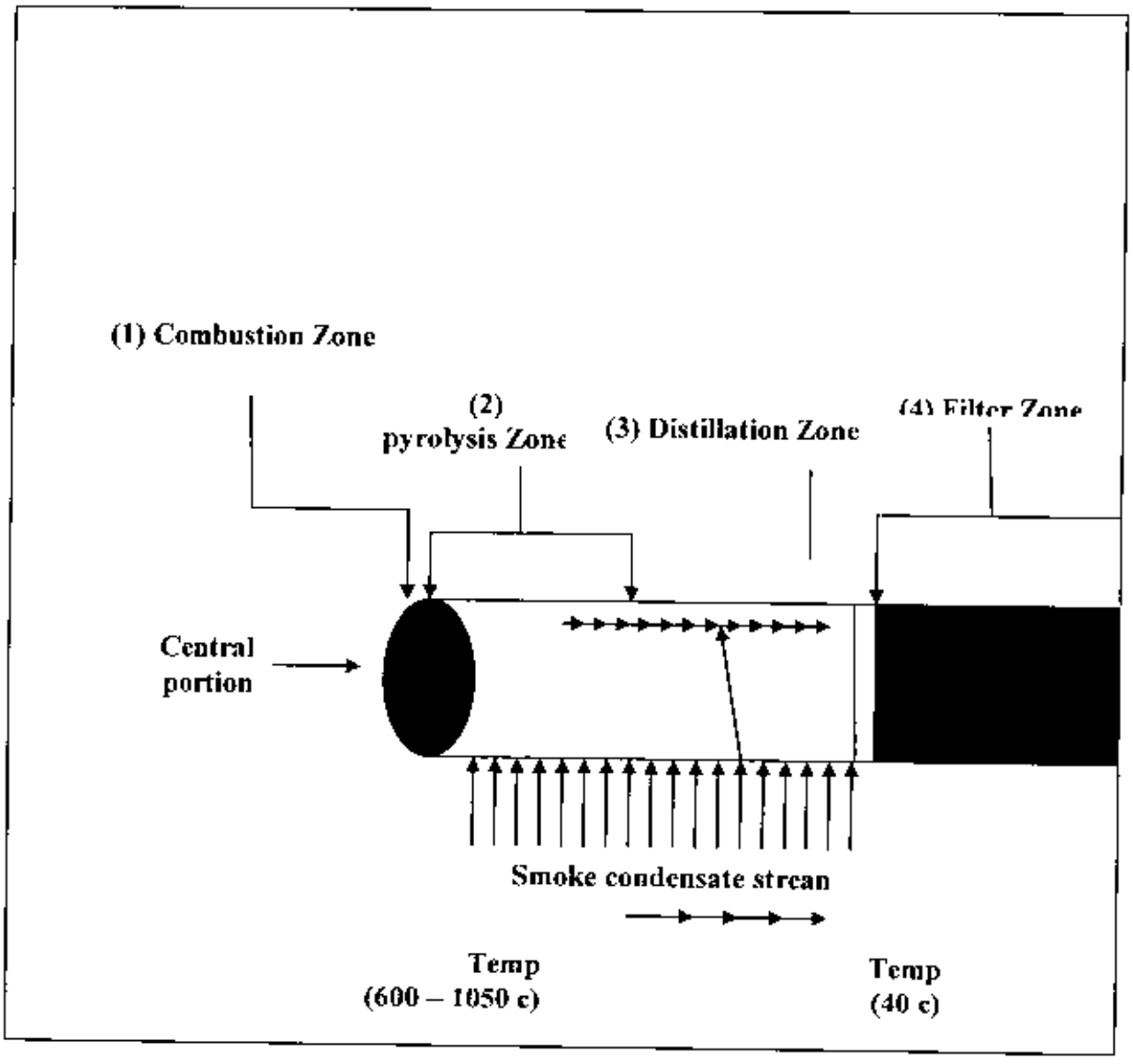

Fig.(7) Functional components of cigarette (Keith, 1982).

CHAPTER I

REVIEW OF LITERATURE

REVIEW OF LITERATURE

More than 400,000 people die in the USA because of smoking and direct expenditures per year for medical purposes regarding smoking–related morbidity exceed 50 million US dollars **(Sopori and Kozak, 1998).**

Among US adults, cigarette smoking has declined from about 42% of the population in 1965 to about 21% in 2005. About 45 million adults smoked cigarettes in 2005. About 23% of men and 19% of women were smokers. Education seems to affect smoking rates, as shown by a steady decrease in the smoking rates in groups with a higher level of education.**(Yolton *et al.*, 2005).**

Fishman *et al.*, (1998) stated that after knowing the harmful components and residues of tobacco we can clarify the fact that tobacco is injurious. Constituents of tobacco smoking are classified into four groups which are :

1. Toxic gases
2. Nicotine
3. Irritant gases
4. Carcinogens

1)Toxic gases

They are numerous as the gas phase accounts for 60% of total cigarette smoke. It was found that 98.9% of the gas phase is made up of he following components:-

- Nitrogen 73%
- Oxygen 10%
- Carbon dioxide 9.5%
- Carbon monoxide 4.2%
- Hydrogen 1%
- Argon 0.6%
- Methane 0.6%

The most important and toxic one is carbon monoxide; other potentially toxic gases include cyanides,and oxides of nitrogen.

2) Nicotine

Nicotine has a lipid solubility property, so it is quickly absorbed via the alveoli in the pulmonary capillary circulation with rapidly increasing level in arterial circulation acceleration its effect on the brain receptors. Mentioned that nicotine withdrawal symptoms include:

- Insomnia
- Anxiety
- Irritability frustrations or anger
- Restlesseness
- Decreased heart rate
- Lack of concentration
- Depressed mode (dysphoria)

Benowitz (1990), nowadays, scientists agreed on the fact that tobacco smoking with its nicotine residue is considered as a sort of addiction and equivalent to different categories of addictive substances. The failure to quite smoking is attributed in large part to the addictive properties of nicotine.

Tobacco can addict resembling the power of substances as alcohol and opium.

3) Irritant gases

They include substances which stimulate coughing, wheezing, and are cilio-static e.g. (acrolein). Both the gas phase and tobacco smoke condensate are cilio-static and broncho-constrictor. Filtering out one phase or another is reducing but not abolishing the hazardous effect of these substances **(Henningfield *et al*., 1990).**

4) Carcinogens

Some of polycylic compounds isolated from the particulate phase of the cigarette smoke have been established to be carcinogenic, the most powerful is the benzo-o-pyrene. Phenols and free fatty acids occur abundantly in tobacco smoke and can function as co-carcinogens **(Bakir, *et al*., 1991).** Smoking cigarettes increases the risk of heart disease. Smokers who have a heart attack are more likely to die within an hour of the heart attack than nonsmokers. Cigarette smoke can cause harm to the heart at very low levels; even levels much lower than needed to cause lung disease **(Pletcher *et al*., 2006).**

COPD is a disease primarily caused by cigarette smoking, which in turn has been shown to affect the susceptibility to and progression of airway infections. After exposure to CSE barrier function, the epithelial cells were more resistant to CSE and responded at doses 20 times higher than T-cells. This study shows that mechanisms, in both T-cells and airway epithelial cells, involved in the defense against infectious agents are modulated by CSE **(Glader *et al*., 2006).**

Plasma cholesterol levels were determined for 51,723 participants of community-based cholesterol screenings in 10 United States cities during 1988. Among white adult men and women under the age of 60 without other cardiovascular disease risk factors, a dose-response relationship was found between the number of cigarettes smoked per day and increasing levels of plasma cholesterol. In men aged 18 to 60 years, average plasma cholesterol increased by 0.33 mg/dl for each cigarette smoked ($P < 0.001$); in women aged 31 to 50 years, average plasma cholesterol increased by 0.48 mg/dl for each cigarette smoked ($P < 0.001$). Plasma cholesterol levels among ex-smokers were found to be similar to those of nonsmokers. No association between cigarette smoking and levels of plasma cholesterol was observed in men and women over age 60. Possible mechanisms for this observed relationship include an intestrogenic effect of cigarette smoking that make the observation more noticeable in younger female, enhanced lipolysis that increases levels of plasma free fatty acids, or differences in dietary intake between smokers and nonsmokers **(Muscat *et al.*, 1991).**

Effect of smoking on the immune system and its parameters is not fully understood and the data about this is limited and somewhat contradictory The studies up till now denoted that the immunotoxin and genotoxin impacts of cigarette arise from the particle phase more than the smoke–phase. The particle phase is composed of thousands of substances, but mainly nicotine. There are a lot of findings about the fact that nicotine is the major immunosuppressive in cigarette and / or smokeless tobacco. Nicotine causes the secretion of chatecolamines that have suppressive effects on immune system by inducing ACTH secretion **(Sopori and Kozak, 1998; Basta *et al.*, 2001).**

Aoshiba *et al.*, (1994) decreased deformability of neutrophils exposed to cigarette smoke is considered a determinant of neutrophil sequestration within the pulmonary microvasculature, which may be a risk for the development of pulmonary emphysema. In this study they examined the effect of nicotine, a major cigarette component, on the reduction of neutrophil deformability, measured as cell filterability, after exposed to cigarette smoke. Neutrophils were exposed to smoke by incubating them in an aqueous solution of smoke extracts. Filterability of neutrophils was studied by a vertical filtration technique by measuring their ability to pass through a micropore membrane and expressed as membrane resistance. There was a negative relationship between membrane resistance after exposure to whole smoke and the nicotine content of the cigarettes tested. Whole smoke increased the membrane resistance less than gas-phase smoke. These results suggest that nicotine prevents the reduction in neutrophil filterability, probably by scavenging oxidants present in the cigarette smoke.

Hematopoiesis, generation of mature blood cells, is vital for life: the white cells fight infections, the red cells carry oxygen throughout the body, and the platelets promote healing and prevent bleeding. The hematopoietic stem cell is a progenitor cell that generates all varieties of mature blood cells throughout life. During adult life, hematopoiesis takes place in the bone marrow. However, colonization of the bone marrow with primitive hematopoietic stem cells must occur during intrauterine development of the fetus. Thus, any changes in the ability of primitive hematopoietic stem cells to egress from the fetal liver, to immigrate into the fetal bone marrow and to establish normal hematopoiesis could be deleterious to the newborn. Nicotine is one such factor, the exposure to which could lead to serious

alterations in the bone marrow development, resulting in a disruption of hematopoietic homeostasis and a changed ratio of mature cells in the blood. Indeed, we have demonstrated that newborn mice exposed to nicotine during intrauterine development have a lower number of hematopoietic stem cells in the bone marrow in comparison to control animals. Furthermore, these mice had severe immunodeficiency during the first month after birth. Based on the preliminary observations, they propose to investigate how nicotine can alter the ability of bone marrow stromal cells to produce soluble factors that mediate migration of hematopoietic stem cells, colonization of the bone marrow and production of mature blood cells. In addition, the investigators plan to examine whether nicotine-induced immunodeficiency in newborn mice is due to inhibition of the functional activity of the immune cells or is a result of impaired colonization of the bone marrow with hematopoietic stem cells and lower production of lymphoid progenitor cells and, consequently, mature immune cells **(Khaldoyanidi and Orlovskaya, 2002).**

Nicotine, one of the major immunosuppressents in tobacco smoke, is responsible for the majority of these deaths. In fact, chronic exposure on cigarette smoke can suppress (AFC) response without affecting the number of lymphocyte, inhibit (TCR) mediated proliferation. Chronic smoking can also inhibit the rise in intracellular Ca^{2+} levels of B and T-cells following the ligation of antigen receptors **(Basta *et al.*, 2001).** Nicotine, also results in decreasing oxygen carrying capacity to the tissue due to increase (COHb), decrease of prostacyclins, which lead to decrease in its dilator effect on the endothelium and increase the platelets aggregation, and finally increase in the blood viscosity potentiating vascular stasis and thrombosis.

However nicotine leads to non-specific oesinophilia and increase serum immunoglobulin "E" (**Bakir *et al.*, 1991**).

Habitual cigarette smoking has also been proven to be associated with a decreased proliferative response to a T-cell mitogen. Cigarette smoke affects a wide range of immunological function in humans and animals including both cell–mediated and humoral immune responses. The results prove that chronic smoking affects the antigen-mediated activation of T-cells in the 8-month nicotine exposed animals, thus suppressing the immune response in these animals (**Goud *et al.*, 1994; Finch *et al.*, 1999**).

Moszczynski *et al.*, (2001) had observed a decrease in CD4/CD8 ratio due to the decrease in the serum concentration of lysozyme and immunoglobulins and a decrease in the number of ($CD16^+$) NK cells particularly in the addicts who had smoked for more than 10 years and an increase in the number of ($CD8^+$) cytotoxic T- lymphocytes.

Studies of the effects of cigarette smoking by pregnant women on intrauterine development of the offspring are foremost, since the development of the child is likely to be hindered. Infants whose mothers smoked during pregnancy have lower size and weight at birth and are not only predisposed to pulmonary and cardiovascular diseases, but also to a severe immunodeficiency (**Khaldoyanidi and Orlovskaya, 2002**). Smoking decreases serum levels of almost all types of immunoglobulins except IgE (IgE increases) (**Gerrard *et al.*, 1980; Burrows *et al.*, 1982**).

