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Study The Relation Between Human Comlement Components C3 and C4 Deficiency and Elevation of C-Reactive Protein (CRP) as an Indicator of Increase Infections.

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Thesis Title

Study The Relation Between Human Complement Components C3 and C4 Deficiency and Elevation of C-Reactive Protien (CRP) as an Indicator of increase Microbial Infection

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Abstract:

The complement system is a part of the immune system that enhances the ability of antibodies and phagocytic cells to clear microbes and damaged cells from an organism through inflammation, and attacks the pathogen's plasma membrane. However, the deficiency in any component of the complement system can lead to immunocompromise and overwhelming infection and sepsis. In Benghazi children hospital this study focused on measuring of complement components C3 and C4 levels in the serum of blood of the hospitalized patients and characterize the relation between the low levels of components C3 and C4 and the increase of C-reactive protein as a marker of microbial infection. In this study the complement C3 and C4 measurements showed that, the rate of components C3 and C4 deficiencies were (11.1%) and (0.7%) respectively. Using C-reactive protein as a marker for microbial infection has provided a clear image of strong correlation between the low levels of C3 and C4 and the increase of microbial infection.

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First of all thanks to Allah the most gracious and the most merciful, for grant me the longanimity and spell to self-realization and to pursue my dream. I would like to express my sincere gratitude to my supervisor Dr. Ismaeel Hussein Bozakouk for his great assistance and advices in my project. Special thanks to stuff of laboratories: serology laboratory of children hospital for providing samples and for their laboratory technical assistant. Great thanks to Dr. Rabeiah AL-Gazal, and amazing frind Najat AL-Reishei for her invaluable support. Also a great thanks to Dr. Amal Eshtewi for medical revision.

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Dedication

I would like to dedicate my project to source of my powerfull my father, to my wonderful Mother , to my husband for his endless love and support, to sweet hearts my children mohammed and wessam, to my brother and sisters and to spirit of my grand mather to all who encouraged me to finish my research.

Abbreviations

Å	Angstrom unit
ANC	Absolute Neutrophil Count
BT	Body Temperature.
BCR	B Cell Receptor.
C3	Complement Protein Number 3
C4	Complement Protein Number 4
C4bp	C4- binding Protein.
CP	Classical Pathway.
CR	Complement Receptor.
CRM	Customer Relationship Management.
CRP	C-Reactive Protein.
DIC	Disseminated Intravascular Coagulation.
DE	Emergency Department.
EID	Emerging Infectious Disease.
ESR	Erythrocyte Sedimentation Rate.
FDC	Follicular Dendritic Cell.
fH	Factor H.
HIV	Human Immunodeficiency Virus.
hs CRP	High- sensitive C-reactive Protein.
HufH	Human factor H.
IC	Immuno Complex.

IFCC	International Federation of Clinical Chemistry.
Ig G	Immunoglobulin G.
Ig M	Immunoglobulin M.
IL-1	Inter Leukin-1.
IL-6	Inter Leukin-6.
LCCD	Late Complement Component Deficiency.
LOS	Lipooligosaccharide.
LPS	Lipopolysaccharide.
LTBI	Latent Tuberculosis Infection.
MAC	Membrane Attack Complex.
MASP	MBL- Associated Serien Proteinase.
MBL	Mannan- Binding Lectin.
PAMPs	Pathogen- Associated Molecular Patterns.
PCT	Procalcitonine.
Por	Porin.
RBCs	Red Blood Cells.
SAA	Serum amyloid A.
SBI	Silent Brain Infection.
SLE	Systemic Lupus Erythromatosus.
WBC	White Blood Cell.

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CHAPTER 1: Introduction

Emerging infectious diseases are one category of emerging risks that could have important consequences for human populations microbial predators are as threatening to human survival and well being as the macro-scale predators. The microbial world is defined by its tiny scale, invisible to the naked eye, and embraces viruses, bacteria, archaea, fungi, prions, and protozoa, Although the evolutionary origin of viruses is uncertain (no fossil record), bacteria have been evolving for 4 billion years. The animal kingdom including the Homo sapiens, was first appeared about 50,000 -100,000 years ago has co-evolved with the microbial world from the outset of its existence (Gerba, *et al*. 1996).

The incidence of infectious diseases is high in some groups such as very young, people the elderly, pregnant women, and the immunocompromised patients, also cancer patients undergoing chemotherapy and organ transplant patients have low defenses.

Before birth, a fetus exists in a sterile environment, during passage through the birth canal, the fetus acquires certain microorganisms that may

become permanently associated with it. Organisms that live on or in the body but don't cause disease are collectively referred to as normal microflora or normal microbiota (Tannock, 1999).

Many such organisms have relationships with ahuman. Most organisms among normal microflora are commensals they obtain nutrients from host secretions, waste substances found on the surface of the skin and mucous membranes. However, there is another relationship which is mutualism, in which both members of the association live together and benefit from the relationship. For example, large numbers of *Escherichia coli* live in the large intestine of humans. These bacteria release useful products such as vitamin K, which we use to make a certain blood-clotting factor. Although the relationship is not obligatory, *E.coli* does make a modest contribution toward satisfying our need for vitamin K. The bacteria, in turn, get a favorable environment in which live and obtain nutrients (Tannock, 1999).

Two categories of microflora can be distinguished; resident microflora and transient microflora:

The resident microflora comprises microbes that are always present on or in the human body. They are found in the mouth, on the skin surface, nose, in the passage of the urinary and reproductive tracts, especially near their

openings. In each of these body regions, resident microflora is adapted to various conditions. Both the mouth and lower part of the large intestine are providing the moisture and warm as well as ample nutrients. On mucous membranes of nose, throat, urethra, and vagina also provide warm and moist conditions but not many nutrients. The skin provides nutrients but less moisture and cooler (Hooper, *et al.* 2001).

Some regions in human body lack the resident microflora because these regions can not provide suitable conditions to microorganisms. For example, The stomuch is too acidic medium and other body fluids as blood, cerebrospinal fluid, saliva in prior, urine in kidneys and in abladder, also muscles (Tannock, 1999).

The resident and transient microflora are usually donen't cause diseases but some species of them cause diseases under certain conditions. These organisms are called opportunists. opportunistic pathogens rarely cause diseases in individuals with intact immunological and anatomical defenses (Poindexter and Washington, 1974). when such defenses are impaired or compromised, as a result of congenital or acquired disease or by the use of immunosuppressive therapy or surgical techniques, then these bacteria become capable to cause diseases. Many opportunistic pathogens, e.g. coagulase negative *staphylococci* and *E.coli*, are part of the normal human

flora and are carried on the skin or mucosal surfaces where they cause no harm and may actually have beneficial effects, by preventing colonization by other potential pathogens. However, the introduction of these organisms into anatomical sites in which they are not normally found, or removal of competing bacteria by the use of broad-spectrum antibiotics, may allow their localized multiplication and subsequent development of disease (Poindexter and Washington, 1974).

1.1 Host and microbial interactions:

The host-pathogen interaction is defined as how microbes or viruses sustain themselves within host organisms on a molecular, cellular, organismal or population level. This term is most commonly used to refer to disease-causing microorganisms although they may not cause illness in all hosts. Because of this, the definition has been expanded to how known pathogens survive within their host, whether they cause disease or not.

On the molecular and cellular level, microbes can infect the host and divide rapidly, causing disease by being there and causing a homeostatic imbalance in the body, or by secreting toxins which cause symptoms to appear. Viruses can also infect the host with virulent DNA, which can affect

normal cell processes (transcription, translation, etc.), protein folding, or evading the immune response.

Pathogens include bacteria, fungi, and viruses. Each of these different types of organisms can then be further classified as a pathogen based on its mode of transmission. This includes the following: food bore, airborne, waterborne, blood borne, and vector-borne. Many pathogenic bacteria, such as *Staphylococcus aureus* and *Clostridium botulinum* are food borne pathogens that secrete toxins into the host to cause symptoms. HIV and Hepatitis B are viral infections caused by blood borne pathogens, and Aspergillus is the most common pathogenic fungi that secrete aflatoxin which acts as a carcinogen and contaminates many foods, especially those grown underground (nuts, potatoes, etc.) (San-Blas and Calderone, 2008).

Within the host, pathogens can do a variety of things to cause disease and trigger the immune response. Microbes and fungi cause symptoms due to their high rate of reproduction and tissue invasion. This causes an immune response, resulting in common symptoms as phagocytes break down the bacteria within the host. Some bacteria, such as *H. pylori*, can secrete toxins into the surrounding tissues, resulting in cell death or inhibition of normal tissue function. Viruses, however, use a completely different mechanism to cause disease. Upon entry into the host, they can do one of two things. Many

times, viral pathogens enter the lytic cycle; this is when the virus inserts its DNA or RNA into the host cell, replicates, and eventually causes the cell to lyse, releasing more viruses into the environment. The lysogenic cycle, however, is when the viral DNA is incorporated into the host genome, allowing it to go unnoticed by the immune system. Eventually, it gets reactivated and enters the lytic cycle, giving it an indefinite "shelf life" so to speak (nas.edu).

Depending on how the pathogen interacts with the host, it can be involved in one of three host-pathogen interactions. Commensalism is when the pathogen benefits while the host gains nothing from the interaction. For example, many microorganisms live on our skin surfaces and utilize those products are released whether or not they are used by microorganisms (Hooper and Gordon, 2001). Mutualism occurs when both the pathogen and the host benefit from the interaction, as seen in the human stomach. Many of the bacteria aid in the breaking down of nutrients for the host, and in return, our bodies act as their ecosystem (Backhed, *et al*. 2005). When the pathogenic microorganism causes diseases in this case exit from commensalism and mutualism relationships and enters parasitism relationship in this relation, the parasite, benefits from the relationship, whereas the other organism, the host, is harmed by it. The parasite has the

capacity to invade tissues, if parasite replicated within or on the host's body, infection occurs, after that if an infection disrupts the normal functions of the host, disease occurs. This can be seen in the unicellular Plasmodium falciparum parasite which causes malaria in humans.

The ability of a pathogen to induce diseases is called pathogenicity, an organism's pathogenicity depends on its ability to invade a host, multiply in the host, and avoid being damaged by the host's defenses. Some disease agents, such as *Mycobacterium tuberculosis,* frequently cause disease upon entering the asusceptible host. Other agents, such as *staphylococcus epidermis,* cause disease only if the immune system is pouring. Most infectious agents exhibit a degree of pathogenicity between these extremes.

Although pathogens do have the capability to cause disease, they do not always do so. This is described as context-dependent pathogenicity. Scientists believe that this variability comes from both genetic and environmental factors within the host. One example of this in humans is E. coli. Normally, this bacteria flourish as a part of the normal, healthy microbiota in the intestines. However, if it relocates to a different region of the digestive tract or the body, it can cause intense diarrhea. So while *E. coli* is classified as a pathogen, it does not always act as such (Clermont, *et al.*

2000). This example can also be applied to *S. aureus* and other common microbial flora in humans.

There is important factor determine the level of pathogenicity which is the number of infectious organisms that enter the body. If only a small number enter, the host's defenses may be able to eliminate the organisms before they can cause disease. If a large number enter, they may overcome the host defenses and cause disease. Other organisms are so highly infectious that *Shigella,* for example, needs only 10 organisms to be ingested to cause a very nasty case of dysentery (Cannon, *et al*. 1995).

So, the intensity of the disease produced by pathogens called (virulence), it varies among different microbial species. Virulence factors are structural or physiological characteristics that help organisms cause infection and disease. These factors include structures such as pilli for adhesion to cells and tissues, enzymes that either help in evading host defenses or protect the organism from host defenses or protect the organism from host defenses, and toxins that can directly cause disease (Casadeval and Pirofski, 1999).

1.2 The Immune System:

Pathogenic microorganisms that are encountered daily in the life of a healthy individual cause disease only sometimes. Most are detected and

destroyed within minutes or hours by defense mechanisms that do not rely on the clonal expansion of antigen-specific lymphocytes. These are the mechanisms of innate immunity. To recognize pathogens, both the innate and adaptive immune systems can distinguish between self and non-self, but they differ in how they do this. Innate immunity relies on a limited number of receptors and secreted proteins that are encoded in the germline and that recognize features common to many pathogens. In contrast, adaptive immunity uses a process of somatic cell gene rearrangement to generate an enormous repertoire of antigen receptors that are capable of fine distinctions between closely related molecules. Nonetheless, the innate immune system discriminates very effectively between host cells and pathogens, providing initial defenses and also contributing to the induction of adaptive immune responses. The importance of innate immunity is shown by the fact that defects in its components, which are very rare, can lead to increased susceptibility to infection, even in the presence of an intact adaptive immune system (MacPherson and Austyn, 2012).