In the study of **Murat *et al.*, (2006)** is assess the impacts of "Maras powder" and cigarette smoking on the parameters of the humoral immune system. Maras powder is a kind of smokeless tobacco that is used by the addicts through buccal mucosa instead of cigarette or in order to give up smoking. It is more addictive than smoking. Its negative impacts on human health could not yet be fully understood. A similar kind of smokeless tobacco used in Sudan is known as Toombak It is reported that Toombak use may play an important role in the etiology of oral sequamosus cell carcinoma of the oral cavity and also may be associated with salivary gland cancers **(Lazarus *et al.*, 1996).**

177 subjects were included in a study, done by **(Murphy *et al.*, 1994),** the IgA, IgG, IgM, C3 and C4 levels were detected via nephelometric method. In 1.4 % of the control group IgM levels were below normal where it was 10.8% and 18.6 % in maras powder group and in cigarette smoking group respectively. The IgM levels of both groups were significantly lower compared to the control group (P<.05). Nonetheless.the IgE levels of maras powder group and smoking group were found to be remarkably higher compared to the control group (P< .01). Nearly all of the individuals (86.5%-95.7%) have levels of IgA, IgG,C3, and C4 within the normal limits. A high number of immune abnormalities, both humoral and cellular, occure either transiently or permanently in type 1 diabetes **(Eisenbarth, 1986).** Despite this abundance of reports, most researchers consider that whether the role of immunological factors is primarily pathogenetic, co-causative, secondary or simply chronologically associated to pathogenic events has not been definitively answered **(Boitard *et al.*, 1997; Stene *et al.*, 2004).** They finding of low levels in both IgG and IgM in smoking

pregnant diabetic sera, leading to humoral immune abnormalities in immunoglobulin estimation by ELISA represents a move in this direction.

CHAPTER II

MATERIAL AND METHODS

MATERIAL AND METHODS

1-Subjects

299 subjects from sirte city, Libya of either sex participated in this study. Full history was taken, from every volunteer. This history included past history of taking drugs, previous illness or any previous surgical operation, any other complaint or abnormal habits. Smoker only were asked about full smoking history such as date of smoking, and number of cigarettes used per day as shown in **(Fig.8)**. In this study, two types of smokers were randomly selected, active smoker and passive smokers. Active smokers were smoking more than 20 cigarettes per day for at least 5 years back to the study. Also, passive smoker, were exposed to active smoker for at least 5 years back to the study. All volunteers were divided into male and female groups. (None of the adult fertile females were pregnant or complaing of any gynecological or obstetrical disturbance). Male and female groups were divided into 4 separate groups according to age, and each group was subdivided into 3 subgroups; non-smoker subgroup, passive smoker subgroup and active smoker subgroup, as in Table **(2)**.

2-Sampling of blood

Venous blood sample was taken from each fellow. It was taken and divided into two portions: a portion on which an anticoagulant was added ethylene diamine tetra acetic acid (EDTA), powder for estimation of hemoglobin and total leukocytic count, and the other portion was collected into a centrifuge tube, containing no anticoagulant. Each tube was then centrifuged for 4-6 minutes in order to separate serum from cells. Sera were separated by fine pipettes to be analyzed, this experimental work was

carried out at analysis lapratoray, blood bank / Ibn Sina Hospital and Zoology labratoray / Al-Tahadi university .

Table (2): Classification of different group

Group	Symbol	Number (n=)	Age (year)	Subgroups
1) Mal groups Early age group	A	48	15-30	A1:Non smoker (n=14) A2:Passive smoker(n=13) A3:Active smoker (n=21)
Middle age group	B	47	30-45	B1: Nonsmoker (n=17) B2:Passive smoker(n=13) B3:Active smoker (n=17)
Late age group	C	39	45-60	C1:Nonsmoker (n=12) C2:Passive smoker(n=13) C3:Active smoker (n=14)
Old age group	D	34	Above 60	D1:Nonsmoker (n=10) D2:Passive smoker(n=11) D3:Active smoker (n=13)
2) Female groups Early age group	E	32	15-30	E1:Nonsmoker (n=10) E2:Passive smoker(n=11) E3:Active smoker (n=10)
Middle age group	F	33	30-45	F1:Nonsmoker (n=11) F2:Passive smoker(n=10) F3:Active smoker (n=12)
Late age group	G	34	45-60	G1:Nonsmoker (n=13) G2:Passive smoker (n=10) G3:Active smoker (n=11)
Old age group	H	32	Above 60	H1:Nonsmoker (n=10) H2:Passive smoker (n=10) H3:Active smoker (n=12)

No.	***Data collected using the questionnaire***	***Answer***
1	Age	
2	Weight	
3	Genus	
4	Smoking (active-passive)	
5	Number of cigarette per day	
6	Types of smoking (Cigarette-Goza or Shisha)	
7	Social state (married/ non-married)	
8	Health state	
9	Pregnancy	

Fig .(8) Questionnaire

All precautions were done to avoid hemolysis of cells. In each sample from second portion, the following parameters were estimated: IgA, IgM,IgG and C3. It is to be noted that blood samples in adult fertile females was taken during mid luteal phase of menstrual cycle.

3-Methods

3.1- Estimation of leukocytes: were counted by the visual method according to **Dacie and lewis (1984).**

3.2- Estimation of hemoglobin concentration: was done by cyanomet hemoglobin method using a photoelectric colorimeter according to **Drapkin (1932):**

- Reagents

Reagent1 potassium ferricyanur 30 mmol/l

Drabkin potassium cyanur 38 mmol/l

Reagent phosphate monopotatium 50 mmol/l

50 fold sterox 25g/l

Toxic Reagent: use automatic pipettor. Store at 20-25°

Sample: blood collected on EDTA.

- Procedure

Work reagent

Drabkin reagent R1.....1 volume

Distilled water.....49 volume

- Stability

1 month at 20-25° C (do not refrigerate)

Wavelength.....540 nm (546 Hg)

Blank .....work reagent

	Standard	Sample
Standard Hg	5ml	
Sample		20µl
Work solution		5ml
Coloration stability		1 hour

(avoid to expose reaction on strong light)

- Calculation

Hemoglobin concentration. g/l =OD sample$^{\times 376}$

- References values (Biochemists handbook 1961)

Newborn : 195±50 g/l (12mmol/l)

Children (1year): 112 g/l (6.95mmol/l)

Children (10 years): 129 g/l (8 mmol/l)

Man : 160± 20 g/l (9.9 mmol/l)

Women : 140± 20 g/l (8.7 mmol/l)

3.3- Estimation of IgA: the estimation was done using radioimmune diffusion (RID) plates (from Astra s.r.I, prodotti diagnostics-using IgA monorid plates) according to **Alexsander, (1980):**

- Principle of the method

In RID, patient specimen in a small well was let to react with an antiserum suspended agarose gel to form antibody antigen complexes. After the incubation, the protein to be examined and the corresponding antibody in the agarose results in a precipitation ring which diameter is proportional to the level of antigen. The value of the measured diameter in millimeters can be compared to a conversion table and the analyte concentration determined as shown in (**Fig.9**) and table (3).

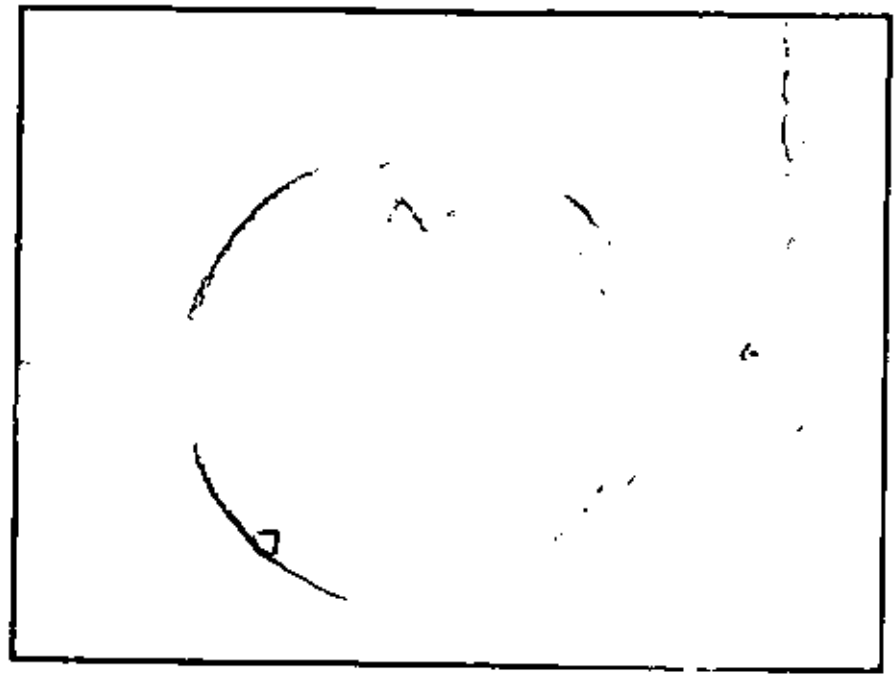

Fig. (9) Immune diffusion plate for IgA

Table (3) Conversion table for Ig A

Ø (mm)	CONC. mg/dl (CRM 470)	Ø (mm)	CONC. mg/dl (CRM 470)	Ø (mm)	CONC. mg/dl (CRM 470)
5	9,20	7,5	196,28	10	458,19
5,1	15,24	7,6	205,32	10,1	470,22
5,2	21,41	7,7	214,48	10,2	482,37
5,3	27,70	7,8	223,75	10,3	494,64
5,4	34,10	7,9	233,15	10,4	507,03
5,5	40,63	8	242,67	10,5	519,56
5,6	47,27	8,1	252,31	10,6	532,18
5,7	54,04	8,2	262,07	10,7	544,93
5,8	60,92	8,3	271,95	10,8	557,80
5,9	67,93	8,4	281,94	10,9	570,79
6	75,05	8,5	292,06	11	583,90
6,1	82,29	8,6	302,30	11,1	597,13
6,2	89,68	8,7	312,65	11,2	610,48
6,3	97,14	8,8	323,13	11,3	623,95
6,4	104,74	8,9	333,73	11,4	637,54
6,5	112,47	9	344,44	11,5	651,25
6,6	120,31	9,1	355,28	11,6	665,08
6,7	128,27	9,2	366,23	11,7	679,03
6,8	136,35	9,3	377,31	11,8	693,09
6,9	144,55	9,4	388,50	11,9	707,28
7	152,87	9,5	399,82	12	721,59
7,1	161,31	9,6	411,25	12,1	736,02
7,2	169,88	9,7	422,80	12,2	750,57
7,3	178,56	9,8	434,48	12,3	765,23
7,4	187,36	9,9	446,27	12,4	780,02

- **Sample:** undiluted serum
- **Volume used**: 10µl into the well
- **Test procedures:** at ambient temperature (18-30°C).

1-Carefully pipette required samples into the center of each well of RID plate.

Note :avoid any overfill or damage to the surrounding agarose gel.

2- Cover RID plate and incubate 48 hours at ambient temperature.

3- Measure diameter of precipitation ring and compare to the corresponding value on the conversion table and record concentration.

-Conversion table IgA Monorid plates

1- Normal values: 90-450 mg/dl

2- Note : carefully measure ring diameters with precision of ± 0.1mm. It is recommended to include ASTRA controls and calibrators in test series.

3.4- Estimation of IgG: the estimation was done using radioimmune diffusion plates (from Astra s.r.I, prodotti diagnostics-using IgG monorid plates), according to **Alexsander, Jr. (1980):**

- **Principle of the method**

As previously mentioned in estimation of IgA

-Conversion table Ig G Monorid plates

Normal values: 800-1800 mg/dl

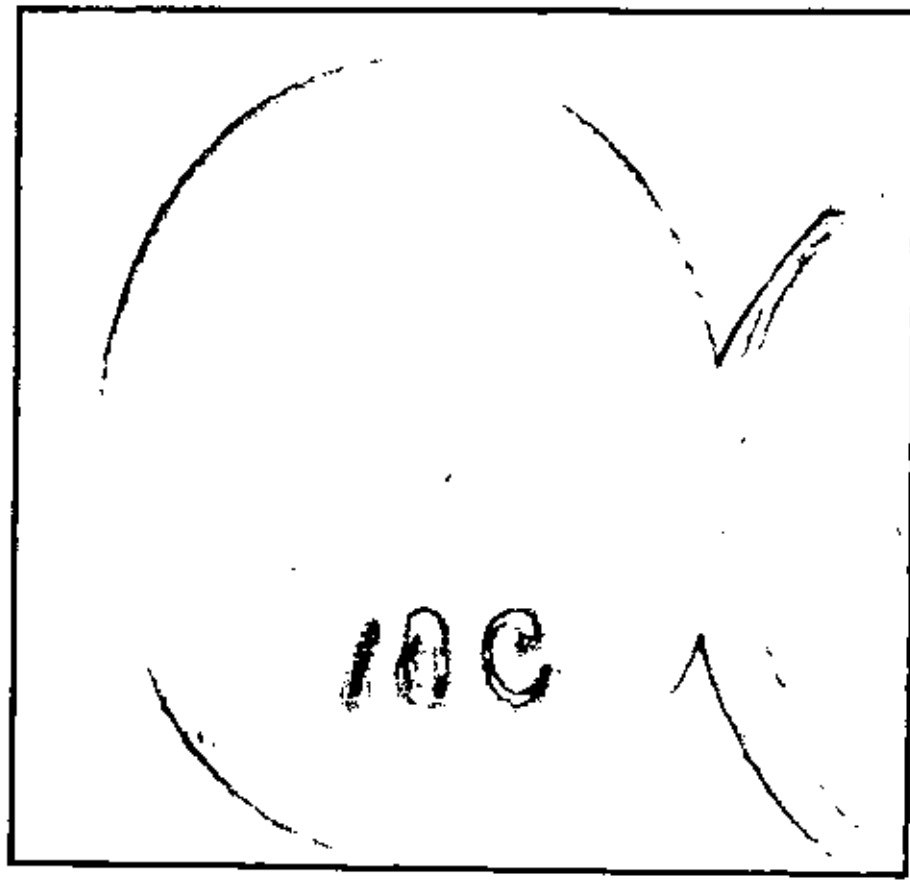

Fig. (10) Immune diffusion plate for IgG

Table (4) Conversion table for Ig G

(mm)	CONC. mg/dl (CRM 470)	(mm)	CONC. mg/dl (CRM 470)	(mm)	CONC. mg/dl (CRM 470)
5,5	389,55	7,7	1310,65	9,9	2538,80
5,6	424,75	7,8	1359,82	10	2601,92
5,7	460,60	7,9	1409,62	10,1	2665,67
5,8	497,07	8	1460,05	10,2	2730,06
5,9	534,18	8,1	1511,12	10,3	2795,08
6	571,93	8,2	1562,82	10,4	2860,74
6,1	610,31	8,3	1615,15	10,5	2927,03
6,2	649,32	8,4	1668,12	10,6	2993,96
6,3	688,97	8,5	1721,73	10,7	3061,52
6,4	729,25	8,6	1775,97	10,8	3129,72
6,5	770,17	8,7	1830,84	10,9	3198,54
6,6	811,72	8,8	1886,35	11	3268,01
6,7	853,91	8,9	1942,49	11,1	3338,11
6,8	896,73	9	1999,26	11,2	3408,84
6,9	940,18	9,1	2056,67	11,3	3480,21
7	984,27	9,2	2114,72	11,4	3552,21
7,1	1028,99	9,3	2173,40	11,5	3624,84
7,2	1074,35	9,4	2232,71	11,6	3698,11
7,3	1120,34	9,5	2292,66	11,7	3772,02
7,4	1166,97	9,6	2353,24	11,8	3846,56
7,5	1214,23	9,7	2414,46	11,9	3921,73
7,6	1262,12	9,8	2476,31	12	3997,54

3.5- Estimation of IgM: was done using radioimmune diffusion plates (from Astra s.r.l, prodotti diagnostics-using IgM monorid plates) according to **Alexsander, Jr. (1980):**

- Principle of the method

As previously mentioned in estimation of IgA.

Note: cover RID plate and incubate 72 hours at ambient temperature.

- Conversion table IgM Monorid plate

Normal values: 60-280 mg/dl

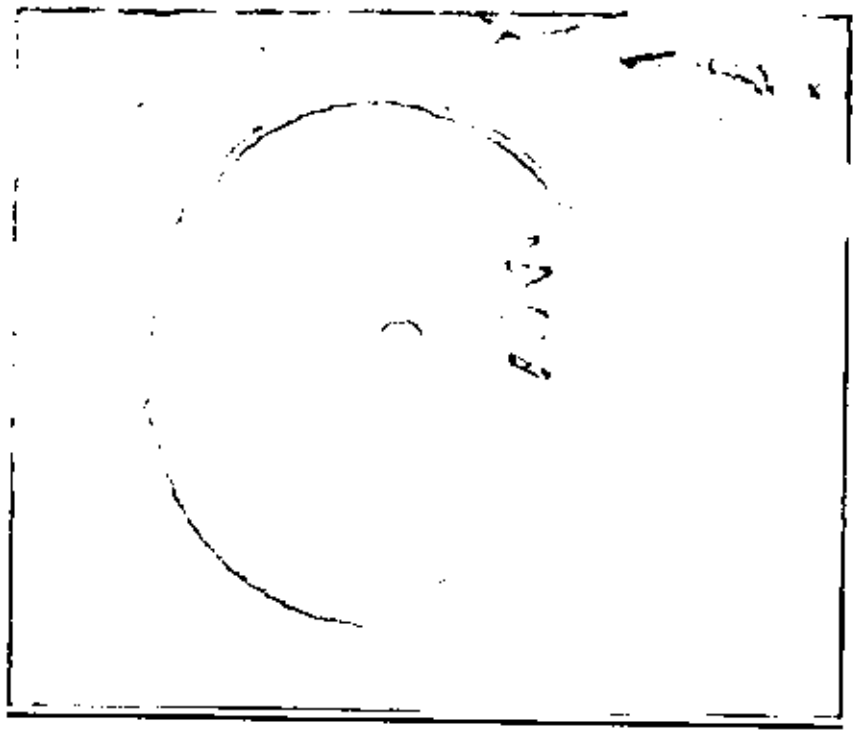

Fig.(11) Immune diffusion plate for IgM

Table (5) Conversion table for IgM

Ø (mm)	CONC. mg/dl (CRM 470)	Ø (mm)	CONC. mg/dl (CRM 470)	Ø (mm)	CONC. mg/dl (CRM 470)
5	22,11	7,2	115,33	9,4	242,16
5,1	25,62	7,3	120,36	9,5	248,72
5,2	29,20	7,4	125,47	9,6	255,36
5,3	32,84	7,5	130,64	9,7	262,06
5,4	36,56	7,6	135,89	9,8	268,83
5,5	40,35	7,7	141,20	9,9	275,67
5,6	44,20	7,8	146,58	10	282,58
5,7	48,12	7,9	152,04	10,1	289,56
5,8	52,12	8	157,56	10,2	296,61
5,9	56,18	8,1	163,15	10,3	303,73
6	60,31	8,2	168,81	10,4	310,92
6,1	64,52	8,3	174,54	10,5	318,18
6,2	68,79	8,4	180,34	10,6	325,51
6,3	73,13	8,5	186,21	10,7	332,91
6,4	77,54	8,6	192,15	10,8	340,37
6,5	82,02	8,7	198,16	10,9	347,91
6,6	86,57	8,8	204,23	11	355,52
6,7	91,19	8,9	210,38	11,1	363,19
6,8	95,88	9	216,60	11,2	370,94
6,9	100,64	9,1	222,88	11,3	378,75
7	105,46	9,2	229,24	11,4	386,63
7,1	110,36	9,3	235,66	11,5	394,59

3.6- Estimation of C3: was done using radioimmune diffusion plates (from Astra s.r.I, prodotti diagnostics-using C3 monorid plates) according to **Alexsander, Jr. (1980):**

- Principle of the method

As previously mentioned in estimation of IgA.

- Conversion table C3 Monorid plates

Normal values: 70-170 mg/dl

Fig.(12) Immune diffusion plate for C3

Table (6) Conversion table for C3

Ø (mm)	CONC. mg/dl (CRM 470)	Ø (mm)	CONC. mg/dl (CRM 470)	Ø (mm)	CONC. mg/dl (CRM 470)
5	30.88	7.2	136.00	9.4	279.04
5.1	34.83	7.3	141.68	9.5	286.45
5.2	38.87	7.4	147.44	9.6	293.93
5.3	42.98	7.5	153.28	9.7	301.49
5.4	47.17	7.6	159.19	9.8	309.13
5.5	51.44	7.7	165.18	9.9	316.84
5.6	55.79	7.8	171.25	10	324.64
5.7	60.21	7.9	177.40	10.1	332.51
5.8	64.72	8	183.63	10.2	340.46
5.9	69.30	8.1	189.94	10.3	348.49
6	73.96	8.2	196.32	10.4	356.60
6.1	78.70	8.3	202.78	10.5	364.78
6.2	83.52	8.4	209.33	10.6	373.05
6.3	88.41	8.5	215.95	10.7	381.39
6.4	93.39	8.6	222.64	10.8	389.81
6.5	98.44	8.7	229.42	10.9	398.31
6.6	103.57	8.8	236.27	11	406.89
6.7	108.78	8.9	243.21	11.1	415.55
6.8	114.07	9	250.22	11.2	424.29
6.9	119.43	9.1	257.31	11.3	433.09
7	124.88	9.2	264.47	11.4	441.98
7.1	130.40	9.3	271.72	11.5	450.95

4- Statistical analysis

The statistical analysis of the obtained data was performed according to **armitage (1983)** using mean (±SE) and (t) test for comparing two parameters.

CHAPTER III
RESULTS

RESULTS

All data of the present study were tabulated in 8 tables (7-14) and represented in 12 figures (13-24).

1) Data of leukocytes count ($x10^3/mm^3$)

1.1- *Male groups (A,B,C and D)* :

Leukocytic count in all age groups of male passive and active smokers was significant elevated when compared to their corresponding values in non-smoker, ($P < 0.01$ and $P < 0.001$) respectively.

It was noticed that data of active smoker showed more significant elevation than in cases of passive smoking. Moreover, leukocytic count in passive and active smokers in old age group (above 60 year) were less significantly changed if compared to groups of younger age (15-30 years).

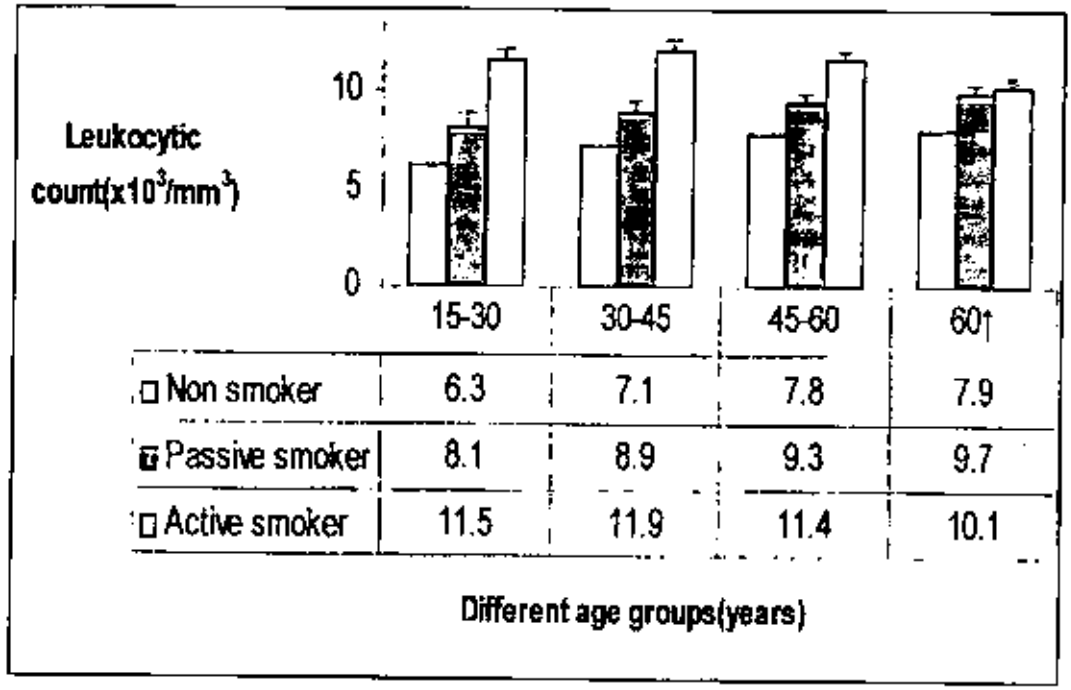

Fig.(13) leukocytes count ($x10^3/mm^3$)in male groups

1.2- Female groups (E,F,G and H)

Nearly, similar results were obtained in female passive and active smokers with only two differences:

- Data of female groups showed more level significant of compared to data of male groups.
- Data of the early female age group showed the most significant change and by increasing the age, the response was decreasing gradually. Therefore, old age female group showed the least significant change.