The response to an encounter with a new pathogen occurs in three phases. When a pathogen succeeds in breaching one of the host's anatomic barriers, some innate immune mechanisms start acting immediately. These first defenses include several classes of preformed soluble molecules present

in blood, extracellular fluid, and epithelial secretions that can either kill the pathogen or weaken its effect. Antimicrobial enzymes such as lysozyme begin to digest bacterial cell walls antimicrobial peptides such as the defensins lyse bacterial cell membranes directly and a system of plasma proteins known as the complement system targets pathogens both for lysis and for phagocytosis by cells of the innate immune system such as macrophages, this phase take (0-4) hours. In the second phase of the response, these innate immune cells sense the presence of a pathogen by recognizing molecules typical of a microbe and not shared by host cells pathogen-associated molecular patterns (PAMPs) and become activated, setting in train several different effector mechanisms to eliminate the infection. By themselves, neither the soluble nor the cellular components of innate immunity generate long-term protective immunological memory, this phase take (4-96) hours. Only if an infectious organism breaches these first two lines of defense, more than 96 hours, will mechanisms be engaged to induce an adaptive immune response the third phase of the response to a pathogen. This leads to the expansion of antigen-specific lymphocytes that target the pathogen specifically and to the formation of memory cells that provide long-lasting specific immunity (MacPherson and Austyn, 2012).

There are types of series of proteins that are always present in blood, these proteins are collectively called the complement system because they can “complement,” or act in conjunction with, the adaptive immune defenses. In response to certain stimuli, the complement proteins become activated, setting off a chain of events that results in removal and destruction of invading microbes (MacPherson and Austyn, 2012).

1.2.1 The Complement system:

The existence of the complement system was first recognized near the end of the nineteenth century 1890s when normal sheep blood was found to possess a mild bactericidal activity that was lost when the blood was heated to 55ºC. This labile bactericidal activity was later termed alexin by Bordet. By 1900 Paul Ehrlich had proposed a scheme for humoral immunity in which he identified the heat-stable immunity sensitizer component of serum as”ambo receptor” (antibody), while the heat-labile factor in serum (Bordet’s alexin) was termed ‘complement’. Since then an impressive number of complement components have been, and are being, added to the list of molecules that make up the complement system as we know it today (Sunyer and Lambris, 2001).

Complement is a system of more than 30 proteins in the plasma and on cell surfaces, amounting to more than 3 g/L and constituting more than 15% of the globular fraction of plasma (Walpart, 2001). In the complement system, activation by proteolysis is used to an even greater degree, because many of the complement proteins are proteases that successively cleave and activate one another. The proteases of the complement system are synthesized as inactive proenzymes, or zymogens, which only become enzymatically active after proteolytic cleavage, usually by another complement protein. The complement pathways are triggered by proteins that act as pattern recognition receptors to detect the presence of pathogens. Detection of pathogen activates an initial zymogen, triggering a cascade of proteolysis in which complement zymogens are activated sequentially, each becoming an active protease that cleaves and activates many molecules of the next zymogen in the pathway. These proteolytic cascades finally generate the effector complement components that aid the removal of the pathogen. In this way, the detection of even a small number of pathogens produces a rapid response that is greatly amplified at each step.

The first proteins discovered belong to the classical pathway and are designated by the letter C followed by a number. The native complement proteins such as the inactive zymogens have a simple number designation, for

example, C1 and C2. Unfortunately, they were named in the order of their discovery rather than the sequence of reactions. The reaction sequence in the classical pathway, for example, is C1, C4, C2, C3, C5, C6, C7, C8, and C9 (note that not all of these are proteases). Products of cleavage reactions are designated by adding a lower- case letter as a suffix. For example, cleavage of C3 produces a small protein fragment called C3a and a bigger fragment, C3b. The larger fragment is always designated by the suffix b, with one exception. For C2, the larger fragment was originally named C2a, because it was the enzymatically active fragment, and this name has survived. Another exception to the general rule is the naming of C1q, C1r, and C1s: these are not cleavage products of C1 but are distinct proteins that together comprise C1. The proteins of the alternative pathway were discovered later and are designated by different capital letters, for example, factor B and factor D.

Complement activation in the fluid phase occurs through three pathways, which are called the classical, lectin, and alternative pathways.

1.2.1.1 The Classical pathway:

Activation of the classical pathway requires antibodies, a component of adaptive immunity. When antibodies bind to the antigen, forming antigen-antibody complexes, the “red flag” portion of the antibody can then interact

with a complement component, activating it. This, in turn, leads to the activation of other complement proteins (Ying, *et al.* 1991).

Initiation of the classical pathway occurs when C1q, in complex with C1r and C1s serine proteases (the C1 complex), binds to the Fc region of complement- fixing antibodies (generally IgG1 and IgM) attached to pathogenic surfaces. Human IgG subclasses also differ in their ability to activate complement; in general, IgG1, IgG2, and IgG3 activate complement in the order IgG3> IgG1> IgG2, while IgG4 does not activate complement. Autocatalytic activation of C1r and C1s, in turn, cleaves C4 and C2 into larger (C4b, C2a) and smaller (C4a, C2b) fragments. The larger fragments as asociate to form C4bC2a on pathogenic surfaces, and the complex gains the ability to cleave C3 and is termed the C3 convertase (Arlaud, *et al.*2002). Generation of the C3 convertase, which cleaves C3 into the anaphylatoxin C3a and the opsonin C3b, is the point at which all complement activation cascades converge (Carroll, *et al.* 1990). When C3 is cleaved into C3b, it exposes an internal thioester bond that allows stable covalent binding of C3b to hydroxyl groups on proximate carbohydrates and proteins. This activity underpins the entire complement system by effectively ‘tagging’ microorganisms as foreign, leading to further complement activation on and around the opsonized surface and terminating in the production of

anaphylatoxins and assembly of the membrane attack complex(MAC), Figure (1) (Ram, *et al*. 2010).

1.2.1.2 The lectin pathway(Mannose-binding lectin pathway[MBL]):

As with the C1 complex of the classical pathway, the lectin pathway also comprises recognition molecules (mannan-binding lectin [MBL]) which is an acute-phase protein, consists of large oligomers assembled from identical polypeptide chains and bears structural similarity to C1q. MBL is a well characterized receptor of the collectin family, so termed because of the fusion of a collagenous domain to a calcium dependent lectin domain, which is synthesized in the liver and secreted into the plasma as a component of the acute-phase response (Dunkelberger and Song, 2010).

MBL in plasma forms complexes with the MBL associated serine proteases MASP-1 and MASP-2, which bind MBL as inactive zymogens. When MBL binds to a pathogen surface, a conformational change occurs in MASP-2 that enables it to cleave and activate a second MASP-2 molecule in the same MBL complex. MASPs are homologs of C1r and C1s of the classical pathway, cleave C2 and C4, forming C4b/C2b C3convertase. From this point, the complement cascade is identical to that of the classical pathway Figure (1) (Dunkelberger and Song, 2010).

1.2.1.3 The Alternative Pathway:

The alternative pathway does not require initiation by antibodies and thus serves to protect the host from invading pathogens prior to the development of specific immune responses. The alternative pathway is capable of autoactivation because of a process termed “tick over” of C3. Spontaneous “tick over” of C3 results in generation of a conformationally altered C3 molecule called C3(H2O) that is capable of binding factor B. Once factor B associated with C3(H2O), factor B itself undergoes a conformational change, which renders it susceptible to cleavage by the serine protease factor D, generating Ba and Bb. The Bb fragment remains associated with C3(H2O) and through its own serine protease domain can cleave the C3a fragment from the N terminus of the α chain of C3 to yield C3b. Cleavage of C3 results in a conformational change in the molecule and exposure of its internal thioester bond. The calculated half-life of the thioester of this metastable C3b molecule is ~60 μs. Within this short period, the nascent C3b must find a suitable electron donor in the form of an —OH or —NH2 group on a biological surface to form a covalent ester or amide bond, respectively; failure to do so will result in reaction of C3b with a water molecule, and inactive C3b will remain in solution. The labile nature of activated C3b ensures that C3b binding occurs only proximate to the site of complement

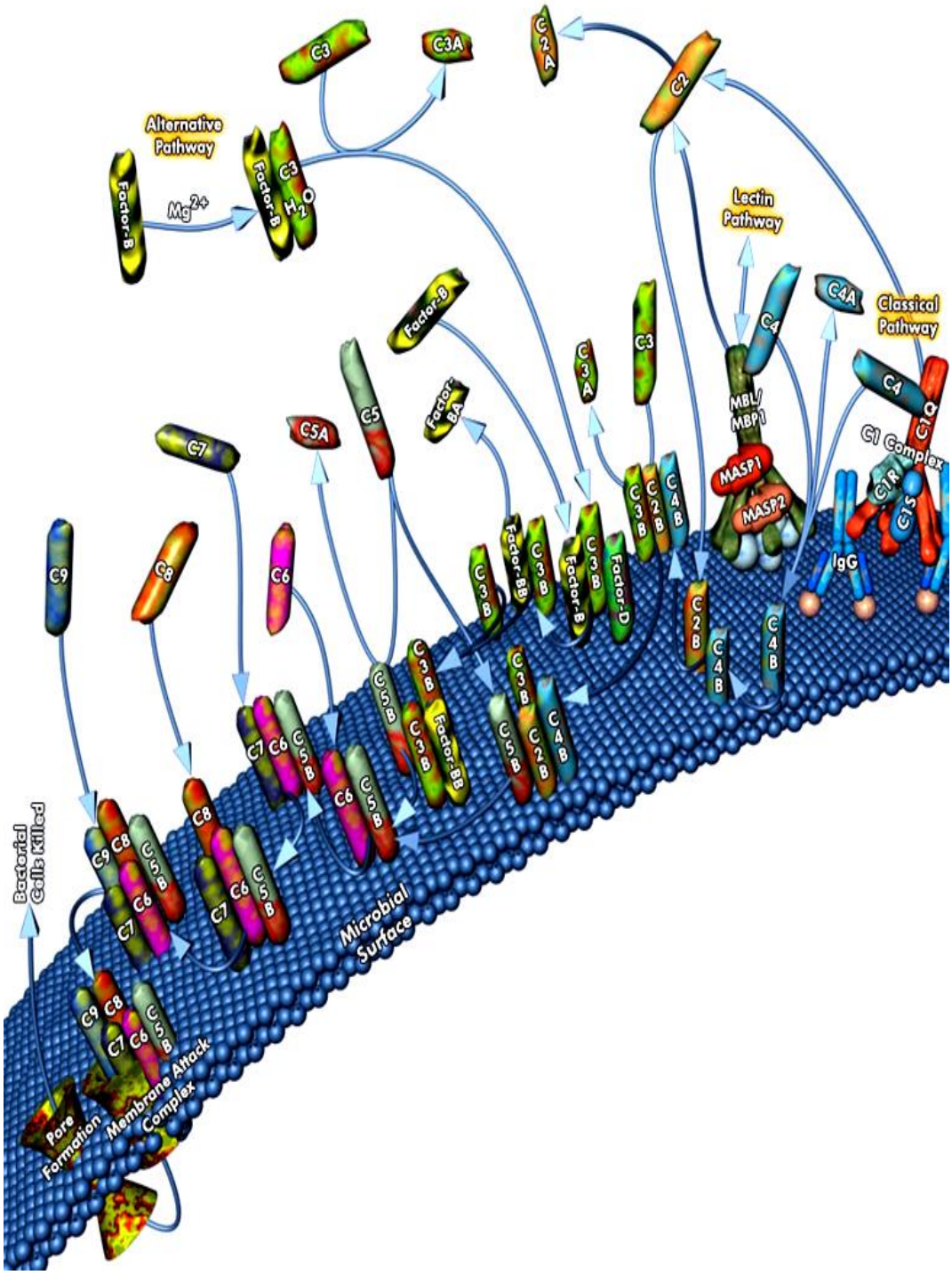
activation, thereby preventing indiscriminate tissue damage. Surface bound C3b can then bind to factor B and generate more C3 convertases and thus set into motion the positive feedback amplification loop that is a feature unique to the alternative pathway. Properdin, the only known positive regulator of the complement system, serves to stabilize the alternative pathway C3 convertase and extends its half life 5- to 10-fold to ~7 min. Recent data suggest that properdin can bind directly to certain surfaces such as zymosan, rabbit erythrocytes (RBCs), and apoptotic cells and to bacteria such as *Neisseria gonorrhoeae* and "rough" *Escherichia coli* (which lack O-antigen repeating units on their lipopolysaccharide [LPS]) and initiate alternative pathway activation. However, commercially available purified properdin preparations, as used in that study, contain aggregates of properdin that result from freeze thawing of the protein, which could result in spuriously high avidity.

The effects of complement activation are the assembly of the terminal components of complement to form a membrane attack complex. The reactions leading to the formation of this complex. The end result is a pore in the lipid bilayer membrane that destroys membrane integrity. This is thought to kill the pathogen by destroying the proton gradient across the pathogen's cell membrane, The membrane attack complex of complement (MAC), apart

from its classical role of lysing cells, can also trigger a range of no lethal effects on cells, acting as a drive to inflammation Figure (1) (Triantafilou, *et al*. 2013).

The first step in the formation of the membrane attack complex is the cleavage of C5 by a C5 convertase to release C5b. after that, C5b initiates the assembly of the later complement components and their insertion into the cell membrane. First, one molecule of C5b binds one molecule of C6, and the C5b6 complex then binds one molecule of C7. This reaction leads to a conformational change in the constituent molecules, with the exposure of a hydrophobic site on C7, which inserts into the lipid bilayer. Similar hydrophobic sites are exposed to the later components C8 and C9 when they are bound to the complex, allowing these proteins also to insert into the lipid bilayer. C8 is a complex of two proteins, C8b and C8a. The C8b protein binds to C5b, and the binding of C8b to the membrane associated C5b67 complex allows the hydrophobic domain of C8a to insert into the lipid bilayer. Finally, C8a induces the polymerization of 10–16 molecules of C9 into a pore forming structure called the membrane-attack complex. The membrane-attack complex has a hydrophobic external face, allowing it to associate with the lipid bilayer, but a hydrophilic internal channel. The diameter of this channel is about 100 Å, allowing the free passage of solutes

and water across the lipid bilayer. The disruption of the lipid bilayer leads to the loss of cellular homeostasis, the disruption of the proton gradient across the membrane, the penetration of enzymes such as lysozyme into the cell, and the eventual destruction of the pathogen Figure (1) (Triantafilou, *et al*. 2013).



https://www.qiagen.com/rb/shop/genes-and-pathways/pathway-details/?pwid=117

Figure (1): The Complement Pathways.

1.2.2 Role of complement system in innate and adaptive immunity

The complement system plays a crucial role in the innate defense against common pathogens. Activation of complement cascades acting after the identification and persecution of the surface identified as foreign and allows complement to recognized to the invading microorganisms, It has the ability to recognize to certain pathogens from common pathogens, and immediately and nappy effect In performing these functions, complement represents a base of the innate defense against infection and provides first line barrier to invading pathogens. The complement cascade terminated in opsonization and lysis of the pathogen as well as in the generation of the classical inflammatory response through the production of potent proinflammatory molecules. More recently, however, the role of complement in the immune response has been expanded due to observations that link complement activation to adaptive immune responses. It is now appreciated that complement is a functional bridge between innate and adaptive immune responses that allows an integrated host defense to pathogenic challenges , complement plays a vital role in shaping adaptive immune responses, functionally integrating it into the ability of the host to combat invasion from a wide range of pathogens (Dunkelberger and Song, 2010).

1.2.3 Complement deficiency associated with infectious diseases.

Deficiencies of the complement components have been reported for most of the constituents. These Deficiencies can be acquired or inherited (Tedesco, 2008) and complete or partial. deficiency of specific complement components has been identified in the classical, alternative and terminal pathways, as well as in regulatory proteins and complement receptors.

Deficiency of early components of classical pathway (C1, C4, C2) related to autoimmune disease, especially systemic lupus erythematosus (SLE), is the most common presentation in patients with early component deficiency. The incidence rates of SLE in individuals with C1q and C4 are reported to be around 90% and 75% respectively. Patients with C2 deficiency develop SLE with alesser frequency (around 15%) (Agrawal, 2015).

MBL deficiency is linked with frequent pyogenic infection, including pneumococcal infection in infants and young children. Severe pneumococcal disease is also reported in patients with MASP-2 deficiency. Inherited MBL deficiency, common in most human populations, predisposes to infectious and autoimmune diseases.

Alternative pathway (properdin, factor B, factor D) deficiency is associated with severe fulminant neisserial infections with a high mortality

rate. (C3, factor H, factor I) deficiency of these factors predisposes individuals to severe pyogenic bacterial infections. Factor H and factor I deficiencies cause secondary C3 deficiency with C3 consumption and impose the same infection risk as aprimary C3 deficiency.

The lack of MAC formation by terminal pathway (C5-C9) deficiency results in severe recurrent infection by *N. gonorrhoea* or *N. meningitides* (Agrawal, 2015).

1.2.3.1 Deficiency of C3 (any pathway)

C3 occupies a central position in the complement cascade and subserves several critical functions. C3 deficiency related with autoimmune disorders, infections or both. Infections tend to be recurrent and severe. Invasive infections (meningitis, bacteremia, pneumonia, and otitis media) with *S. pneumonia, N. miningitides,* and *H. influenza* have been reported (Farhoudi, *et al.* 1988). Complement deficiency of C3 has been correlation with ahistory of recurrent Pyogenic infections (Ghannam, *et al.* 2008). The presence of even small amounts of C3 reduces the severity and frequency of infections. Deficiency of factors (factor I, factor H, CR1, CD59) predisposes individuals to severe pyogenic bacterial infections. Factor H and factor I deficiencies cause secondary C3 deficiency with C3 consumption and impose the same infections risk as aprimary C3 deficiency (Agrawal, *et al*. 2015).

1.2.3.2 Deficiency of Classical pathway (C4).

Infections reported in persons with complete deficiency of the components of the classical pathway (C4) include those with encapsulated bacteria such as *S. Pneumoniae, Haemophilus influenzae, Neisseria meningitidis.* It is not surprising because classical pathway components form the "effector arm" of antibodies against these bacteria. Partial C4 deficiency of C4a or C4b is the most common inherited immune deficiency in humans, but complete deficiency of either C4A or C4B is relatively common and occurs in about 6% of the population (Blanchong, *et al.* 2001).

Arole for C4 in protection against certain fungal infections was suggested by the observation that C4b and C4a deficiencies were both associated with increased susceptibility to paracoccidioidomycosis (de Messias, *et al.* 1991). Also, at mortality level, properdin deficiency is associated with ahigh mortality rate due to fulminant infection with *N meningitidis*. Primary and secondary C3 deficiency present with severe recurrent pyogenic infections early in life, similar to those observed in patients with hypogammaglobulinemia, leading to high comorbidity (Agrawal, *et al.* 2015).

1.3 C-Reactive protein(CRP)

C-reactive protein (CRP) was so named because it was first discovered as a substance in the serum of patients with acute inflammation that reacted with the C-(capsular) polysaccharide of *Pneumococcus* (Bone, *et al*. 1992). It is discovered by Tillett and Francis in 1930, it was initially through that CRP might be a pathogenic secretion as it was elevated in people with a variety of illnesses including cancer (Pepys, *et al*. 2003). However, the discovery of synthesis demonstrated that it is a native protein.

CRP is phylogenetically a highly conserved plasma protein, with homolog in vertebrates and many invertebrates that participates in the systemic response to inflammation. Its plasma concentration increases during inflammatory states, acharacter that has long been employed for clinical purposes. CRP is a pattern recognition molecule, binding to specific molecular configurations that are typically exposed during cell death or found on the surfaces of pathogens. Its rapid increase in synthesis within hours after tissue injury or infection suggests that it contributes to host defense and that it is part of the innate immune response (Black, *et al*. 2004).

CRP involved in several host defense related functions based on its ability to recognize foreign pathogens and damaged cells of the host and to

initiate their elimination by interacting with humoral and cellular effector systems in the blood. Consequently, the level of this protein in plasma increases greatly during acute phase response to tissue injury, infection, or other inflammatory stimuli. It is induced by IL1/interleukin-1 and IL6/interleukin-6 (Black, *et al*. 2004). Since the CRP is a general test, a positive CRP may indicate any of number of things: Rheumatoid arthritis, Rheumatic fever, Cancer, Tiberculosis, Pneumococcal pneumonia, Myocardial infection, SLE, Heart attack.

1.3.1 Role of CRP in microbial infection:

Patients presenting with fever, tachycardia, hyper-ventilation, and leukocytosis with no known stimulus such as trauma, burns, or pancreatitis are usually presumed to have sepsis, a typical body response to infection (Bone, *et al*. 1992). Sepsis is a major cause to morbidity and mortality, and early institution of anappropriate antimicrobial regimen is associated with improved survival (Kreger, *et al*. 1982). Clinical signs of infection and routine laboratory tests are, however, not specific and can be misleading. Infection can be present in some patients without sepsis, particularly in the debilitated and elderly. Acutely ill patients, however, frequently present signs of sepsis, even when no bacterial infection can be demonstrated. The widespread use of antibiotics for all these patients presents problems of

antibiotic resistance, drug toxicity, and increased medical costs. Identifying patients who are likely to benefit from an antimicrobial agent is apriority. Since sepsis response involves the release of a wide array of inflammatory mediators (Pinsky, *et al*. 1993).

As these mediators may be increased in other inflammatory conditions, none is specific for infection. C-reactive protein (CRP), in addition to the white blood cell (WBC) count, is currently the most widely used parameter to support a diagnosis of infection. CRP is an acute-phasereactant produced by the liver. Plasma concentrations are normally below 10 mg/l but increase several fold after trauma, infection, inflammation, and other stimuli involving tissue damage (Pepys, *et al*. 1983). C-reactive protein (CRP) induces adhesion molecule expression by endothelial cells. However, the effects of CRP on chemokine expression by endothelial cells are not known (Pasceri, *et al*. 2001). Similarlly, Serum Procalcitonin and C-Reactive Protein Levels as Markers of Bacterial Infection, the elevation of these compounds indicating to bacterial infection (Simon, *et al*. 2004).

Quantitative CRP concentration is a valuable laboratory test in the evaluation of febrile young children who are at risk for occult bacteremia and SBI, with a better predictive value than the WBC or ANC (Pulliam, *et al*. 2001). CRP may be useful in diagnosing the onset of sepsis in acutely ill

patients and for indicating successful treatment during follow-up of the clinical course. Although large increases can occur in response to infection, no definite correlation between infection and changes in CRP has been documented, and using a low CRP level to exclude the presence of infection remains controversial. This investigation evaluated the feasibility of using CRP levels, as compared to clinical signs of infection and routine laboratory tests, for detecting bacterial infection (Matson, *et al*. 1991).

1.4 Aims of the Study

1. Analysis the destribution of the complement components C3 and C4 defeciency at children in the children hospital - Benghazi.
2. Study the possible relation between the complement components C3 and C4 deficiencies and increase the microbial infection in the children using CRP as a marker for infections.

CHAPTER 2: Litterature Review

In a study about the Global trends in emerging infectious diseases, they have found that; Emerging infectious diseases (EIDs) are a significant burden on global economies and public health. Their emergence is thought to be driven largely by socioeconomic, environmental and ecological factors, but there has been no comparative study has explicitly analyzed these linkages to understand global temporal and spatial patterns of EIDs. It has been demonstrated that; EID events have risen significantly over time after controlling for reporting bias, with their peak incidence (in the 1980s) concomitant with the HIV pandemic. EID events are dominated by zoonoses (60.3% of EIDs): the majority of these (71.8%) originate in wildlife and are increasing significantly over time. From their study they concluded that; 54.3% of EID events are caused by bacteria or rickettsia, reflecting a large number of drug-resistant microbes. (Kate, *et al*. 2008).

To investigate the role of complement proteins in immune deficiency, a study has been carried out on the hexameric complement, they found: complement activation is an immediate and potent immune defense mechanism, but how immunoglobulin G (IgG) antibodies activate complement at the molecular level is poorly understood. Using high-

resolution crystallography, Diebolder *et al*. showed that human IgGs form hexameric structures by interacting with neighboring IgG molecules, and this complex then activates complement. Therefore, IgG molecules and the complement system can coexist in the blood because complement activation will only be triggered after IgG senses a surface antigen and begins to aggregate (Diebolder, *et al*. 2014). another study on the role of complement in immune defense has revealed that complement is a key system for immune surveillance and homeostasis, it has been demonstrated that: Nearly a century after the signing of the human complement system was recognized, they have come to realize that its functions extend far beyond the elimination of microbes. Complement acts as a rapid and efficient immune surveillance system that has distinct effects on healthy and altered host cells and foreign invaders. By eliminating cellular debris and infectious microbes, orchestrating immune responses and sending 'danger' signals, complement contributes substantially to homeostasis, but it can also take action against healthy cells if not properly controlled (Daniel, *et al*. 2010).

A study was carried out on the role of complement and complement receptors in induction and regulation of immunity, they have found that: covalent attachment of activated complement C3 (C3d) to antigen links innate and adaptive immunity by targeting antigen to follicular dendritic cells

(FDC) and B cells via specific receptors CD21 and CD35. Recent characterization of knockout mice deficient in complement components C3, C4, or the receptors CD21 and CD35, as well as biochemical studies of the CD21/CD19/Tapa-1 coreceptor on B cells, have helped to elucidate the mechanism of complement regulation of both B-1 and B-2 lymphocytes. Interestingly, a natural antibody of the adaptive immune system provides a major recognition role in activation of the complement system, which in turn enhances activation of antigen-specific B cells. Enhancement of the primary and secondary immune response to T-dependent antigens is mediated by ligation of the coreceptor and the B cell antigen receptor, which dramatically increases follicular retention and B cell survival within the germinal center (Michael, 1998). Also in a role of the complement system in innate immunity, there is another study they have found that; complement is a major component of the innate immune system involved in defending against all the foreign pathogens through complement fragments that participate in opsonization, chemotaxis, and activation of leukocytes and through cytolysis by C5b-9 membrane attack complex. Bacteria and viruses have adapted in various ways to escape the complement activation, and they take advantage of the complement system by using the host complement receptors to infect various cells. Complement activation also participates in clearance of

apoptotic cells and immune, complexes. Moreover, at a sublytic dose, C5b-9 was shown to promote cell survival. Recently it was also recognized that complement plays a key role in adaptive immunity by modulating and modifying the T cell responses. these data suggested that complement activation constitutes a critical link between the innate and acquired immune responses (Rus, *et al*. 2005).