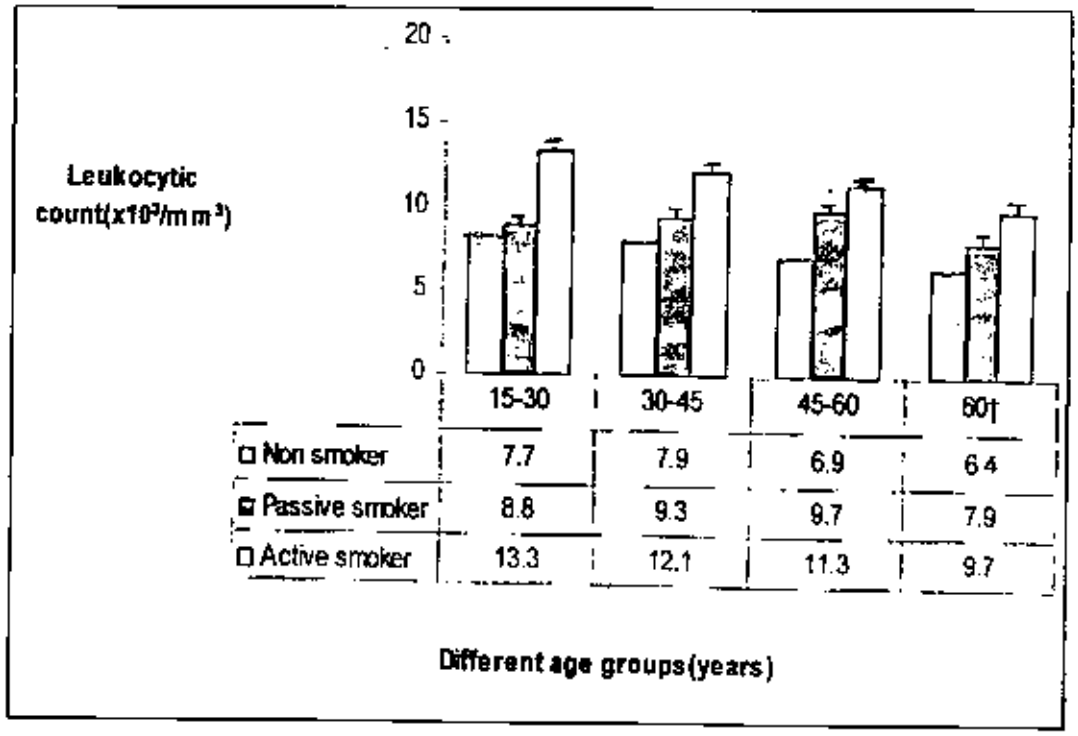

Fig.(14) leukocytes count (x10^3/mm^3)in female groups

2) Data of hemoglobin concentration (g/dl)

2.1- Male groups

Hb concentration in active smokers more significantly decreased ($P<0.001$) compared to Hb concentration of the non-smoker of the same age group and less significantly lowered ($P<0.01$) if compared to passive smoker. Howerover, Hb concentration in passive smokers was more significantly decreased if compared to non-smokers of the same age group ($P<0.01$).

Hb concentration in non-smoking males in early age group was more significantly increased ($P<0.05$) if compared to Hb concentration in females non-smoker, passive smokers and active smokers of the same age group ($P<0.01$ and $P<0.001$) respectively. By increasing the age, the response was decreasing to reach maximal decreasing effect in old age group.

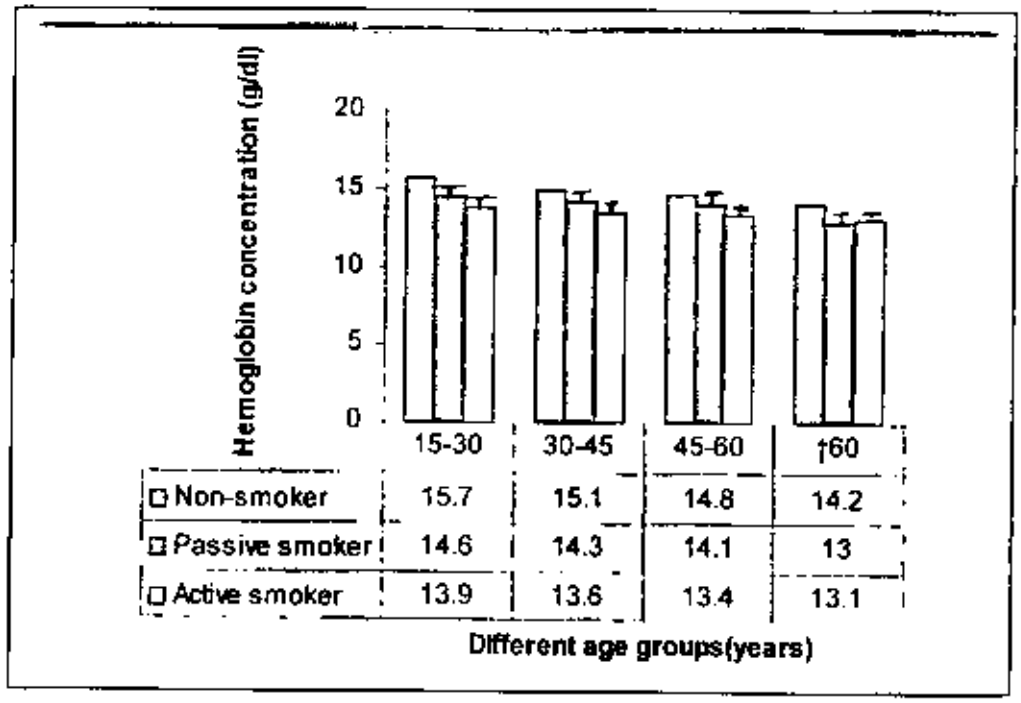

Fig.(15) Concentration of Hb (g/dl) in male groups

2.2- Female groups

- Hb concentration in female non-smokers, passively smokers and active smokers run nearly in the same manner like corresponding values in male groups.
- Also, active smoking affects more Hb concentration rather than passive smoking but both have significant effect.
- Moreover, old age female group illustrated the maximal significant ($P<0.001$) effect but gradually this effect become less significant ($P<0.01$) in early age female group.

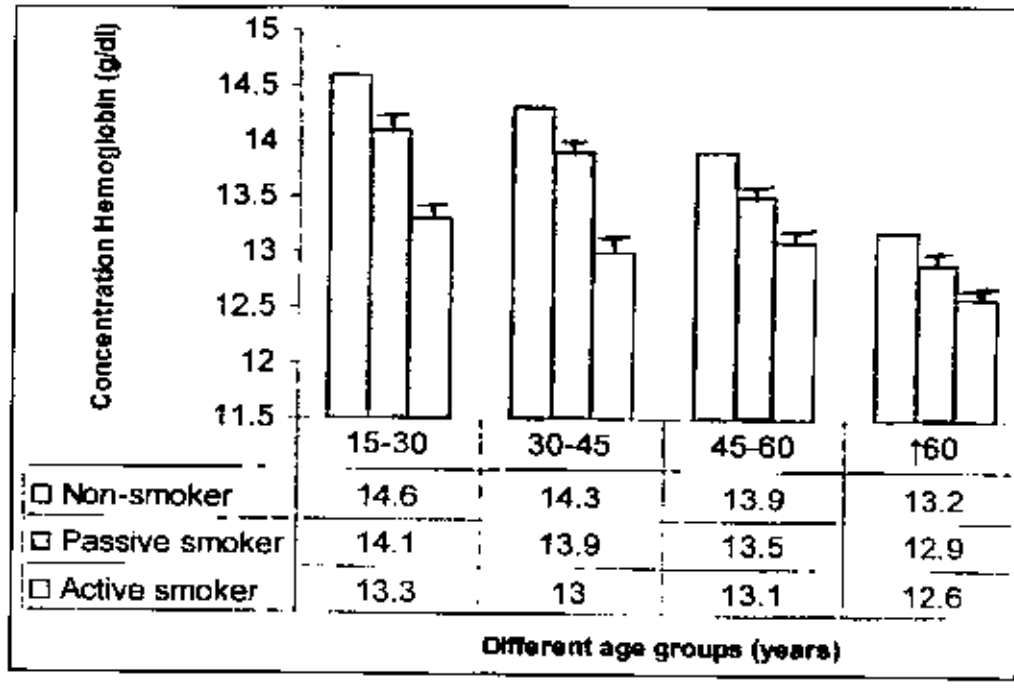

Fig.(16) Concentration of Hb (g/dl) in female groups

3) Data of IgA concentration (mg/dl)

3.1- Male groups

- IgA concentration showed significant increase in passive smokers ($P<0.01$) and also significant increase in active smokers ($P<0.001$) if compared to their corresponding values of non-smokers of group A (younger age male groups).
- Other groups of males (B,C and D) showed parallel changes with lower different level of significant.
- Old age group D showed the least significant response.

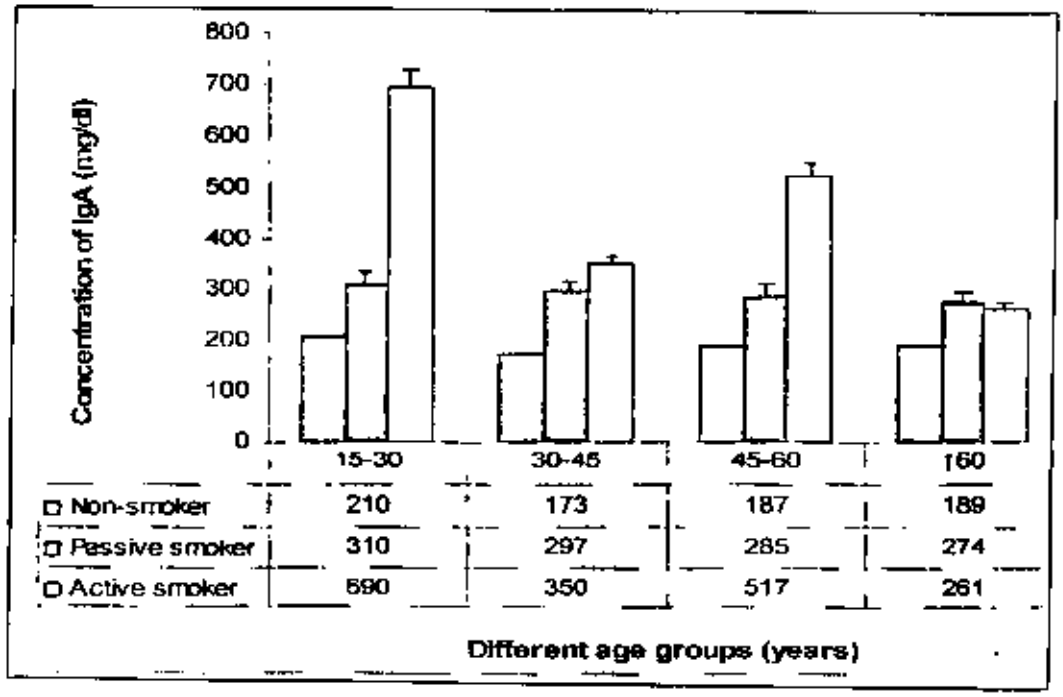

Fig.(17) Concentration of IgA (mg/dl) in male groups

3.2- Female groups

- Changes in IgA concentration of early age female group E showed nearly similar effects regarding effect of smoking on passive and active smokers, like similar male group A. However, female group was more reactive.

- Other female groups (F,G and H) showed the same significant changes in IgA concentration. If these female groups are compared regarding IgA concentration to similar groups in males, these groups showed more significant increase.

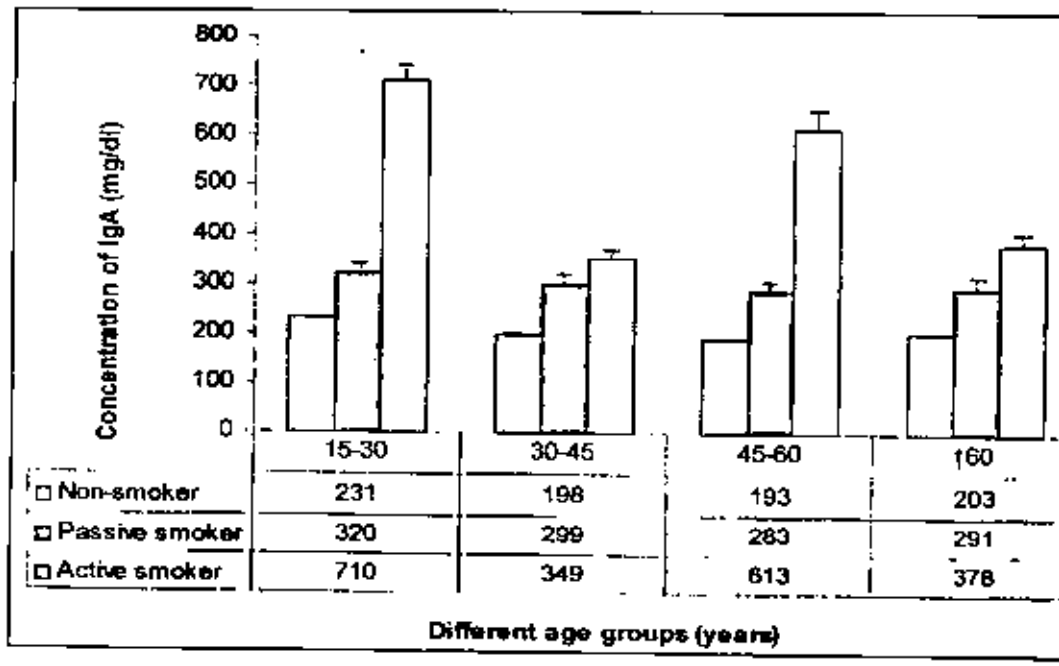

Fig.(18) Concentration of IgA (mg/dl) in female groups

4) Data of IgG concentration (mg/dl)

4.1- Male groups

- IgG concentration in passive and active smokers was significantly increased if compared to non-smoker male ones ($P < 0.01$ and $P < 0.001$ respectively), in all age groups.

- The increase in IgG concentration in male passive and active smokers was less significant than the increase in IgG concentration in females of the corresponding group.

- The maximal significant increase was observed in younger age group but the least significant increase was observed in old age group.