In order to investigate in the study of the complement and its role in innate and adaptive immune responses, has revealed that: The complement system plays a crucial role in the innate defense against common pathogens. Activation of complement leads to robust and efficient proteolytic cascades, which terminates in opsonization and lysis of the pathogen as well as in the generation of the classical inflammatory response through the production of potent proinflammatory molecules. More recently, however, the role of complement in the immune response has been expanded due to observations that link complement activation to adaptive immune responses.

It will known that appreciated that complement is functional bridge between innate and adaptive immune responses that allows an integrated host defense to pathogenic challenges. As such, a study of its functions allows insight into the molecular underpinnings of host pathogen interactions as well as the organization and orchestration of the host immune response

(Dunkelberger, *et al.* 2010). To understand the interaction between human defenses and fungal infection, a study was carried out on the role of complement that attacks against *Aspergillus*, they have found that; the low levels of complements or poor recognition of *Aspergillus* surface may support the fungal infection (Speth and Rambach, 2012). Also, a study has been carried out in meningococcal diseases also participated in complement system strategies, they have found that: in an understanding of the pathogenesis of the meningococcal disease, the infection remains a major cause of morbidity and mortality globally (Lisa and Sanjay, 2014).

The role of the complement system in innate immune defenses against invasive meningococcal disease is well established. Individuals deficient in components of the alternative and terminal complement pathways are highly predisposed to invasive, often recurrent meningococcal infections. Genome-wide analysis studies also point to a central role of complement in disease pathogenesis. Indeed, pathophysiologic events pertinent to the complement system that accompany meningococcal sepsis in humans. Meningococci use several often redundant mechanisms to evade killing by human complement. Capsular polysaccharide and lipooligosaccharide glycan composition play critical roles in complement evasion (Lisa and Sanjay, 2014).

In order to investigate the role of complements in inhibiting meningococcal diseases a study has been carried out on the Binding of the Complement Inhibitor C4bp to Serogroup B *Neisseria meningitides*, they found: The host defenses against neisseriae require the complement C as indicated by the fact that individuals deficient in properdin or late C components (C6-9) have an increased susceptibility to recurrent neisserial infections. Because the classical pathway is required to initiate efficient complement activation on neisseria, meningococci should be able to evade it to cause disease. To approve that, a study was carried out to understand the interaction between meningococci infection and the classical pathway C4b-binding protein, they found that; all bacterial strains that expressing Por (A) interacted with C4bp, whereas stranding that lacking Por A are not bind with C4bp, therefore, they were concluded that the binding of C4bp to bacterial cell helps *N. meningitides* to escape complements activation of classical pathway (Hanna, *et al*. 2005).

In a study carried out on the elevation *Neisseria gonorrhoeae* to complement system, they have revield that: Complement forms a key arm of innate immune defenses against gonococcal infection. Sialylation of gonococcal lipo-oligosaccharide enables *N. gonorrhoeae* to bind the alternative pathway complement inhibitor, factor H (fH), leading to evade

killing by human complement. Also, they have found that; Lipo-oligosaccharide sialylation of *N. gonorrhoeae* resulted in classical pathway regulation as indecated by decreased C4 binding in human, chimpanzee, and Rhesus serum but was accompanied by serum resistance only in human and chimpanzee serum (Jutamas, *et al*. 2008).

Most of the complement proteins and regulators are inherited as autosomal recessive genes; this means that there are two copies of each gene present, one contributed by each parent. There are two exceptions: Adeficiency of Properdin, is inherited as an X-linked recessive trait, and C1 inhibitor deficiency (or hereditary angioedema) requires the presence of only one abnormal gene out of the two genes for this protein to produce the disease. When the presence of one abnormal gene "dominates" over the normal gene, it is called autosomal dominant inheritance. In this case, the presence of the one normal gene does not produce sufficient C1 inhibitor to prevent patients from having Hereditary Angioedema attacks.

In order to investigate of Inheritance of complement deficiency and disease associations a study has been carried out on the study of the Complement genetics, deficiencies, and disease associations, they have screened 45 genes encoding proteins of complement component, these genes are carried on different chromosomes, with 19 genes comprising three

significant complement gene clusters in the human genome. their results revealed that the deficiency of any early component of the classical pathway (C1q, C1r/s, C2, C4, and C3) is associated with autoimmune diseases due to the failure of clearance of immune complexes (IC) and apoptotic materials, and the impairment of normal humoral response (Karine, 2012).

Deficiencies of mannan-binding lectin (MBL) and the early components of the alternative (factor D, properdin) and terminal pathways (from C3 onward components: C5, C6, C7, C8, C9) increase susceptibility to infections and their recurrence. While the association of MBL deficiency with a number of autoimmune and infectious disorders has been well established, the effects of the deficiency of other lectin pathway components (ficolins, MASPs) have been less extensively investigated due to incomplete knowledge of the genetic background of such deficiencies and the functional activity of those components (Karine, 2012). For complement regulators and receptors, the consequences of their genetic deficiency vary depending on their specific involvement in the regulatory or signaling steps within the complement cascade and beyond (Karine, 2012).

The role of complements in the inflammatory network was clarified by Knut, *et al*. (2009), they have found that: The Complement component C5 is crucial for experimental animal inflammatory tissue damage; however, its

involvement in human inflammation was incompletely understood. Also, they provide important insight into the comprehensive role of complement in human inflammatory responses to Gram-negative bacteria (Knut, *et al*. 2009).

In order to investigate the association between complement deficiencies and increase of the infectous disease, Figueroa and Densen, 1991, have concluded that: The increase of infectous diseases can be distinguished by unique epidemiology, clinical, and microbiological feature, also by the disruption of four aspects of the complement cascade which include: activation of the classical pathway, activation of the alternative pathway, C3 convertase formation, and C3 deposition, and membrane attack complex assembly and insertion. In general, mechanisms evolved by pathogenic microbes to resist the effects of complement are targeted to these four steps (Figueroa and Densen, 1991). Ethnic background and the incidence of infection are important cofactors determining this prevalence. Although complement undoubtedly plays a role in host defense against many microbial pathogens, it appears most important in protection against encapsulated bacteria, especially *Neisseria meningitidis* but also *Streptococcus pneumoniae*, *Haemophilus influenzae*, and, to a lesser extent, *Neisseria gonorrhoeae* (Figueroa and Densen, 1991).

Also, another study of Infections of People with Complement Deficiencies, they have found that; Deficiencies of components of the classical pathway lead to the development of autoimmune disorders and predispose individuals to recurrent respiratory infections and infections caused by encapsulated organisms, including *Streptococcus pneumonia*, *Neisseria meningitides*, and *Haemophilus influenza*. C3 is the point at which all complement pathways converge, and complete deficiency of C3 invariably leads to severe infections, including those caused by meningococci and pneumococci. Deficiencies of the alternative and terminal complement pathways result in an almost exclusive predisposition to invasive meningococcal disease (Sanjay, *et al*. 2010).

Also, another study has been carried out of Complement deficiency states and meningococcal disease, he has found that; Analysis of complement deficiency states has supported the role of complement in host defense and elucidated diseases associated with defective complement function. Although neisserial infection plays a prominent role in these deficiency states, examination of individuals with late complement component deficiency (LCCD) reveals a particular propensity for recurrent meningococcal disease and provides important clues to the role of complement in neisserial infections. In response to meningococcal disease, LCCD individuals produce

significantly greater amounts of antilipooligosaccharide (LOS) antibody which can kill group B meningococcus in a complement-sufficient in vitro system, further studies of antibody cross-reactivity to other meningococci has led to a clearer understanding of its epitopic specificity. Nevertheless, epidemiologic evidence is consistent with the relative absence of protective immunity in LCCD persons following an episode of infection and supported by quantitation of antibody to capsular polysaccharide. However, compared to anti-LOS antibodies, anticapsular antibodies can offer immune protection to LCCD individuals via complement-dependent opsonophagocytosis--the only form of complement-mediated killing available to these persons. Thus vaccination of LCCD persons with capsular antigens is considered an important means of protecting these high-risk individuals against meningococcal disease (Figueroa, 1993).

In order to investigate the role of CRP as an indicator for microbial infection, a study has been carried out on the role of C-reactive protein as biochemical indicator of bacterial infection in neonates, they have found that; CRP concentrations were found to be elevated high levels (>80 mg/L) during the course of infectious diseases in all neonates with proven bacterial infection (septicemia, pneumonia, multiple micro-abscesses, urinary tract infection) (Jean-Claude, *et al*. 1986). Serial measurement of CRP

concentrations is shown to be valuable in detecting bacterial infection in neonates as well as in following the efficacy of antimicrobial therapy (Jean-Claude, *et al*. 1986). Another study was carried out on the role of C-Reactive Protein in Febrile Children 1 to 36 Months of Age With Clinically Undetectable Serious Bacterial Infection, they have found that: Quantitative CRP concentration is a valuable laboratory test in the evaluation of febrile young children who are at risk for occult bacteremia and SBI, with a better predictive value than the WBC or ANC (Pulliam, et al. 2001).

C-Reactive Protein can be used as indicator for bacterial infection also in adults, a study has been carried out by Yi-Ling Chan, *et al*., 2002, approved that, where the approved that; CRP is a better indicator of bacterial infection than either BT or WBC count for adult atraumatic Emergency Department ED patients (Yi-Ling Chan, *et al*. 2002).

In order to investigation of complement proteins and C-reactive protein as markers to microbial infection a study has been carried out In the study of C-reactive protein and complement components C3 and C4 in children with latent tuberculosis infection, they have found that; The concentration of hsCRP can be used in the follow up of latent tuberculosis infection (LTBI) patients to evaluate response to isoniazid prophylaxis and the level of disease activity (Slavica, *et al*. 2008).

CHAPTER 3: Materials and Methods

Decreased levels of C3 occur in individuals with a congenital deficiency or immunologic diseases (where complement is consumed at an increased rate). C3 and/or complement C4 (C4) levels may be decreased in cases of: systemic lupus erythematosus (SLE) (especially with lupus nephritis), acute and chronic hypocomplementemic nephritis, infective endocarditis, disseminated intravascular coagulation (DIC) (especially with hemolytic uremic syndrome form), and partial lipodystrophy (with associated nephritis-like activity in serum). Cases of hereditary C3 deficiency, while rare, are characterized clinically by recurrent infection and by immune complex disease, in particular, membranoproliferative glomerulonephritis. The central role of C3 in both classical and alternate pathways results in C3 deficient patients being at risk for especially severe infections by encapsulated bacteria such as *S. pneumoniae*, *H. influenzae*, and *N. meningitidis*. Bacteremia, sinopulmonary infections, meningitis, paronychia, and impetigo may occur. Deficient C3 levels have also been found in cases of uremia, chronic liver diseases, anorexia nervosa, and celiac disease (Jacobs, *et al.* 1996).

Decreased levels of C4 occur in individuals with a congenital deficiency or immunologic diseases (where complement is consumed at an increased

rate). C4 levels may be decreased in hereditary and acquired angioedema, complement activation due to immune complex diseases, decreased synthesis due to liver disease, increased consumption in glomerulonephritis, systemic lupus erythematosus (SLE), rheumatoid arthritis, respiratory distress syndrome, autoimmune hemolytic anemia, cryoglobulinemia, and sepsis. Total congenital C4 deficiency is rare, but partial C4 deficiency is common. Partial and complete congenital C4 deficiencies have been associated with immune complex diseases, SLE, autoimmune thyroiditis, and juvenile dermatomyositis. Infections associated with C4 deficiency include bacterial or viral meningitis, *Streptococcus* and *Staphylococcus* sepsis, and pneumonia (Tietz, 1995).

This study was designed to investigate the relationship between deficiency of C3, C4 proteins with an elevation of C- Reactive Protein (CRP). In this study, we focused on children stage from a pediatric hospital of Benghazi city, Libya. Children were chosen randomly from all of the units of the hospital also from OPD patients, This study has included about (588) sample from both genders, collected in period (January- April).

The pateints who are chosen for CRP were (324) and they selected based on medical history and by a referral from a medical consultation to the serology department.

3.1 Samples Collection and Preparations of Complement Components C3, C4, and CRP Testing.

All blood samples were collected in accordance with a standard medical technics. In order to obtain blood serum, all whole blood samples were collected in a covered test tubes, tubes were allowed to clot by leaving them undisturbed at room temperature for 15-30 minutes, tubes were then centrifuged (5000 rpm for 5 minutes) the resulting supernatant is serum. Following centrifugation, immediately serum components were transferred into a clean polypropylene tubes using a Pasteur pipettes, serum samples were maintained at 2-8 C˚prior analyzing, if serum is not analyzed immediately serum should be stored at -20 C˚ it is important to avoid serum components destructive by not use freeze-thaw cycles if so mix thawed samples thoroughly by low-speed vortexing 10 times, visually inspect the samples, if layering or stratification is observed, continue mixing until samples are visibly homogeneous. after thawing, bring to room temperature and mix well by gently shaking .