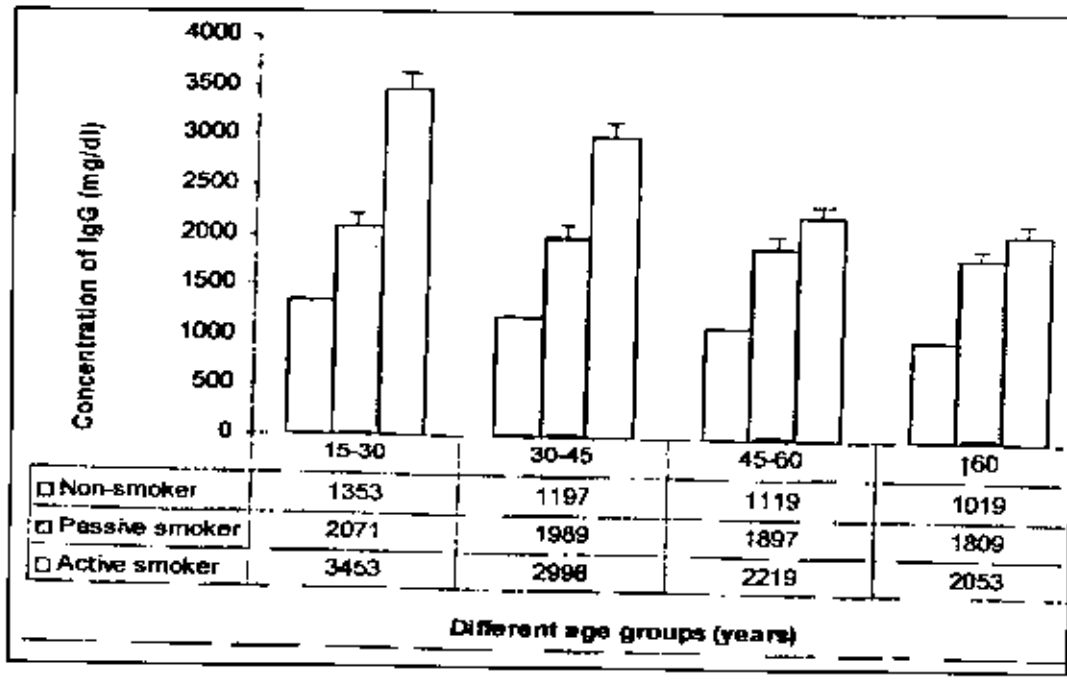

Fig.(19) Concentration of IgG (mg/dl) in male groups

4.2- Female groups

- Similar changes were observed in all female groups, if compared to their corresponding data in male groups. Also, the maximal increase was observed in younger female age group with least significant increase in old female age group.

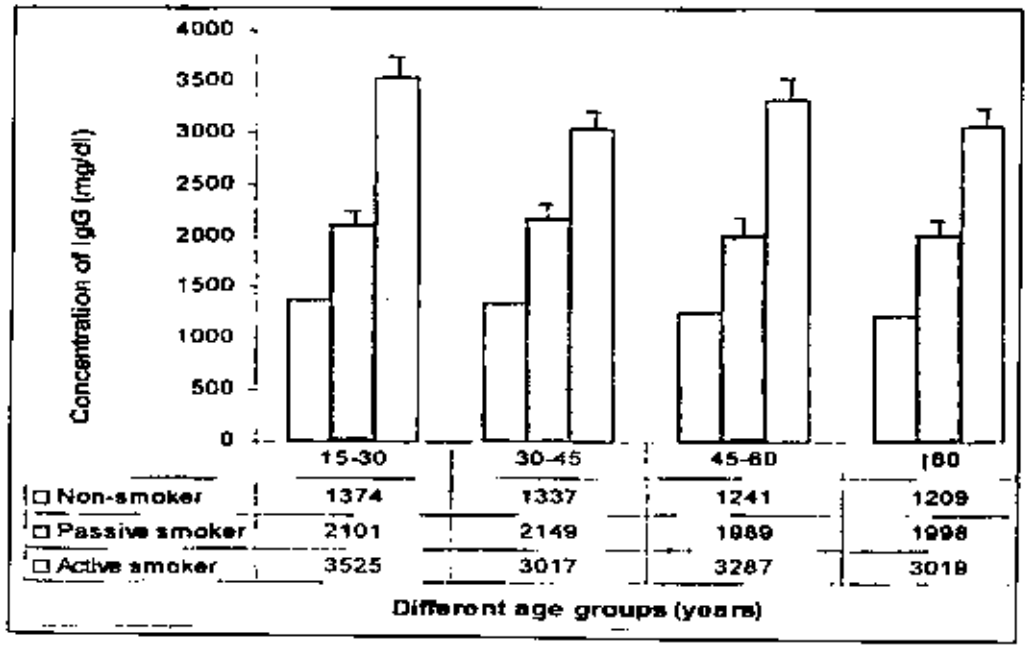

Fig.(20) Concentration of IgG (mg/dl) in female groups

5) Data of IgM concentration (mg/dl)

5.1- Male groups

- Concentration of IgM in passive and active smokers was also significantly increased ($P < 0.01$ and $P < 0.001$ respectively) in all male groups, if compared to their corresponding values of non-smoker male groups. Howvered, maximal significant increase effect of active smoking was observed in younger age group while minimal significant increase effect was observed in old age group.

- On the other hand, the effect of passive smoking on concentration of IgM was nearly the same in all groups (A,B,C and D) if compared to non-smoking groups.
- Male groups showed less increasing effect in concentration of IgM, if compared to concentration of IgM in the corresponding female groups.

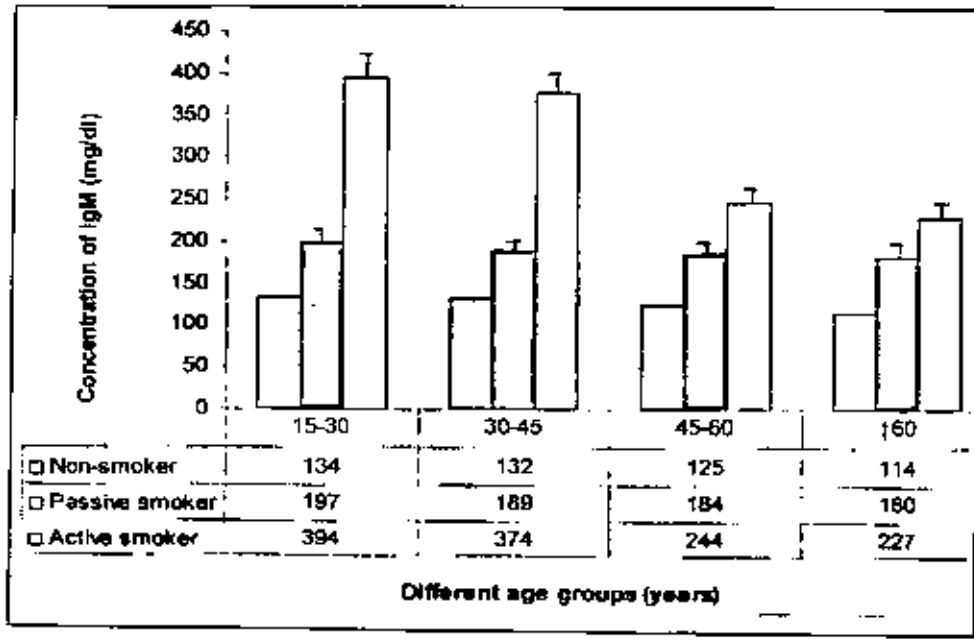

Fig.(21) Concentration of IgM (mg/dl) in male groups

5.2- Female groups

- Similar results in all groups obtaind in female passive and active smokers if compared to non-smokers female ones.
- There is only one difference between male and female data of active smokers. The maximal increasing significant effect of active smoking on IgM concentration was observed as usual in younger age group but the least significant effect was observed in middle age group, this might be due female hormonal interference.

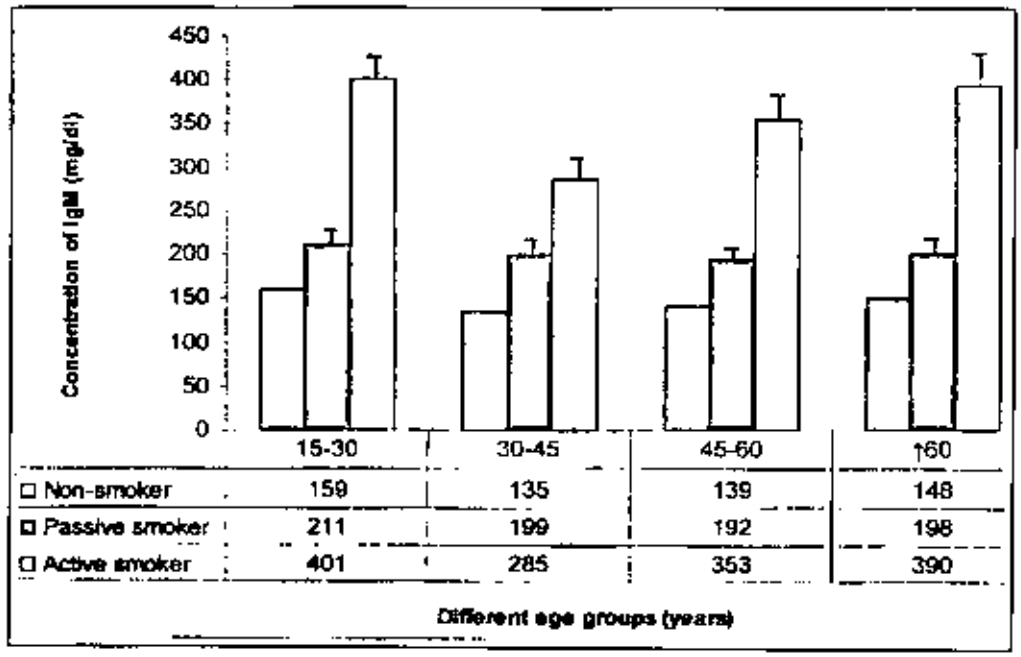

Fig.(22) Concentration of IgM (mg/dl) in female groups

6) Data of C3 concentration (mg/dl)

6.1- Male groups

- Concentration of C3 in all male groups of either passive or active smokers was significantly increased ($P< 0.01$ and $P< 0.001$ respectively) if compared to corresponding values in groups of male non-smoking individuals.
- The significantly increasing effect observed in smoker males was less if compared to corresponding values in smoker females.
- Also, younger age male group showed maximal significant effect while the old age group showed the least significant effect (in both passive and active smoker).

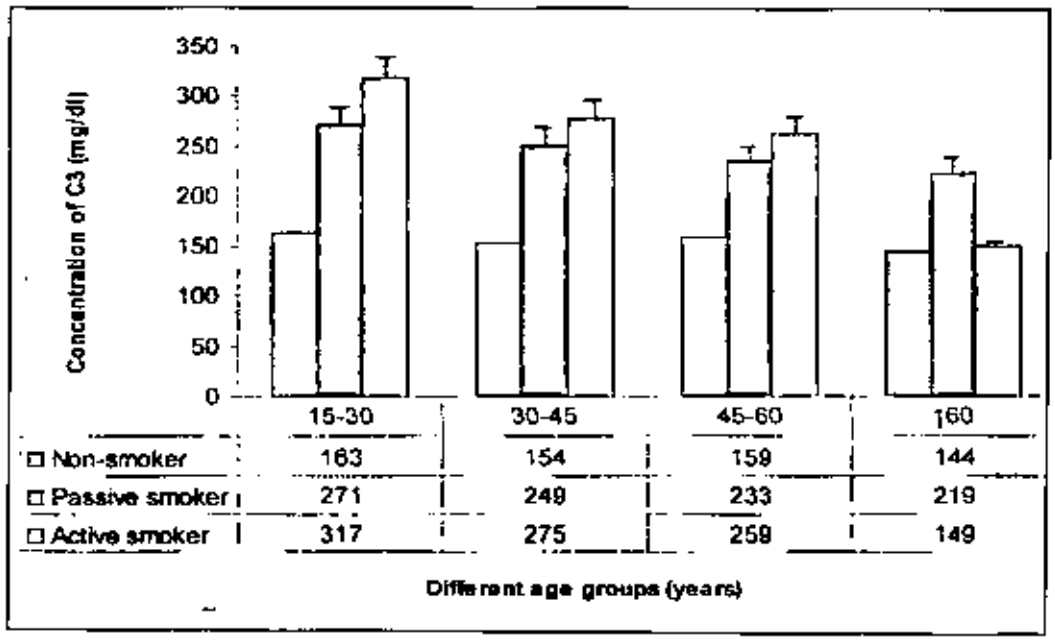

Fig.(23) Concentration of C3 (mg/dl) in male groups

6.2- Female groups

Effect of passive and active smoking on C3 concentration in female groups was nearly similar to its effect in corresponding values in male groups but with more reactivity.

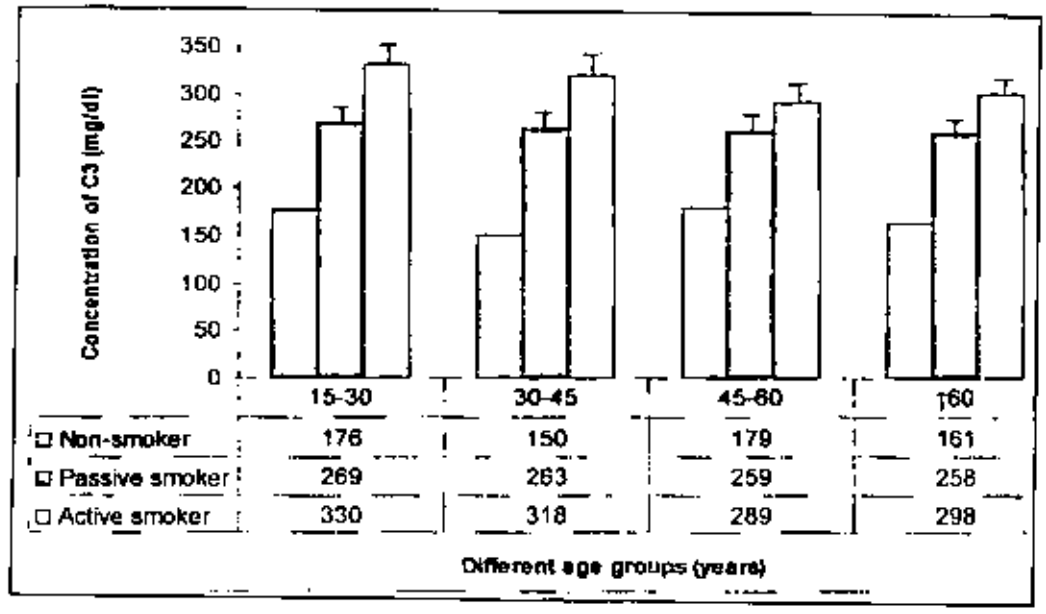

Fig.(24) Concentration of C3 (mg/dl) in female groups

From the above results, it could be concluded that:

1- Smoking, either passive or active has definite stimulatory effect on immune response, represented in this study in the concentrations of IgA, IgM, IgG , C3 and in total leukocytic count.

2- Old age individuals of either sex , have less reactive immune response than members of younger age .

3- Both males and females show nearly an apparent reactive response to smoking.

4- Females are more reactive to smoking than males.

5- IgM shows less significant effect than IgG and IgA.

6- Persons who are not actively smoking but exposed to smoke (=passive smoker), are also affected by smokes of active smoker, but to a lesser extent than active ones .

CHAPTER IV
DISCUSSION

DISCUSSION

This study was designed to investigate the effect of smoking; active or passive on some main immunological parameters specially the humoral type. Results of the present study revealed a significant decrease in hemoglobin concentration in both passive and active smokers of all groups studied but the decrease in Hb was more in active smoker than in passive ones, in females more than males, and in older age groups more than younger age groups.

Also the results of the current study showed a significant increase in total leukocytic count, which was proven to be higher in female groups than male groups, in younger more than older age groups, and in the active smokers more than the passive ones. Therefore, age, sex and mode of exposure to smoke are contributing factors so that, old, senile active female smokers showed the most significant changes while, the young, passive male smoker showed less effects. Regarding immunoglobulins studied and C3, it was found that smoking increases all these parameters significantly. More prominent significant changes were observed in IgG, IgA,and C3, but less significant changes were observed in IgM, the IgM showed the least significant increase while IgG, IgA and C3 on the other hand showed the highest increase. These results run parallel to other very few studies, but are contradictory to other reports.

In a study done by (**Cederqvist *et al.*, 1984**), to investigate the effect of smoking on fetal and maternal humoral immune parameters in cord and maternal blood. There were higher levels of IgA ($P < 0.01$) IgM ($P < 0.001$),

and IgG ($P < 0.001$) in cord sera of children of mothers who smoked than in the offspring of mothers who did not smoke, and these results run parallel to results of my study. However, mothers themselves who smoked had higher levels of IgM ($P < 0.001$) and IgG ($P < 0.001$), but not IgA, as compared to control mothers who did not smoke. Since cord IgA and IgM are produced by fetus, these results could be interpreted as being due to either a higher incidence of infection in utero or due to metabolic differences in the children of mothers who smoke. Either of these possibilities may explain the increased frequency of postpartum endometritis, increased incidence of fetal distress, and the characteristic of meconium. Stained amniotic fluid in mothers who smoke.

(Hersey *et al.*, 1983 and Onari *et al.*, 1978), in their study about the effects of cigarette smoking on bronchial fluids, they found that the IgG content of bronchial fluids from smokers twice as higher than non-smokers.

On the other hand, many investigators postulated the cigarette smoke affects a wide range of immunological functions in humans and animals, including both cell-mediated and humoral immune responses **(Goud *et al*, 1994; Finch *et al*, 1999).**

Chronic tobacco smoke exposure also inhibits T-cell receptor mediated proliferation of PLC-δ1 **(Geng *et al.*, 1995; 1996)**. However. After stoppage of smoking, the resulting immunosuppression could still persist **(Basta *et al.*, 2001)**. Chronic exposure to nicotine, the major component of 4,000 chemicals of tobacco smoke, inhibits the AFC response, and this immunosuppression is casually related to the impairment of antigen-

mediated signaling in T and B cells. Moreover, habitual cigarette smoking has also proven to be associated with a decreased proliferative response to a T or B cell mitogen. **(Finch *et al*., 1999).**

In a recent study done by **(Murat *et al.*, 2006)** to investigate and do a comparison of the effects of cigarette smoking and smokeless tobacco. Maras powder (used in Turkey), they found that IgM level was decreased significantly in those using maras powder and in cigarette smoker. Nonetheless, the IgE levels of maras powder user and in cigarette smokers were significantly higher. However, nearly most of the individuals had levels of IgA, IgG, C3 and C4 within normal. Effects of mars powder on humoral immune response were found to be similar to that of smoking.

Moszczynski *et al*., (2001) had observed a decrease of CD4/CD8 ratio due to the decrease in serum concentration of lysozyme and immunoglobulin and a decrease in the number of (CD16+) NK cells particularly in the addicts who had smoked for more than 10 years an increase in the number of CD8 Cytotoxic T lymphocytes.

There are also some articles that defend the idea that cigarette smoking has no particular effect on immunoglobulin levels. Most of studies mentioned that their effects arise from the particular phase more than the smoke-phase. The particular phase is composed of thousands of substance, but mainly nicotine. There are a lot of findings about the fact that nicotine is the major immune effectors in cigarette **(Sopori and Kozak, 1998).**

In the present study, a significant leukocytosis was observed in smoker. In a number of studies carried out for finding out the possible effects of cigarette smoke on lymphocytes, a leukocytosis accompanying the increase in all lymphocytic populations is mentioned. **(Holt, 1987 and Larramendy and knuutla, 1991)**. As well as **(Tollerud, 1989 and Mili, 1991)**, they proven that white blood cell counts in peripheral blood was elevated in smokers, about 30% higher than non-smoker.

From the above results and discussion, are could be concluded that:

1- Most of researchers stated that smoking affects the immune system.

(a) In the present study, these effects were found to be positive. IgG and IgA showed the highest significant increases in smokers but lowest significant increases were observed in IgM.

(b) In few other studies, these effects were found to be negative. These discrepancies might be attributed as mentioned before to the influence of multifactorial interacting points as age, sex, dosage, duration of exposure or inhalation of smoke, ethnic origin or any other unknown factor, which might be the hidden infections associating. All effects of smoking on immune system smoking are conducted due to presence of nicotine inside cigarette. This nicotine might :

- Release catecholamines
- Release more ACTH (adrenocorticotropic hormone) and more cortisol.
- These substances might alter or affect antibody forming cell (AFC) response alter tyrosine phosphorylation of PLC- δ1.

2- Not only, the immune response is affected in active smoking, but also it is definitely charged on exposure to smoke (= passive smoking). This gives

us an idea about how smoking is dangerous for the person who is smoking and for others who are exposed to it.

3- Smoking affects females more than males. This means the immune system in females is more reactive.

4- Smoking has a more depressive effect on older age, as it has been shown in this study, that all Igs measured were lowered in old age group of people.

Recommendation

It is recommended for all individuals not to be exposed to smoke and for actively smoking individuals to stop smoking as early as possible to avoid their hazardous disturbing effects on immune response.

References

Abbas AK, Lichtman AH, and Pober JS, (1994): Cellular and Molecular Immunology, 2nd ed. Saunders.

Abraham SN, and Malaviya R, (1997): Mast in infection and immunity. Infect Immunology, 65, 3501-8.

Alexander JR, (1980): Comparison of radial immunodiffusion and laser nephelometry for quantitating some serum proteins. Clinical Chemistry, Feb; 26: 314-317.

Allison AC, (1972): Immunological tolerance. Elin Immunology 1:113.

Arany I, Tyring SK, (1996): Status of local cellular immunity in interferon-responsive and -nonresponsive human papillomavirus-associated lesions. Sex Transm Diseases. 23:475-480.

Aoshiba K, Nagai A, and Konno K, (1994): Nicotine prevents a reduction in neutrophil filterability induced by cigarette smoke exposure, American Journal of Respiratory & Critical Care Medicine, 150(4):1101-7.

Armitage P, (1983): Statistical methods in medical research. Blachwell Scientific Publications, Oxford, London 4th Printing.

Baker R.R, (1984): Temperature distribution inside a burning cigarette. Nature, P: 247, 405, 406.

Bakir M.I, Masoud H.M, and Sharaf El-Din, M.M, (1991): Goza smoking and various aspects of pulmonary function. Master thesis, Faculty Medicine, Cairo University, p: 12, 17, 81-87.

Bassing CH, Swat w, and Alt FW, (2002): The mechanism and regulation of chromosomal V (D) Journal of recombination. S45-55.

Basta P. V, Navarro H. H, Seidler F.J, and Slotkin T. A, (2001): Adolescent nicotine: deficits in immune function. Developmental Brain research, 130, 253-256.

Benowitz N. L, (1990): Pharmacological aspects of cigarette smoking and nicotine addiction. The New England. Journal of Medicine. (17): 1318-1330.

Bjorkman PJ. M, (1997): C restriction in three diniew of T cell receptor/ligand interaction. Cell.89:167-70.

Boitard C, Timsit J, Larger E, and Dubois D, (1997): Immune mechanisms leading to type1 insulin-dependent diabetes mellitus. Horm. Res. 48, 58-63.

Boyle AH, and Waters H.F, (2000): COPA, Focus on Prevention: Recommendations of the National Lung Health Education Program. Heart and Lung: The Journal of Acute and Critical care, 29(6). 446-449.

Brown GC, and Borutaite V, (2001): Nitric oxide, mitochondria, and cell death. IUBMB life. 52:189-95.

Burrows B, Halonen M, Lebowitz MD, Knudson RJ, and Barbee RA, (1982): The relationship of serum immunoglobulin E, allergy skin tests, and smoking to respiratory disorders. The Journal of Allergy and Clinical Immunology. 70(3):199-204.

Burton DR, Delves PJ, and Roitt IM, (1998): Immunoglobulin, functions. Encyclopedia of immunology. 2nd ed. Vol. 3. London: Academic Press: 1315-9.

Cederqvist LL, Eddey G, Abdel-Latif N, and Litwin SD, (1984): The effect of smoking during pregnancy on cord blood and maternal serum immunoglobulin levels. American Journal of Obstetrics and Gynecology, April 15; 148 (8): 1123-6.

Dacie J.V, and Lewis S.M, (1984): In practical haematology, 6th edition, Churchill living stone, New York, 38.

David D, and Birmingham, Ala, (2003): Overview of the immune response. Journal of Allergy Clinical Immunology. 111(2):S442-S459.

Drapkin D. L, (1932): Journal of Biological chemistray.98, 719.

Eisenbarth G.S, (1986): Type1 diabetes mellitus, A chronic autoimmune disease. Nature England Medicine Journal, 314, 1360-1368.

Finch G. L, Kalra, R. Savage, S. M, Singh, S. P, and Sopori, M. L, (1999): Effects of cigarette smoke impairs antigen-mediated signaling in T cells and depletes IP3-Sensitive Ca^{2+} stores. The Journal of Pharmacology and Experimental Therapeutics, 293(1), 166-171.

Fishman A, Pelias J.A, and Grippl M.A, (1998): Quoted from: a textbook of internal medicine, McGraw 3rd edition "Fishman s pulmonary diseases and disorders". (Eds.) Fishman A, Pelias J.A, Grippi M.A, USA. (109): 1719-1725.

Gabay C, and Kushner I, (1999): Acute-phase proteins and other systemic responses to inflammation. North England Journal of Medicine. 340:448-454.

Gal P, and Ambrus G, (2001): Structure and function of complement activating enzyme complexes: C1 and MBL-MASP. Carries protein Peptide Science. 2:43-59.

Geng Y, Savage SM, Seagrave J, Johnson L and Sopori ML, (1995): Effects of nicotine on immune response. I. Chronic exposure to nicotine impairs the antigen receptor- mediated signal transduction in lymphocytes. Toxicology Applied Pharmacology 135: 268-278.

Geng Y, Savage SM, Razani S, and Sopri ML, (1996): Effects of nicotine on the immune response. II. Chronic nicotine treatment induces T cell energy. Journal of Immunology 156: 2384-2390.

Gerrard JW, Heiner DC, Ko CG, Mink J, Meyers A, and Dosman JA, (1980): Immunoglobulin levels in smokers and non-smokers. Annals of Allergy. 44(5): 261-262.

Ghetie V, and Ward ES, (1997): FcRn: the MHC class I-related receptor that is more than an IgG transporter. Immunology Today 18:592-598.

Glader P, Moller S, Lilja J, Wieslander E, Lofdahl CG, and Von Wschenfeldt K, (2006): Cigarette smoke extract modulates respiratory defence mechanisms through effects on T-cells and airway epithelial cells.100(5):818-27.