3.2 Sample Storage:

Most of the serum samples were examined for protein analysis not affected by along time of storage.

3.3 Cobas Integra:

COBAS INTEGRA	100Tests
Tina-quanta complement C3 VER.2	
Calibrator f.a.s. Proteins	5×1 ml
Calibrator f.a.s. Proteins (for USA)	5×1ml
Precinorm Protein	3×1ml
Precipath Protein	3×1ml
NaCl Diluent 9%	6×22ml

3.3.1 Reagents – work Solutions:

R1 TRIS buffer: 100m mol/l, pH 8.0; polyethylene glycol: 3.0%; preservative (liqued)

R2 Anti-human C3 antibody (goat): dependent on titer; TRIS buffer: 33mmol/l; preservative (liquid)

3.3.2 Additional material required for the analysis:

NaCl 9% (10-fold concentrated isotonic saline solution) for automatic sample dilution and standard serial dilutions.

Material used is NaCl Diluent 9%, Cat. No. 20756350, System-ID 075635 0, or prepare the 9% NaCl solution with commercially available sodium chloride tablets or concentrated saline solutions. The NaCl solution is placed in its predefined rack position and is stable for 28 days on-board COBAS INTEGRA 400/400 analyzers.

3.3.3 Application of serum and plasma:

Measuring mode	Absorbance
Abs. Calculation mode	Endpoint
Reaction mode	D-RI-S-SR
Reaction direction	Increase
Wavelength A/B	340/659nm
Calc. First/last	33-60
Typical test range	0.3-0.5 g/l (30-500 mg/dl)
with rerun	0.15-10.0g/l (15-1000 mg/dl)
Typical prozone effect	>13.6 g/l (>1360 mg/dl)
Antigen excess check	No
Predilution factor	21
Postdilution factor	2 recommended
Postconcentration factor	2 recommended
Unit	g/l

3.3.4 Pipetting parameters:

		Diluent (H_2O)
R1	90µl	
Sample	10µl	10µl
SR	17µl	10µl
Totalvolume	137µl	

3.4 Method of Complements C3, C4 analysis:

The complement C3 and C4 assay are an immunoturbidimetric procedure that measures increasing sample turbidity caused by the formation of insoluble immune complexes when antibody to C3 and C4 component is added to the sample. Sample containing C3 and C4 components are incubated with a buffer and a sample blank determination is performed prior to the addition of C3 and C4 antibodies. In the presence of an appropriate antibody in excess, the C3 and C4 concentration are measured as a function of turbidity.

3.4.1 Calibration method:

Calibrator	Calibrator f.a.s. proteins
Calibration dilution ratio	1:10, 1:20, 1:50, 1:100, 1:200, and 0g/L performed automatically by the instrument
Calibration mode	Logit /log 5
Calibration replicate	Duplicate recommended
Calibration interval	Each lot and as required following quality control procedures.

Enter the assigned lot-specific C3 value of the undiluted calibrator, indicated in the package insert of the calibrator f.a.s. Proteins.

Traceability: This method has been standardized with regard to the IFCC/BCR/CAP reference preparation CRM 470(RPPHS 91/0619) for 14 serum proteins.

The reference preparation CRM 470 contains only the C3c fragment, whereas fresh serum samples, contain mainly C3. In fresh serum samples lower C3c values have to be considered because the COBAS INTEGRA C3c test is directed against the C3c is directed against the C3c fragment.

3.5 Calculations and Expected Values:

Be long to the automated system the calculation was carried out in COBAS INTEGRA analyzers automatically calculate the analyte concentration of each sample.

Conversion factor: g/l × 100 = mg/dl

Reference Value for C3: 0.9-1.8 g/l (90-180 mg/dl)

Reference Value for C4: 0.1-0.4g/l (10-40 mg/dl)

3.6 Analytical sensitivity (lower detection limit)

≤ 0.3 g/l (≤ 30 mg/dl)

The detection limit represents the lowest measurable analyte level that can be distinguished from zero. It is calculated as the value lying three standard deviations above that of a zero sample (zero sample +3 SD, within run precision, n=21)

3.7 Statistical Analysis:

In this study used the Chi-sequer, Person test for founded the relations between C3, C4 deficiency with gender type, hospital departments, and CRP elevation.

Figure(2) Complement C3 Casstte.

Figure(3) Complement C4 Casstte.

Figure(4) COBAS INTEGRA 400 PLUS TEST DEFINITION

3.8 CRP Identification Method

In this study, Latex-Agglutination-Test for Qualitative and Semiquantitative Determination of C-reactive Protein was used. The CRP-latex is a slide agglutination test for the qualitative and semi- quantitative detection of C- Reactive Protein (CRP) in human serum. Latex particles coated with goat IgG anti-human CRP are agglutinated when mixed with samples containing CRP. When latex particles complexed human anti-CRP are mixed with a patient's serum containing C reactive proteins, a visible agglutination reaction will take place within 2 minutes.

3.8.1 Reagent Concentrations

R1:

Latex particles coated with anti-CRP

preservative

R2:

Protein containing solution with C-Reactive protein

preservative

R3:

Protein containing solution with C-Reactive protein

preservative

Figure: (5) CRP Test Latex and Positive Negative Reagents.

3.8.2 Procedure and Results of Qualitative Test

1. All reagents and specimens were left at room temperature before use.

2. For will homogeneity, the latex reagent got gently resuspended.

3. Placed one drop (40µl) of each test specimen and if a reference is necessary, one drop (40µl) of the pos./neg. control on separate fields of the reaction slide.

4. Added one drop Latex Reagent (40µl) to each sample. For spreading, reaction mixture over entire test field used a fresh spatula dispenser for each field.

5. For got optimal results have rotated the slide for 2 minutes and read immediately under optimal light conditions.

A negative reaction is indicated by a uniform milky suspension with no agglutination as observed with the negative control. A positive reaction is indicated by any observable agglutination in the reaction mixture. The specimen reaction should be compared with the positive control.

3.8.3 Procedure and Results of Semi-Quantitative Test

1. All reagents and specimens were left at room temperature before use.

2. For will homogeneity, the latex reagent got gently resuspended.

3. For diluted the specimens 1:2, 1:4, 1:8, 1:16 or as needed were saline used.

4. placed one drop (40µl) of the test specimen followed by the corresponding prepared dilutions and as reference one drop (40µl) of the pos./neg. control on separate fields of the reaction slide.

5. Added one drop Latex Reagent (40µl) to each sample. For spreading, reaction mixture over entire test field used a fresh spatula dispenser for each field.

6. For getting optimal results were rotated the slide for 2 minutes and read immediately under optimal light conditions.

The CRP concentration of the serum lies between the highest dilution which just shows a positive reaction (1:4) and the following dilution which shows a negative reaction (1:8). For calculating the serum concentration multiply the corresponding dilution factor by 6mg/l (cut off of the test).

last positive signal 1:4 4×6mg/l= 24mg/l

first negative signal 1:8 8×6mg/l= 48mg/l

The CRP conc. Of the sample is between 24 and 48mg/l.

Interperation:

After complete the positive and negative reaction seen the results, in figure (6):

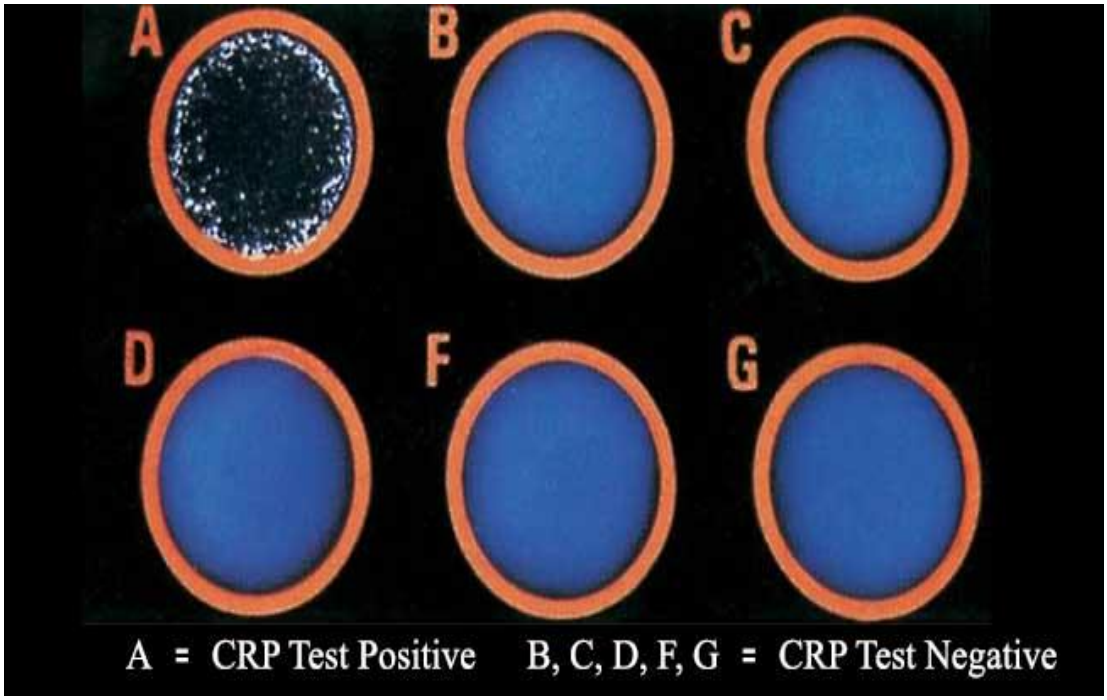


Figure (6) Result Interpretation of CRP Test.

3.8.4 Reference Values of CRP Test:

The more than (6mg/l) results are related to microbial infections occurance.

CHAPTER 4: Results

Many physicians rely on their diagnosis of microbial diseases on the measurement of the concentration of CRP in the blood and they believe that gives an indication of the absolute presence of microbial infection. Although high concentrations of C-Reactive protein in the blood is used to detect any inflammation or disorders occurred within the body and not a specific medical tool to detect the microbial infections, however, many of microbial infection diagnoses depend on the measurement of the CRP. And undoubtedly the complement components are proteins in the blood that play an important role in the innate immune system in order to control the spread of pathogens that invade the body, and is also known that the lack of these proteins may lead to increased risk for microbial infections, therefore, numerous studies and modern scientific methods were proposed to measure complement components levels in the blood. This study was designed to investigate the relationship between human complement C3 and C4 deficiency and C-Reactive protein (CRP) as an indicator of microbial infection.

4.1 Analysis of complement components C3 and C4 levels according to genders.

The results obtained for C3 and C4 levels measurements on 446, In C3, the number of patients has low C3 are 51 patient, where the results show (37% for male, 63% for female). 88.5% of the recorded results showed normal of high C3 concentrations for both genders, a number of patients with the low C3 concentration in females are higher than males.

In C4 the number of samples has low C4 are 4 patients, where the results recorded (25% in female and 75% in males). 99% of the recorded results showed normal or high C4 concentrations for both genders. A number of patients with low C4 in males is higher than females. The gender type is not significant correlated with C3, C4 deficiency (P-value= 0.05)(r=+0.01). Table(1), Figure (7) show that:

Table: (1) Analysis of complement components C3 and C4 levels according to genders.

Levels of C3,C4	Low				Normal				High			
	C3		C4		C3		C4		C3		C4	
	NO.	%	NO.	%	NO.	%	NO.	%	NO.	%	NO.	%
Male	19	4.3%	3	0.7%	148	33%	133	30%	50	11.2%	81	18.1%
Female	32	7.2%	1	0.2%	160	36%	176	39.5%	37	8.2%	52	8.7%
Total	51	11.5%	4	0.9%	308	69%	309	69.5%	87	19.4%	133	26.8%

- This percentage according to total number of samples.

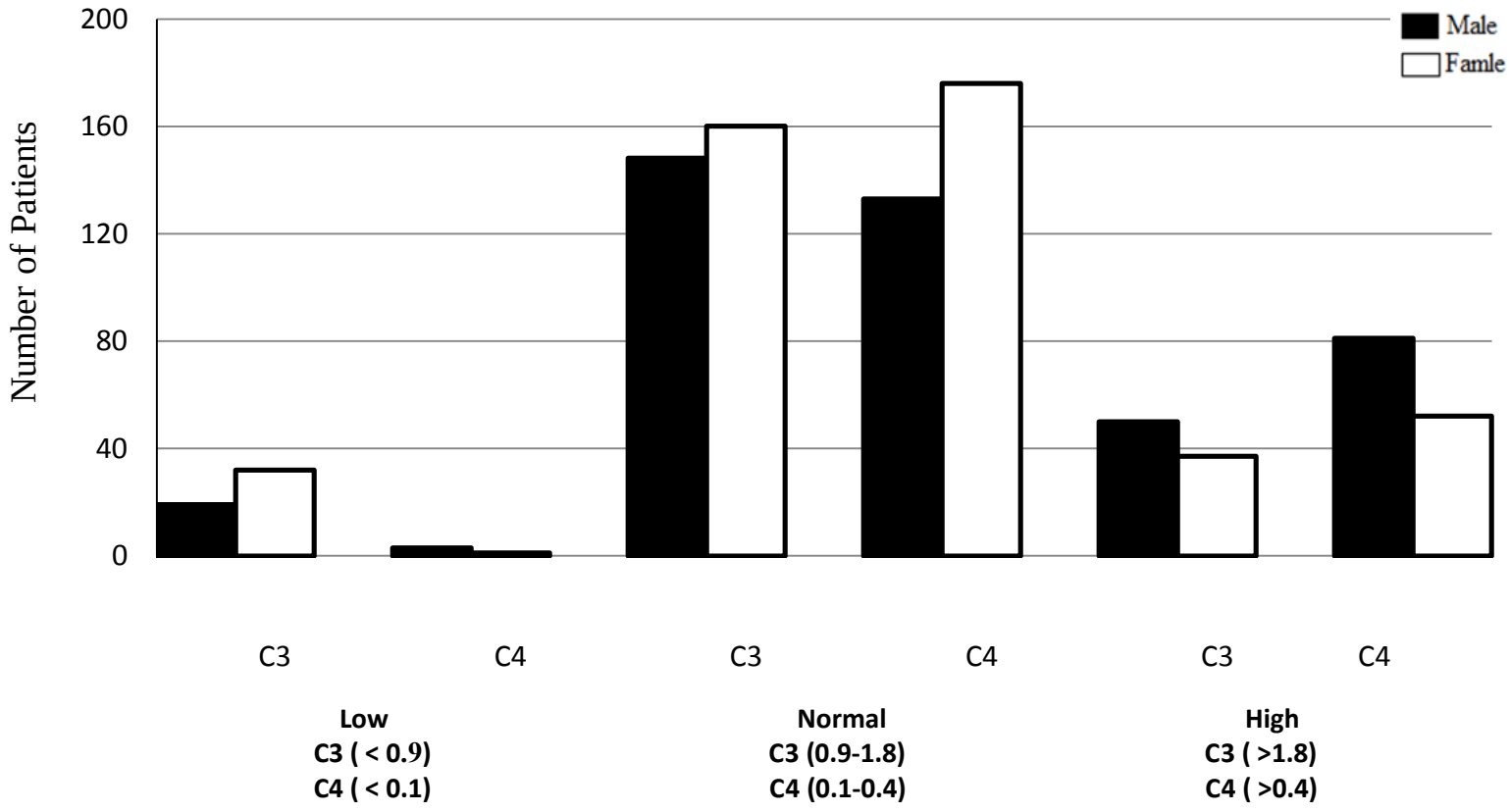


Figure:(7) Analysis of complement components C3 and C4 levels according to genders.

4.2 The distribution of complements C3 and C4 deficiency at the hospital department.

In order to investigate the distribution of complements C3 and C4 deficiency at hospital departments, 588 are the total serum samples collected from different hospital words and sections including: inpatients wards A,B, and C; Isolation department (Iso.); Intensive Care Unit (I.C.U.); Nephrology department (Neph.); New Nate department (N.N.); Gastrology department (Gast.) and surgery department (CHsw). Also, this study has included samples for an outpatient department (O.P.D.). The results of C3 and C4 deficiency measurements have reviled that, the size of the sample which has a date about hospital departments and C3, C4 results are (390). the distribution of C3 deficiency at hospital departments was variable, the C3 deficiency in females are in all departments, however, C3 deficiency in the male has just appeared in ICU, OPD, units (A, B, C), Iso. The rate of C3 deficiency in females is higher than males.

Unit (A,B,C). And I.C.U. Departments have recorded the highest rate of deficiency Unit (A,B,C) (36.3%) and I.C.U. (22.2%) respectively, the hospital units not has astrong significant correlation with C3, C4 deficiency (P- value= 0.02) (r=-0.3), (P-value=0.01) (r=-0.4) table (2), figure (8) show the distribution of C3, C4 deficiency at hospital units.

Table: (2) The distribution of complements C3 and C4 deficiency at the hospital department.

Hospital Departments	C3				C4			
Gender	**Male**		**Female**		**Male**		**Female**	
	NO	**%**	**NO**	**%**	**NO**	**%**	**NO**	**%**
Units.(A,B,C)	6	13.6%	10	22.7%	0	0%	0	0%
Iso.	4	9%	4	9%	0	0%	1	50%
Neph.	0	0%	1	2.2%	0	0%	0	0%
I.C.U.	4	9%	7	16%	1	50%	0	0%
N.N.	0	0%	1	2.2%	0	0%	0	0%
O.P.D	1	2.2%	3	6.8%	0	0%	0	0%
Gast.	0	0%	1	2.2%	0	0%	0	0%
CHsw	0	0%	2	5%	0	0%	0	0%
Total	1 5	33.8%	29	66.2%	1	50%	1	50%

• This percentages according to each result C3, C4 deficiency.

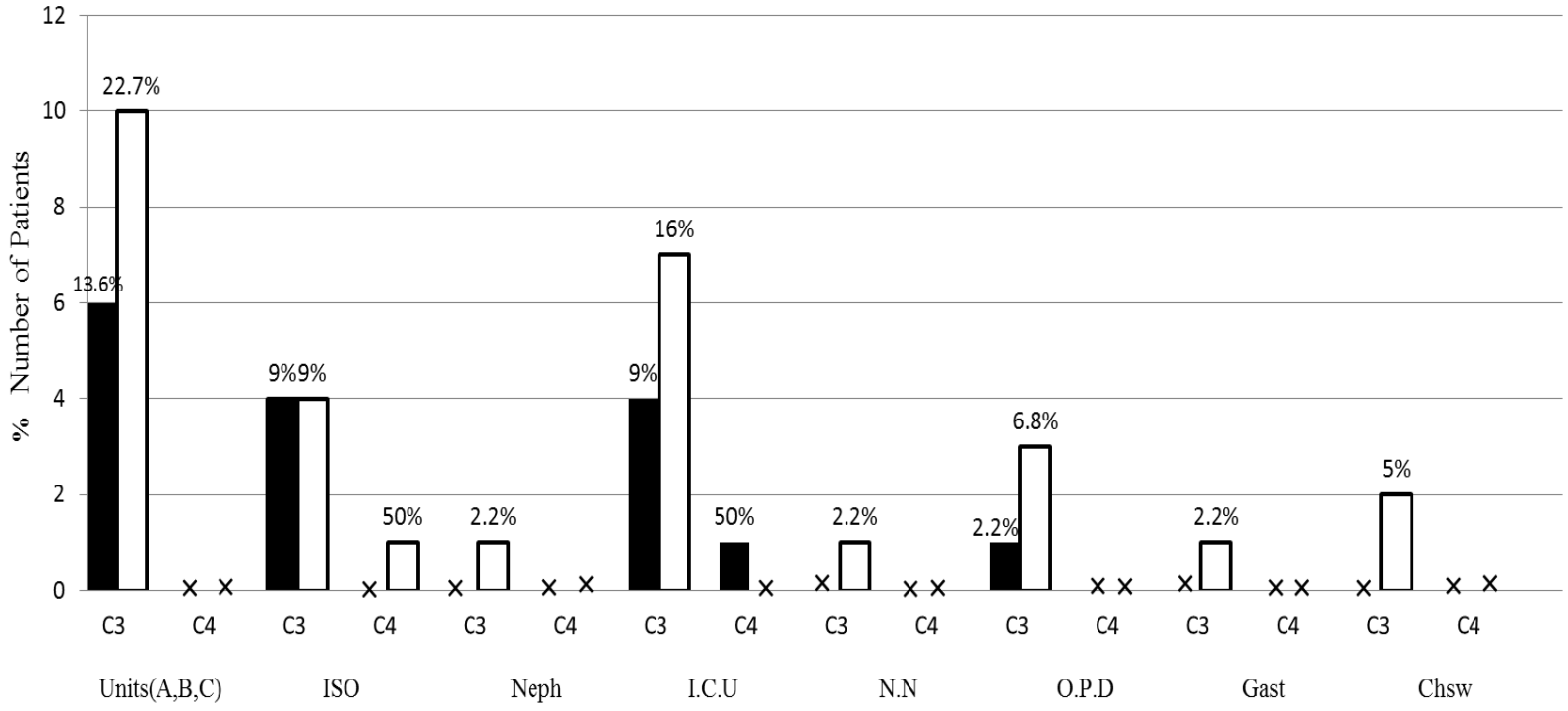


Figure: (8) The Distribution of C3 and C4 Deficiency at Hospital Departments

The distribution of complement C4 deficiency at hospital departments has did not show C4 deficiency in all hospital departments with the exception of the isolation and I.C.U. departments were there was deficiency in just one patient in each unit. Table (2) and figure (8). The results of total percentage of C3 and C4 deficiency for all departments shows that, the deficiency with C3 (11.1%) was much higher than C4 (0.7%).Table (3), Figure (9).

Table: (3) Total Percentage of C3,C4 Deficiency

Type of measurement	C3	C4
Percentage of Deficiency	11.1%	0.7%

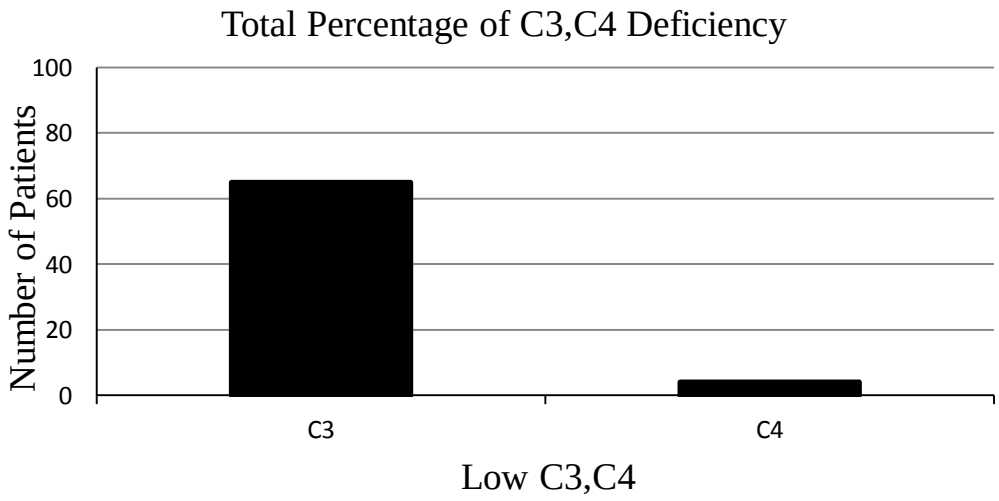


Figure: (9) Total Percentage of C3 and C4 Deficiency

4.3 Analysis of C-Reactive protein test (CRP) in hospital departments according to the gender.

In order to investigate the microbial presence and pathogenesis in the body, many routinely microbial diagnosis relies on the increase of inflammation, and the inflammation can be detecting by using C-Reactive Protein test. Therefore CRP test was used as an indicator for microbial infection.

To test the CRP levels in the serum, 299 random serum samples were collected as a second portion of the serum that used to measure the complements C3 and C4 complement levels. The tested samples were collected from different hospital words and sections including inpatients words A,B and C, Iso., I.C.U., Neph., N.N., Gast., Chew., O.P.D. The results of CRP elevation (positive CRP) have revealed that, the distribution of positive CRP test was variable, the CRP elevation in females are in all departments, CRP elevation in males has not appeared in Gast. and CHsw.

Department have recorded the highest rate of elevation for both genders unit(A,B,C) are 41 male (24.5%) and 38 female (23%). table(4), figuer(10) show that:

Table: (4) Distribution of C-Reactive protein test (CRP) in hospital departments according to the gender.

	(+ve) CRP				(-ve) CRP			
Gender	**Male**		**Female**		**Male**		**Female**	
	NO.	**%**	**NO.**	**%**	**NO.**	**%**	**NO.**	**%**
Units(A,B,C)	41	24.5%	38	23%	38	28.8%	27	20.4%
Iso.	13	7.8%	13	7.7%	7	5.3%	3	2.2%
Neph.	4	2.4%	0	0%	3	2.2%	5	3.8%
I.C.U.	15	9%	21	12.5%	10	7.5%	11	8.3%
N.N.	4	2.4%	1	0.6%	2	1.5%	7	5.3%
O.P.D.	10	6%	5	2.9%	6	4.5%	11	8.3%
Gast.	0	0%	1	0.6%	0	0%	0	0%
CHsw.	0	0%	1	0.6%	1	0.7%	1	0.7%

- This percentage according to each CRP result.