Goud N, Kaplan, A. M, and Sopori, M.L, (1994): Effects of tobacco smoke on the immune system. Immunotoxicology and Immunopharmocology 2nd ed. New York : Raven Press.

Gulsvik A, and Fagerhoi MK, (1979): Smoking and immunoglobulin levels. Lancet. 1(8113):449.

Hayakawa K, Asano M, and Shinton SA, (1999): Positive selection of natural autoreactive B cells, Science 285:113-116.

Heaney ML, and Golde DW, (1998): Soluble receptors in human disease. Journal of Leukocytes Biology 64:135-146.

Henningfield J.E, Steplton J.M, and Benowitz N.L, (1990): Higher levels of nicotine in arterial than in venous blood after cigarette smoking. Drug alcohol dependence (33): 23-29.

Heresy P, Prendergast D, Edwards A. (1983): Effect of cigarette smoking on the immune system:follow-up studies in normal subjects after cessation of smoking. Medical Journal Aust.2:425-429.

Holt P.G, (1987): Immune and inflammatory function in cigarette smokers. Thorax. 42(4):241-249.

Janeway CA Jr, Travers P, (1994): Immunobiology Current Biology /Garlan.

Kamradt T, and Mitchison NA, (2001): Advances in immunology: tolerance and autoimmunology. New England Journal of medicine 344:655-664.

Kay AB, (2001): Advances in immunology allergy and allergic disease (parts I and II). New England Journal of medicine 344:303-37, 109-113.

Keilholz U, Conradt C, and Legha SS, (1998): Results of interleukin-2 based treatment in advanced melanoma: a case record-based analysis of 631 patients. Journal of Clinical Oncology 16:2929.

Keith C.H, (1982): Modification of tobacco smoke, the chemistry of tobacco and tobacco smoke. Plenum press. New York 6th Ed, pp 149-166.

Khaldoyanidi S, and Orlovskaya L, (2002): Exposure to nicotine during gestation affects trafficking of fetal hemopoietic stem cells. American Society of Hematology, Vol: 100, Nbr: 16.

Khoury J. C, Miodovnik, M. Buncher, C. R, Kalkwarf H, McElvy S, Khoury, P. R and Sibai B, (2004): Consequences of smoking and caffeine consumption during pregnancy in women with type 1 diabetes. Journal Maternal and Fetal Neonatalology Medicine. 15, 44-50.

Kiefer J.E, (1982): Filtration of tobacco smoke, the chemistry of tobacco and tobacco smoke. Plenum press, New York, 6th Ed. P: 149-166.

Klein J, (1993): Immunology. Blackwell. Life, death and the immune system, Scientific American.

Klein J, (2000): Akiesato, The HLA system-first of two parts. Neng of Journal Medicine 343:702-709.

Krajci P, Kvale D, and Brandtzaeg P, (1995): Cloning, chromosomal localization, and linkage analysis of the gene encoding human transmembrane secretory component (the poly-Ig receptor). Advanced Excerpta Medical Biology 371:617-623.

Lacy P, and Moqbel R, (2001): Immune effector function of eosinophils in allergic airway inflammation Current Opinion Allergy Clinical Immunology.1:79-84.

Larramendy ML, Knuutila S.(1991): Increased frequency of micronuclei in B and T8 lymphocytes from smokers. Mutation Research. 259(2): 189-195.

Lazarus P, Idris AM, Kim J, Calcagnotto A, and Hoffmann D, (1996): P53 mutations in head and neck squamous cell carcinomas from Sudanese snuff (toombak) users. Cancer Detection and Prevention. 20(4):270-278.

Lidia Arcavi MD, Neal L, Benowitz MD, (2004): Cigarette smoking and infection. Archives of Internal medicine, Vol;164 (20)

Liszewski MK, Farries TC, Lublin DM, Rooney LA, and Atkinson JP, (1996): Control of the complement system. Adv Immunology. 61:201-83.

McSharry C, McKay I.C, Lewis C, Galloway E, Ross C, and McMenamin J, (1994): Cigarette smoking, effects on peripheral blood cytology and immunoglobulins in a Glasgow cohort of HIV- infected subjects. AIDS, Vol 8 (Suppl 4); 31.

Medhat H.(2006): The influence of tobacco smoking on humoral immune response in insulin dependent diabetic pregnancy. Indian Journal of clinical Biochemistry, 21(2)95-99.

Mescher MF, (1995): Molecular interactions in the activation of effector and precursor cytotoxic T lymphocytes. Immunology Review. 146:177-210.

Mili F, Flanders WD, Boring JR, Annest JL, Destefano F. (1991): The associations of race, cigarette smoking, and smoking cessation to measures of the immune system in middle-aged men. Clinical immunology immunopathology.59:187-200.

Miller C. G, Cook D . N, and Kotwal G. J, (1996a): Two chemotactic factors, C5a and MIP-lalpha, dramatically alter the mortality from zymosan-induced multiple organ dysfunction syndrome (MODS): C5a contributes to MODS while MIP-lalpha has a protective role. Molecular. Immunology. 33, 1125-1127.

Miller F, Freeman W. J, and Stedman R. J, (1988): Effect of additives on the combustion temperature of cigarettes. Betir tobak forsch 4:269-274.

Miller JF, and Basten A, (1996b): Mechanisms of tolerance to self. Current Opinion of Immunology 8:815-821.

Miller JP, (2002): The discovery of thymus function and of thymus-derived lymphocytes. Immunology Review. 185:14.

Miller LG, Goldstein G, Murphy M, Ginns LC, (1982): Reversible alterations in immunoregulatory T cells in smoking: analysis by monoclonal antibodies and flow cytometry. *Chest.* ;82:526-529.

Mire-Sluis AR; Thorpe R, and eds, (1998): Cytokines. San Diego, Calif: Academic Press.

Moszczynski P, Zabinski Z, Moszczynski P. Jr, Rutowski J, Slowinki S, and Tabarowski Z, (2001): Immunological findings in cigarette smokers. Toxicology Letters.118 (3):121-127.

Murat A, Hasan C, Mustafa C, Pina C, and Mustafa G, (2006): Comparison of effects of smoking and smokeless tobacco "Maras powder" use on humoral immune system parameters. Mediators inflammation, V.2006 (3):85019.

Murphy SE, Carmella SG, Idris AM, and Hoffmann D, (1994): Uptake and metabolism of carcinogenic levels of tobacco-specific nitrosamines by Sudanese snuff dippers. Cancer Epidemiology, Biomarkers & Prevention. 3(5): 423-238.

Muscat JE, Harris RE, Haley NJ, Wynder EL. Am Heart J, (1991): Cigarette smoking and plasma cholesterol , 121:141-7.

Omer S, and Sadek I, (1995): Smoking profile; A study of a developing country (Egypt) as an example. In Karen Slama (Eds.) Tobacco and health. Plenum press, New York p:705-710.

Onari K, Seyama A, Inamizu T. (1978): Immunolgical study on cigarette smokers, part I:serum protein pattern in smokers. Hiroshima Journal Medical Scientific. 27:113-118.

Paul WE, and Benacerraf B, (1977): Functional specificity of thymus dependent lymphocytes. Science 195:1293.

Peter J, Delves, Ivan M, and Roitt, (2000a): The Immune System - First of Two Parts, North England Journal Medicine 343(1):37-49.

Peter J, Delves, Ivan M, and Roitt, (2000b): The Immune System - second of Two Parts, North England Journal Medicine 343(2):108- 117.

Pletcher MJ, Hulley BJ, and Houston T, (2006): Menthol cigarettes, smoking cessation, atherosclerosis, and pulmonary function: The Coronary Artery Risk Development in Young Adults (CARDIA) Study. Archive of Internal Medicine. 166:1915-1922.

Qian Z, Wasowska BA, Behrens E, Cangello DL, Brody JR, Kadkol SS, Horwitz L, Liu J, Lowenstein C, Hess AD, Sanfilippo F, and Baldwin WM, (1999): C6 produced by macrophages contributes to cardiac allograft rejection. American Journal of Pathology. 155:1293-1302.

Randolph GJ, (1999): Differentiation of phagocytic monocytes into lymph nodes dentritic cells in vivo, immunity; 11:753.

Rasch V, (2003): Cigarette, alcohol, and caffeine consumption: risk factors for spontaneous abortion. Acta. Obstetrics and Gynecology. Scandinavia. 82, 182-188.

Roitt I , (1994): Essential Immunology, 8th ed. Blackwell.

Roitt IM.(1988): Essential immunology, Blackwell Scientific Puplications, Oxford.

Rossetti G, Collinge M, Bender JR, Moltein R, and Pardi R, (2002): Integrin-dependent regulation of gene expression in leukocytes. Immunological Review. 186:189-207.

Ruslan M, and Charles J, (2000): Innate Immunity, North England Journal of Medicine 343(5):338-344.

Ruslan M, and Charles J, (1997): Innate immunity: impact on the adaptive immune response. Current Opinion Immunology 9:4-9.

Salem E.S. (1973): Goza versus cigarette smoking among patients with pulmonary disease. Egypt Journal chest dis and tuber 16: 121-139.

Salem E.S, (1979): Smoking and chest. Journal Egypt medicine association. 62: 286-306.

Sargent J.D, Dalton M.A, and Beach M.L, (2002): Viewing tobacco use in movies, does it shape attitudes that mediate adolescent smoking (1) American Journal Prev Medicine April, 22 (3): 137-145.

Scofied RM, (2004): Autoantibodies as predictors of disease. Lancet 363:1544-1546.

Sedlacek TV, (1999): Advances in the diagnosis and treatment of human papillomavirus infections. Clinical Obstetrics Gynecology. 42:206-220.

Shevach EM, (2002): $CD4^+$ & $CD25^+$ suppressor T cells: more questions than answers. Natural Review of Immunology 2:389-400.

Skoskiewicz MJ, Colvin RB, Schneeberger EE, and Russell PS, (1985): Widespread and selective induction of major histocompatibility complex-

determined antigens in vivo by gamma interferon. Journal of Excerpta Medical, 62.

Sopori ML, and Kozak W, (1998): Immunomodulatory effects of cigarette smoke. Journal of Neuroimmunology. 83(1-2):148-156.

Stene L. C, Barriga K, Norris, J. M, Hoffman M, Erlich H. A, Eisenbarth G. S, Meduffie R. S, Jr. and Rewers M, (2004): Perinatal factors and development of islet autoimmunity in early childhood: the diabetes autoimmunity study in the young. American Journal Epidemiol. 160, 3-10.

Thomas CA, Li Y, Kodama T, Suzuki H, Silverstein SC, El Khoury J, (2000): Protection from lethal gram-positive infection by macrophage scavenger receptor-dependent phagocytosis. Journal Excerpta Medical. 191:147-156.

Tollerud DJ, Clark JW, and Brown LM, (1989): Association of cigarette smoking with decreased numbers of circulating natural killer cells. The American Review of Respiratory Disease.139(1): 194-198.

Tollerud DJ, Clark JW, and Brown LM, (1989): The effects of cigarette smoking on T cell subsets. A population-based survey of healthy Caucasians. The American Review of Respiratory Disease. 139(6): 1446-1451.

Tough DF, Sprent J, (1995): Lifespan of lymphocytes. Immunological response, 14:252.

Uhr JW, and Finkelestein, MS, (1967): The kinetics of antibody formation, Allergy 10:37.

Van Ewijk, (1991): T-cell differentiation is influenced by thymic microenvironments. Annular Review of Immunology. 9:591-615.

Van Eeden SF, Hogg JC, (2000): The response of human bone marrow to chronic cigarette smoking. Eurbean Respiratory Journal ;15:915-921.

Von Andrian UH, and Mackay CR, (2000): Advances in Immunology: T-cell function and migration-two sides of the same coin. New England Journal of medicine 343:1020-1034.

Walport, MJ, (2001): Advances in immunology: Complement (parts I and II). New England Journal of Medicine 343:1058-1066, 1140-1144.

Wynde E.L, and Haffman D, (1987): Tobacco and tobacco. New York academic press, 4th Ed (8):625.

Yang D, Biragy A, Kwak LW, and Oppenheim JJ, (2002): Mammalian defenser in immunity: more than just microbieidal. Trends. 23; 291-6.

Yolton K, Dietrich K, and Auinger P, (2005): Exposure to environmental tobacco smoke and cognitive abilities among U.S. children and adolescents. Environ Health Perspect. 113:98-103.

APPENDIX

Table (7) : All parameters in male group A.

Group	A(n=48)									
Age (year)	15-30 years									
Subgroup	A1 Non-smokers(n=14)	A2 Passive smokers (n=13)				A3 Active smokers (n=21)				
	Mean ±SE	Mean ±SE	P with A1	P with B2	P with E2	Mean ± SE	P with A1	P with A2	P with B3	P with E3
(1) Hb(gm/dl)	15.7± 1.01	14.6±0.95	*	*	*	13.9±0.87	***	**	*	*
(2) leukocytic count ($\times10^3$/mm^3)	6.3±0.63	8.1±0.71	**	NS	*	11.5±0.85	***	**	*	***
(3)IgA (mg/dl)	210±9.4	310±11.4	**	*	*	690±21.4	***	**	***	***
(4) IgG(mg/dl)	1353±41.7	2071±50.3	**	*	*	3453±71.7	***	***	***	**
(5) IgM (mg/dl)	134±5.7	197±12.8	*	*	*	394±13.9	***	***	**	**
(6) C3(mg/dl)	163±6.6	271±9.9	**	**	*	317±12.9	***	***	***	*

-Where:- NS : Non significant.
* : Significant (P<0.05).
** : Significant (P<0.01).
*** : Very highly significant(P<0.001).