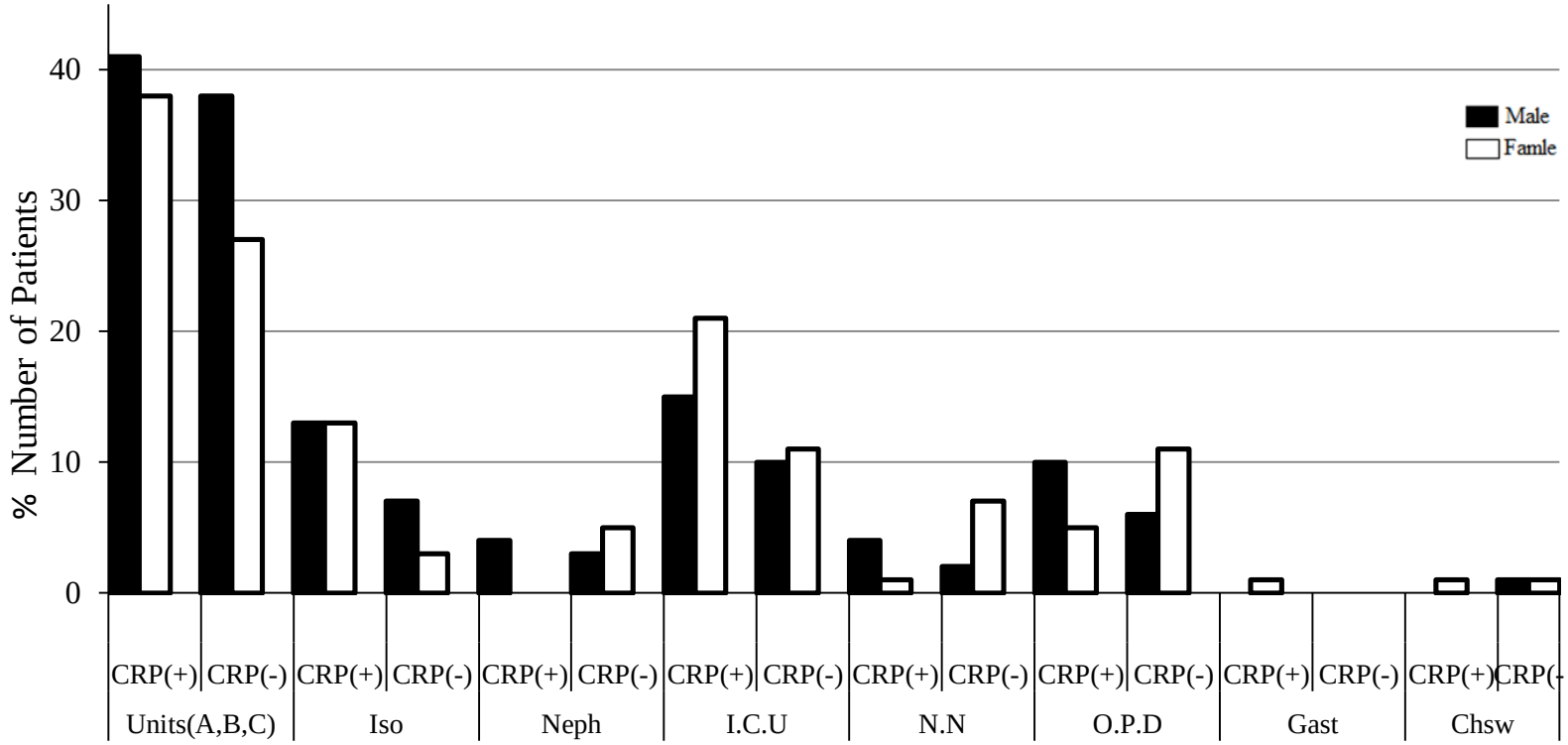


Figure: (10) Distribution of C-Reactive Protein (CRP) in Hospital Departments According to The Gender

4.4 Analysis the Association between C-Reactive Protein and C3,C4 Component Levels.

To determine the ability to increase of microbial infection, Complements C3 and C4 were measured in comparison with the increase of CRP concentration. 324 are the total samples that used to investigate the interaction between the increase of CRP levels in the blood and Complements C3 and C4 concentrations were represented as (low- Normal- High) and CRP test represented as (positive and negative).

The results of C3 measurements showed that all CRP levels were variable, the samples that detected low C3 and (+ve) CRP are (8.6%), where as samples have low C3 and (-ve) CRP were (5%). table (5), figure (11) show that: The results of C4 measurments have revealed that, most of the serum samples showed normal and high levels of C4 concentrations. For both genders, only two patients were having low C4 component and positive CRP.table (6), figure (12) show that:

However, normal levels of C3 and C4 with (+ve) CRP showed the highest rate of total percentage (35.2% and 40%) respectively.

The statistical analysis for correlation between C3, C4 deficiency and positive (CRP) : the correlation is significant, conversely relationship (p-value=0.00, r=-0.8) (p-value=0.00, r=-0.57) respectivly for C3,C4.

Table: (5) Association between C-Reactive Protein and C3 Component Levels

C3 Levels	LOW (<0.9 g/l)				NORMAL (0.9 - 1.8 g/l)				HIGH (>1.8 g/l)			
Gender	**M**		**F**		**M**		**F**		**M**		**F**	
	NO	**%**	**NO**	**%**	**NO**	**%**	**NO**	**%**	**NO**	**%**	**NO**	**%**
(+ve) CRP	9	2.8%	19	5.9%	62	19.1%	52	16%	26	8%	18	5.5%
(-ve) CRP	6	1.8%	10	3%	46	14.2%	51	15.7%	16	4.9%	9	2.8%
Total	15	4.6%	29	9%	108	33.3%	103	31.8%	42	13%	27	8.3%

- This percentage according to C3 and CRP Tests.

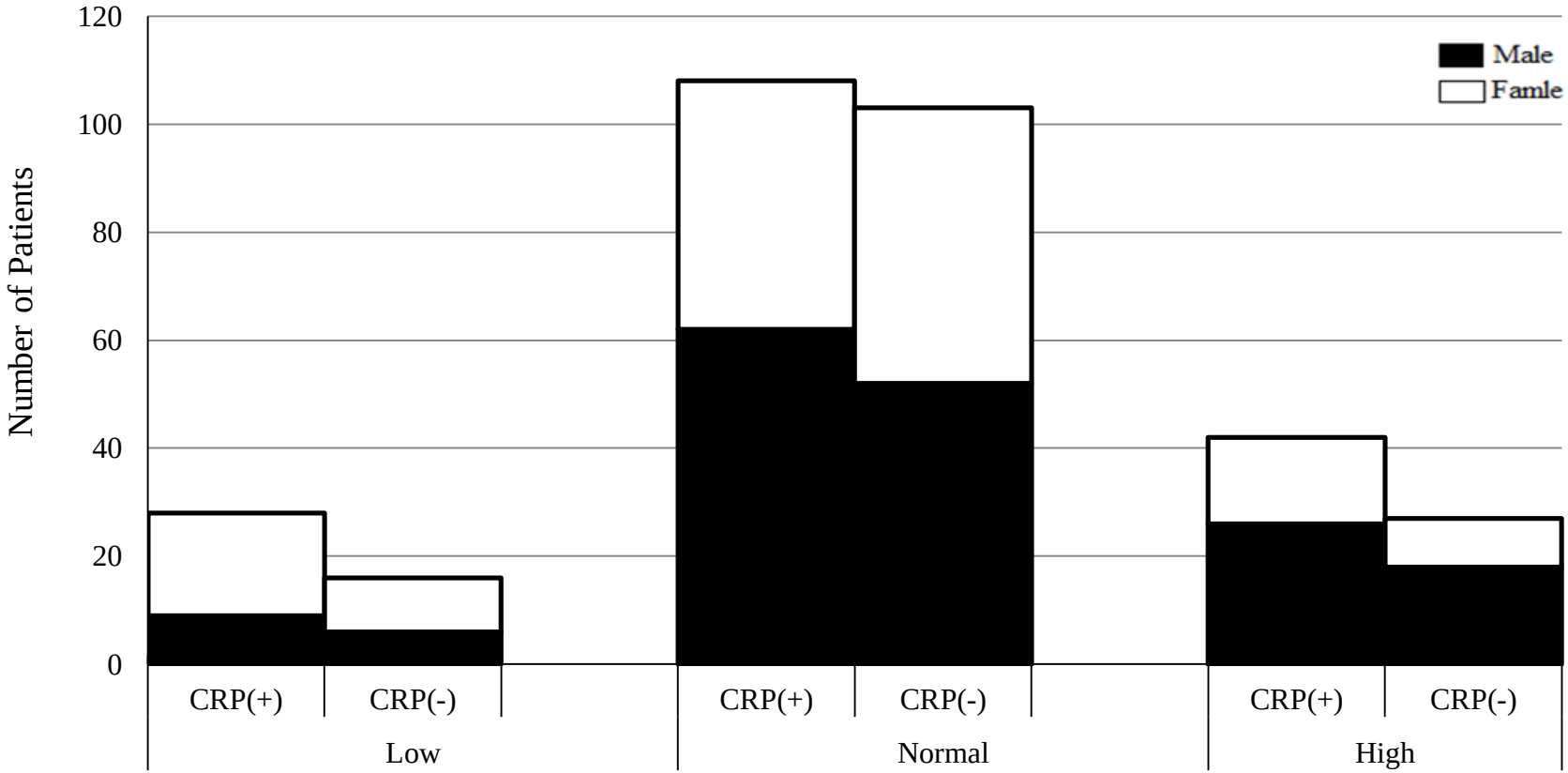


Figure: (11) Association between C-Reactive Protein and C3 Component Levels

Table: (6) Association between C-Reactive Protein and C4 Component Levels.

C4 Levels	LOW (<0.1 g/l)				NORMAL (0.1 – 0.4 g/l)				HIGH (>0.4 g/l)			
Gender	**M**		**F**		**M**		**F**		**M**		**F**	
	NO	**%**	**NO**	**%**	**NO**	**%**	**NO**	**%**	**NO**	**%**	**NO**	**%**
(+ve) CRP	1	0.3%	1	0.3%	63	19.4%	67	20.6%	33	10.1%	21	6.7%
(-ve) CRP	1	0.3%	0	0%	41	12.6%	56	17.3%	26	8%	14	4.3%
Total 0f number of percentage	2	0.6%	1	0.3%	104	32.1%	123	38%	59	18.2%	35	10.8%

- This percentage according to C4, CRP tests.

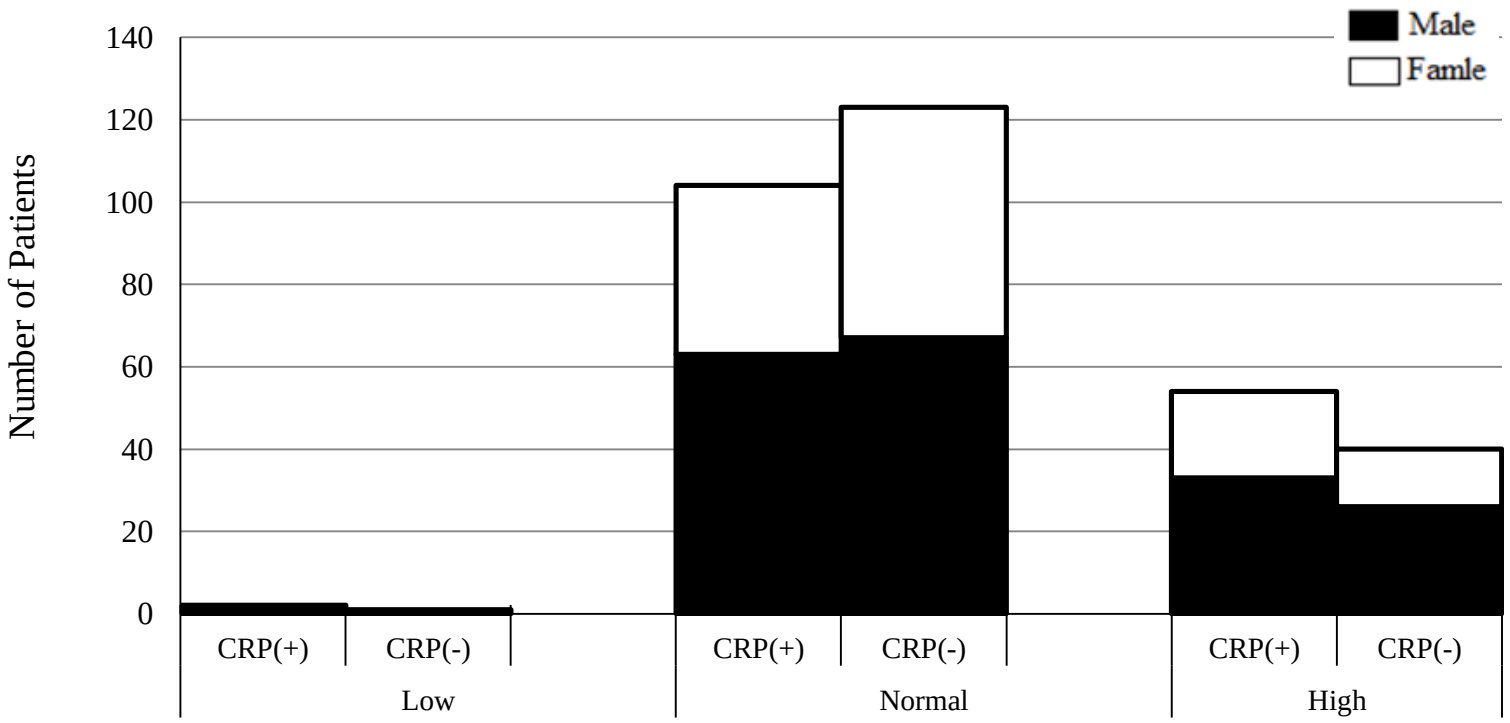


Figure: (12) Association Between C-Reactive Protein and C4 Component Levels.

4.5 Distribution of Low C3 and C4 Vs Positive CRP at Hospital departments.

Because of the positively of CRP as an indicator for infections, 299 samples from the different hospital words and have +ve CRP was introduced to investigate the interaction between C3 and C4 deficiencies and positive CRP. The results showed that the highest hospital words and departments that involved in low C3 and +ve CRP are I.C.U., Units A, B, C, Isolation department (43.5%, 34.8%, 13%) respectively. Where as C4 deficiency and +ve CRP appeared just in 1 patient in Isolation department. Table (7), Figure (13) show that:

Table: (7) Distribution of Low C3 and C4 Vs Positive CRP at Hospital departments.

	Low C3 Vs (+ve) CRP		Low C4 Vs (+ve) CRP	
	NO.	**%**	**NO.**	**%**
Units(A,B,C)	8	34.8%	0	0%
Iso.	3	13%	1	100%
Neph.	0	0%	0	0%
I.C.U.	10	43.5%	0	0%
N.N.	0	0%	0	0%
O.P.D.	0	0%	0	0%
Gast.	1	4.3%	0	0%
CHsw.	1	4.3%	0	0%
Total	23	100%	1	100%

- This percentages accourding to CRP test

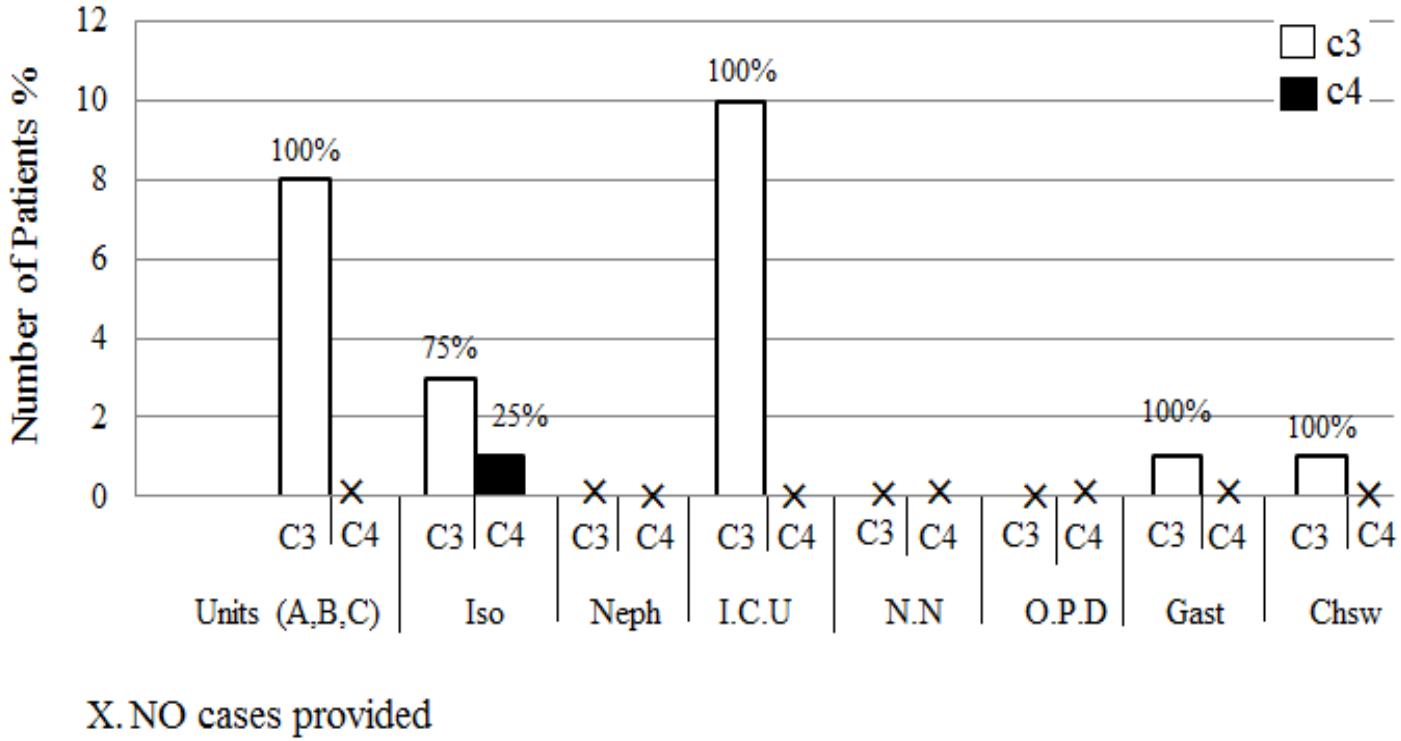


Figure: (13) Distribution of Low C3 and C4 Vs Positive CRP at Hospital departments.

CHAPTER 5: Discussion

It is clear that infections lead to increase of the mortality and morbidity rates, so that it is nessesary to establish a new reliable recognition test to detect and identify infection diseases. From the routine laboratory tests which acting as indicators for microbial infection are; WBCs acounts, (ESR) erythrocyte sedimentation rate, (PCT) Procalcitonin, (CRP) C-Reactive protein, (SAA) Serum amyloid A, Plasma viscosity.

CRP production is part non specific acute-phase response of most forms of infections, infection and tissue damage, increase of CRP in serum used clinically as amarker of chronic inflamatory disorder, and used for monitaring autoimmune diseases and infections (Pepys, *et al.* 2002). Also, it has been shown that CRP has an important role in host defence by opsonization and complements action (Szalai, *et al*. 2002).

Because of CRP test availability and low cost, phesicent in children hospital used as routeinly relaible test to investigate of abroad of infecious diseases and inflamations.

CRP well known kinetics, it has been used to detect septic infections.Other scientist have attempted to evaluate the role of CRP in the diagnosis of sepsis in different patients (Van Gestel, *et al.* 2004).

The analysis of CRP in children hospital demonstrated that, the hospital words (A,B, and C), Iso and ICU departments showed the highest rate in CRP concentrations, and this is quite expected because of the medical conditions of the patients.

The measurements of CRP concentration according to the gender were variable at all hospital departments.

The increase of CRP in blood serum of the admeted patients in ISO and ICU could be used to predect bacteraemia. This is come as aresults of astudy showed that, CRP and inflamations can be used as amonter for bacteramia during 48 hours of fever in neutropenic patients (Presson, *et al.* 2005).

Huang, *et al*. (2000), investigated 123 acutely ill ED patients with suspected infection and reported that a combination of CRP and WBC count provided a satisfactory predictive value for infection. In this investigation, however, only patients with specific complaints, including fever, chills, or acute distress such as dyspnea, chest pain, consciousness change, abdominal pain, arthralgia, or upper gastrointestinal bleeding, were investigated. Many

patients were dropped from the investigation because their CRP levels were unchecked, either because the patients had no fever or seemed uninfected, or because the diagnosis of infection was straightforward. The investigation's results might, therefore be hampered by selection bias and sampling error and should be interpreted with caution.

Measurments of complement components C3 and C4 has provided initial image of complement concentration among patients, Arround 11.4% of patients have low concentrations of C3, which make them were suscebtable to be infected. Studies has aproved that deficiency of C3 component have a corellation with both invasive infection (meningitis, bacteremia, pneumonia) and related with autoimmune disorders (Agrawal, 2014).

In our study using C3, C4 deficiency also as a marker to microbial infections which demonstrated with many previous studies related to meningococcal disease (Lisa and Sanjay, 2014), Aspergillosis (Speth and Rambach, 2012), increasing susceptibility to recurrent Neisserial infectious (Hanna, *et al*. 2005), increasing to susceptibility to recurrent respiratory infections and infections caused by encapsulated organisms as*: S. Pneumoniae, N. meningitidis,* and *H. Influenzae.*

The patients have showed deficiency in complements also showed 11% of increase in CRP levels and this support increase the probability of infections diseases and inflamation.

This study demonstrated that, found the relation between CRP as an indicator to microbial infection and complement proteins deficiency. There are a few studies about it. In Libya, there is no previous study included. a few knowledge of prevalence of C3 and C4 deficiencies and study of microbial isolation may assist physicians to make a clear image on the posibility of increase of microbial infection.

Conclusion:

With increasing of microbial diseases and spreading it, from importance depending on perfect test acting as an indicator of microbial infection in the body, in this study looking for complement proteins as an indicator of microbial infection founded. The previous studies indecated to deficiency of complement proteins in the blood related to microbial infections occurrence compared with indicators as body temperature, WBC account, lymphocytes accounts, and many tests, in this study demonstrated this relation by using C-Reactive Protein (CRP) test as an indicator of microbial infection in children of children hospital of BENGHAZI LIBYA.

Where this study included 588 sample, from all hospital units to recognize of complement proteins deficiency percentage among children, and this deficiency indicator to microbial infection occurence. This study indicated the deficiency percentage of complement proteins C3 and C4 are (11.1%) (0.7%) respectively, the females have deficiency percentage more than males. Also demonstrated the relation between complement proteins deficiency and CRP elevation is a strong relationship (p-Value=0.00), which gave the confidence to using these proteins as an indicator to microbial infection diagnosis.

الملخص:

مع تزايد الأمراض الميكروبيه وانتشارها فمن الضروره الإعتماد على إختبار مناسب يعمل كمؤشر دقيق لوجود الإصابه الميكروبيه، تمت الإشاره في هذه الدراسه للبروتينات المكمله في الدم وأهميتها كمؤشر لوجود الإصابه الميكروبيه في الجسم.

أشارت الدراسات السابقه ان نقص البروتينات المكمله في الدم مرتبط بوجود الأمراض الميكروبيه مقارنة بمؤشرات كإرتفاع درجة الحراره وزيادة عدد كرات الدم البيضاء والخلايا الليمفاويه وعدة إختبارات اخرى فأجرينا هذه الدراسه لدراسة هذه العلاقه باستعمال اختبار ال C-Reactive Protein (CRP) كمؤشر لوجود الإصابه الميكروبيه عند الاطفال بمستشفى الاطفال في بنغازي – ليبيا.

حيث شملت الدراسه على 588 طفل لجميع وحدات المستشفى ، لمعرفة مدى نسبة النقص للبروتينات المكمله C3 و C4 بين الأطفال، ومعرفة ما إذا كان نقص هذه البروتينات مؤشرا على وجود الإصابه الميكروبيه.

واتضح أن نسبة النقص للبروتينات المكمله C3, C4 كانت (11.1%) و(0.7%) على التوالي . وكانت الإناث الأكثر إصابة بالنقص من الذكور، كما إن الدراسة أكدت أن العلاقه بين نقص البروتينات المكمله وارتفاع معدل ال CRP بأنها علاقه قويه جدا، وهذا ما يعطي الثقه بإستخدام البروتينات المكمله كدليل لتشخيص وجود الإصابه الميكروبيه.

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جامعة بنغازي **كلية العلوم**

قسم علم النبات

بحث مقدم لكلية العلوم بجامعة بنغازي كجزء من متطلبات الحصول على درجة الاجازه العاليه الماجستير في علم النبات بعنوان :

دراسة العلاقه بين نقص المكونات المكمله للانسان C3 و C4 وإرتفاع معدل البروتين التفاعلي C (CRP) كمؤشر لزيادة الإصابات الميكروبيه

مقدم من الطالبه : منى المهدي المبروك القطيط

تحت إشراف : د. إسماعيل حسين بوزعكوك

خريف 2017

جامعة بنغازي

كلية العلوم

قسم النبات

رسالة بعنوان:

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مقدم من الطالبه:

منى المهدي المبروك القطيط

اللجنه المشرفه : التوقيع:

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د. إسماعيل حسين بوزعكوك. **----------**

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معتمده من:

د. علي محمد البكوش. **د.حسين البرعصي.**

(رئيس قسم النبات) **(عميد كلية العلوم)**

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MUNA .ELMAHDI .E. ELGUTAIT

Study The Relation Between Human Comlement Components C3 and C4 Deficiency and Elevation of C-Reactive Protein (CRP) as an Indicator of Increase Infections.