Table (8) : All parameters in male group B.

Group	B(n=47)									
Age (year)	30-45 years									
Subgroup	B1 Non-smoker(n=17)	B2 Passive smokers (n=13)				B3 Active smokers (n=17)				
	Mean±SE	Mean ± SE	P with B1	P with C2	P with F2	Mean ± SE	P with A1	P with B2	P with C3	P with F3
(1) Hb(gm/dl)	15.1±0.94	14.3±0.76	**	NS	*	13.6±0.91	***	**	NS	*
(2) leukocytic count($\times10^3$/mm^3)	7.1±0.59	8.9±0.69	**	*	*	11.9±0.83	***	**	*	**
(3)IgA (mg/dl)	173±13.2	297±11.3	**	***	NS	350±11.1	***	***	**	NS
(4) IgG(mg/dl)	1197±61.2	1989±1.49	**	**	**	2998±16.3	***	***	***	*
(5)IgM(mg/dl)	132±8.9	189±903	**	**	*	374±11.4	***	***	***	*
(6) C3(mg/dl)	154±10.3	249±11.2	**	**	**	275±7.7	***	***	***	**

- Where:- NS : Non significan.
* : Significant (P<0.05).
** : Significant (P<0.01).
*** : Very highly significant (P<0.001).

Table. (9) : All parameters in male group C.

Group	C(n=39)									
Age (year)	45-60 years									
Subgroup	C1 Non-smoker(n=12)	C2 Passive smokers (n=13)				C3 Active smokers (n=14)				
	Mean ± SE	Mean ± SE	P with C1	P with D2	P with G2	Mean ± SE	P with C1	P with C2	P with D3	P with G3
(1) Hb(gm/dl)	14.8±1.03	14.1±0.7	**	**	*	13.4±0.89	***	**	NS	NS
(2) leukocytic count($\times 10^3$/mm^3)	7.8±0.55	9.3±0.83	**	NS	NS	11.4±0.9	***	***	**	NS
(3)IgA (mg/dl)	187±14.2	285±19.4	**	*	NS	517±21.2	***	***	***	**
(4) IgG(mg/dl)	1119±72.4	1897±60.9	**	**	**	2219±71.2	***	***	***	**
(5)IgM(mg/dl)	125±9.4	184±11.7	*	*	**	244±12.9	***	***	***	**
(6) C3(mg/dl)	159±10.7	233±13.7	**	*	**	259±14.1	***	***	**	**

- Where :- NS : Non significant.
  * : Significant (P<0.05).
  ** : Significant (P<0.01).
  *** : Very highly significant (P<0.001).

Table. (10): All parameters in male group D.

Group	D(n=34)							
Age (year)	Above 60 years							
Subgroup	D1 Non-smokers (n=10)	D2 Passive smokers (n=11)			D3 Active smokers (n=13)			
	Mean ± SE	Mean ± SE	P with D1	P with H2	Mean ± SE	P with D1	P with D2	P with H3
(1) Hb(gm/dl)	14.2±1.07	13±0.87	**	**	13.1±0.79	***	*	*
(2) leukocytic count($\times 10^3/mm^3$)	7.9±0.63	9.7±0.77	**	***	10.1±0.93	***	**	**
(3)IgA (mg/dl)	189±13.7	274±18.3	**	**	261±19.5	***	**	**
(4) IgG(mg/dl)	1019±61.4	1809±59.5	**	***	2053±63.1	***	***	***
(5)IgM(mg/dl)	114±10.3	180±12.6	*	**	227±13.7	***	***	***
(6) C3(mg/dl)	144±9.8	219±14.5	**	***	149±14.5	***	***	***

-Where:- NS : Non significant.
* : Significant (P<0.05).
** : Significant (P<0.01).
*** : Very highly significant (P<0.001).

Table. (11): All parameters in female group E.

Group	E(n=31)							
Age (year)	15-30 years							
Subgroup	E1 Non-smoker(n=10)	E2 Passive smokers (n=11)			E3 Active smokers (n=10)			
	Mean ± SE	Mean ± SE	P with E1	P with F1	Mean ± SE	P with E1	P with E2	P with F3
(1) Hb(gm/dl)	14.6±0.95	14.1±0.71	*	NS	13.3±0.64	***	**	NS
(2) leukocytic count($\times 10^3$/mm^3)	7.7±0.71	8.8±0.87	**	**	13.3±0.97	***	**	**
(3)IgA (mg/dl)	231±7.9	320±17.3	**	***	710±21.8	***	***	***
(4) IgG(mg/dl)	1374±65.7	2101±50.5	**	**	3525±85.5	***	***	***
(5)IgM(mg/dl)	159±0.5	211±12.5	*	**	401±15.8	***	***	***
(6) C3(mg/dl)	176±10.3	269±11.2	**	*	330±12.9	***	***	*

-Where:- NS : Non significant.
* : Significant (P<0.05).
** : Significant (P<0.01).
*** : Very highly significant (P<0.001).

Table. (12): All parameters in female group F.

Group	F(n=33)							
Age (year)	30-45years							
Subgroup	F1 Non-smoker(n=11)	F2 Passive smokers (n=10)			F3 Active smokers (n=12)			
	Mean± SE	Mean ± SE	P with	P with G1	Mean ± SE	P with F1	P with F2	P with G3
(1) Hb(gm/dl)	14.3±0.89	13.9±0.76	*	NS	13.0±0.68	***	**	NS
(2) leukocytic count($\times 10^3/mm^3$)	7.9±0.49	9.3±0.68	**	NS	12.1±0.79	***	***	*
(3) IgA (mg/dl)	198±9.3	299±15.5	**	*	349±23.7	***	***	***
(4) IgG(mg/dl)	1337±50.5	2149±53.7	**	***	3017±77.5	***	***	***
(5) IgM(mg/dl)	135±10.3	199±13.6	*	*	285±16.6	***	***	***
(6) C3(mg/dl)	150±11.3	263±12.7	**	**	318±13.8	***	***	***

-Where:- NS : Non significant.
* : Significant (P<0.05).
** : Significant (P<0.01).
*** : Very highly significant (P<0.001).

Table. (13): All parameters in female group G.

Group	G(n=34)							
Age (year)	45-60 years							
Subgroup	G1 Non-smoker (n=13)	G2 Passive smokers (n=10)			G3 Active smokers (n=11)			
	Mean ± SE	Mean ± SE	P with G1	P with H2	Mean ± SE	P withG1	P with G2	P with H3
(1) Hb(gm/dl)	13.9±0.82	13.5±0.68	*	*	13.1±0.83	**	NS	*
(2) leukocytic count($\times 10^3/mm^3$)	6.9±0.49	9.7±0.71	**	***	11.3±0.96	***	**	***
(3)IgA (mg/dl)	193±904	283±13.4	**	*	613±29.4	***	***	***
(4) IgG(mg/dl)	1241±63.5	1989±73.3	**	NS	3287±97.4	***	***	***
(5)IgM(mg/dl)	139±8.9	192±11.5	**	*	353±18.3	***	***	***
(6) C3(mg/dl)	179±10.5	259±13.2	**	NS	289±15.6	***	***	*

- Where:- NS : Non significant.
* : Significant (P<0.05).
** : Significant (P<0.01).
*** : Very highly significant (P<0.001).

Table. (14): All parameters in female group H.

Group	H(n=32)					
Age (year)	Above 60					
Subgroup	H1 Non-smoker(n=10)	H2 Passive smokers (n=10)		H3 Active smokers (n=12)		
	Mean ± SE	Mean ± SE	P with H1	Mean ± SE	P with H1	P with H2
(1) Hb(gm/dl)	13.2±0.72	12.9±0.43	*	12.6±0.39	***	NS
(2) leukocytic count($\times 10^3/mm^3$)	6.4±0.39	7.9±0.38	**	9.7±0.54	***	**
(3) IgA (mg/dl)	203±13.3	291±14.4	**	378±15.7	***	***
(4) IgG(mg/dl)	1209±71.4	1998±75.1	**	3019±79.9	***	***
(5) IgM(mg/dl)	148±9.6	198±10.8	**	390±15.6	***	***
(6) C3 (mg/dl)	161±11.5	258±13.6	**	298±14.8	***	**

- Where:- NS : Non significant.
* : Significant (P<0.05).
** : Significant (P<0.01).
*** : Very highly significant(P<0.001).

الخـــــــــــــــلاصة

يهدف هذا البحث لدراسة تأثير التدخين على بعض العوامل والمقاييس الخاصة بجهاز المناعة في الإنسان.

أجريت هذه الدراسة علي عدد 299 من الأشخاص المتطوعين من شعبية سرت من الجنسين من مختلف الأعمار.

حيث قسم هؤلاء الأفراد إلى 4 مجموعات للذكور و4 مجموعات مقابلة للإناث. وذلك طبقا للعمر كالتالي :

أولا) مجموعة الذكور (A,B,C,D) على النحو التالي :

- المجموعة (A) شملت الأعمار من سن 15 ــ 30 عاما.
- المجموعة (B) شملت الأعمار من سن 30 ــ 45 عاما.
- المجموعة (C) شملت الأعمار من سن 45 ــ 60 عاما.
- المجموعة(D) شملت الأعمار ما بعد 60 عاما.

ثانيا) مجموعة الإناث (E,F,G,H) على النحو التالي :

- المجموعة (E) شملت الأعمار من سن 15 ــ 30 عاما.
- المجموعة (F) شملت الأعمار من سن 30ــ45 عاما.
- المجموعة (G) شملت الأعمار من سن 45 ــ 60 عاما.
- المجموعة (H) شملت الأعمار ما بعد 60 عاما .

وقد شملت كل مجموعة من المجموعات الثمانية السابقة 3 أقسام اصغر منها، قسم شمل غير المدخنين أو غير المتعرضين لدخان السجائر واعتبروا مجموعة ضابطة ، والقسم الثاني شمل الأشخاص المتعرضين لدخان السجائر أما القسم الثالث فقد شمل الأشخاص المدخنين.

وقد تم قياس نسبة الهيموجلوبين والعدد الكلي لكريات الدم البيضاء ونسبة الأجسام المضادة (A,G,M) وأيضا تقدير نسبة C3 في كل شخص من الأشخاص المتطوعين.

وبمقارنة نتائج الأفراد المدخنين والمتعرضين للتدخين بغير المدخنين من نفس المجموعة أو المجموعات الأخرى ذات الأعمار السنية المختلفة والجنس المختلف تبين الأتي:

1- يؤثر التدخين تأثير بالغا و واضحا على ارتفاع نسب الأجسام المضادة الأمر الذي يشير إلى أثاره الواضحة في الجهاز المناعي.

2- يؤثر التدخين في الإناث كتأثيره في الذكور وان كان التأثيرفى الإناث أكثر وضوحا.

3- تأثير التدخين كان أكثر ظهورا في الأجسام المضادة من النوع IgG واقل ظهورا في الأجسام المضادة من النوع IgM .

4- يرفع التدخين من نسب عدد كريات الدم البيضاء ، الأمر الذي يشير إلى إن التدخين بالنسبة للجسم شئ مثير للأجهزة ومهيج لها.

5- إن التدخين يؤثر على المستنشق بنفس التأثير على المدخن ولكن بدرجة اقل ، الأمر الذي يجعلنا نصل إلى نتيجة مؤكدة وهى إن التدخين له تأثير على المدخن والمتعرض للتدخين أيضا لذا ننصح المدخن بالإقلاع عن التدخين فورا والناس جميعا بالابتعاد عن دخان السجائر لما له من تأثير ضار على صحة المدخن والمتعرض للتدخين .

6- تأثير التدخين يكون اقل وضوحا كلما تقدم الشخص (من الجنسين) في الـن

الجماهيرية العربية الليبية الشعبية الاشتراكية العظمى
جامعة التحدي
سرت

إن الدراسة تعبث نقية في حد ذاتها
وإنما الغاية هي خلق الإنسان المتوازن المتجدد

التاريخ : .....
الموافق : 26/7/2006
الرقم الإداري : .....

كلية العلوم

قسم الأحياء

حفل مناقشة البحث

دراسة حول تأثير التدخين على بعض عناصر جهاز المناعة

مقدمة من الطالبة
انتصار حمد عبد الله

* * لجنة المناقشة :

1 – د.ماهر نجيب ابراهيم .....
(مشرفاً رئيساً)

2 – د.أحمد الصغير دبوب .....
(مشرفاً مساعداً)

3 – د.فهيمة حامد هماك .....
(ممتحناً داخلياً)

4 – د.علي محمد عثمان .....
(ممتحناً خارجياً)

أمين اللجنة الشعبية لكلية العلوم

جامـــــعة التحدي

كلية العـــــلوم

قسم علم الحيوان

دارسة حول تاثير التدخين علي بعض عناصر جهاز المناعة

مقدمة من

انتصار حمد عبدالله حمد

وذلك للحصول على الإجازة العليا (الماجستير) في الأحياء

تحت إشراف

د. ماهر نجيب إبراهيم

د. احمد الصغير دبوب

(2007-2008)