بسم الله الرحمن الرحيم

كلية نبتة
NAPATA COLLEGE

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Napata College

Medical Laboratory program

Department of Microbiology

Determine C-Reactive protein as a Biomarker For the severity of Bacteria Infections in Younger and Older Sudanese Patients

تحديد بروتين سي التفاعلي كمؤشر حيوي لشده العدوى البكتيريه لدي المرضي السودانين الصغار وكبار السن

A graduation project submitted to Napata College in partial of fulfillment of BSc. Degree (Honor) in Microbiology

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الأيه

بسم الله الرحمن الرحيم

أَمَّنْ هُوَ قَانِتٌ آنَاءَ اللَّيْلِ سَاجِدًا وَقَائِمًا يَحْذَرُ الآخِرَةَ وَيَرْجُو رَحْمَةَ رَبِّهِ قُلْ هَلْ يَسْتَوِي الَّذِينَ يَعْلَمُونَ وَالَّذِينَ لا يَعْلَمُونَ إِنَّمَا

(يَتَذَكَّرُ أُولُواْ الأَلْبَابِ)

.[سورة الزمر:9]

DEDICATION

Firstly, we dedicated this thesis to Allah the Almighty, thank you for the guidance, strength, power of mind, protection and skills and for giving us healthy life.

Secondly we would like to dedicate this Work to our family to our wonderful friends who really helped us through tough times, and also we would like to thank whom they gave up their rest to raise us, to our teachers.

Acknowledgement

We would like to give our warmest thanks to our supervisor ***Dr. Abdalkalig Elkhider*** who made this work possible. Guidance and advice carried us through all the stages of writing our project.

With all our pleasure and gratitude, I would like to thanks ***Dr. Reel Gamal*** for her greatsupport and no lasting support and encouragement.

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ABSTRACT

Background :C-reactive protein (CRP) is frequently utilized in younger populations, although its efficacy in suspecting of bacterial infections in elderly populations is less clear. This study looked at the effectiveness of serum CRP levels in the suspecting of bacterial infections in elderly adults and younger patient at an early stage.

Method :In a prospective cross-sectional study from July to September 2022, 50 serum and various samples were taken from patients at a private military hospital in Omdurman, Sudan, including blood, urine, wound swabs, throat swabs, and sputum. Cobas c 311 used the quantity method to measure the serum C-RP level and collect samples for culture and processing according to stander bacteriological techniques.

Result :A total of 50 patients 27 male 54% and 23 female 46% were recruited over a period of 3 months with ratio of (1:1.7). CRP levels in patients less than 20 years show 56.5±50.8mg/l (mean±SD), in 20-40 years 100.7±66.5 mg/l (mean±SD), in 41-60 years show higher CRP level 124.87±81.8 mg/l (mean±SD), in More than 60 years 128.1±94.8 (P-value 0.467), higher CRP was found in Respiratory tract infection by Ps.aeruginose bacteria and we found no association between the result of C-RP and demographic data across the younger and older groups (Age group), also we conclude that there is no association between the result of C-RP and males and females (Sex group).

Conclusion: In this study CRP level alone as biomarker indicator of inflammation and infection cannot use alone to suspect the type of bacterial infection, and there were no significant different in CRP level as response to bacterial infection in different patients ages and genders of patients.

الأطروحه

الخلفيه : كثيرا ما يستخدم بروتين سي التفاعلي في الفئات العمريه الأصغر سنا، على الرغم من أن فعاليته في تحديد الألتهابات البكتيريه لدي كبار السن أقل وضوحا . نظرت هذه الدراسه في فعاليه بروتين سي التفاعلي في مصل الدم في تحديد الألتهابات البكتيريه لدي كبار السن وصغار السن في مرحله مبكره

الطريقه :- في دراسه مقطعيه مستقبليه من يوليو الى سبتمبر 2022 ، تم أخذ 50 مصل وعينات مختلفه من المرضي في مستشفي عسكري خاص في أم درمان ، السودان بما في ذلك عينه (دم – بول – مسحات جروح – مسحات حلق – و بلغم) أستخدم جهاز الكوباس سي 311 لقياس بروتين سي التفاعلي وجمعت العينات للتزريع

النتيجه :- تم جمع 50 مريض 27 ذكور 54% و23 اناث 46% خلال فتره 3 أشهر ، تظهر مستويات بروتين سي التفاعلي في الفئه العمريه الأقل من 20 عام وسط الحسابي +_ الانحراف المعياري 50 $^{+-}$ 56.5 (8. مجم / لتر في الفئه العمريه 20-40 عاما , وسط الحسابي $^{+-}$الأنحراف المعياري 66.5 $^{+-}$ 100.7 (ومن 40 – 60 عام مجم / لتر) ووسط حسابي $^{+-}$ الانحراف المعياري , 81.8 $^{+-}$124.87 (أكثر من 60 عام مجم / لتر) ووسط حسابي $^{+-}$ الانحراف المعياري 94.8 $^{+-}$ 128.1 (نستنتج أن بروتين سي التفاعلي ليس له ارتباط بين البيانات الديموغرافيه عبر الفئات الأصغر والأكبر سنا (الفئه العمريه) كما نستنتج أنه لايوجد ارتباط بين نتيجه البروتين وبين الذكور والأناث (مجموعه الجنس)

الخلاصه :- في هذه الدراسه لا يمكن أستخدام مستوي البروتين سي التفاعلي وحده كموشر للعلامات الحيويه للالتهاب والعدوي للأشتباه في نوع العدوي البكتيريه ، ولم يكن هنالك اختلاف كبير في مستوي بروتين سي التفاعلي استجابه للعدوي البكتيريه في مختلف أعمار المرضي وأجناسهم

Table of contents:

CHAPTER ONE

CHAPTER ONE

INTRODUCTION AND LITERATURE REVIEW

Introduction:

Bacterial infection and multi drug resistance are a major cause of morbidity and mortality in the older and younger population in hospitals. The older patient is generally susceptible to infections because of a decline in host defense mechanisms that occurs with ageing, and concomitant medical co- morbidities. [2]

C-reactive protein (CRP) is an acute-phase protein that serves as an early marker of inflammation or infection. The protein is synthesized in the liver and is normally found at concentrations of less than 10 mg/L in the blood. During infectious or inflammatory disease states, CRP levels rise rapidly within the first 6 to 8 hours and peak at levels of up to 350–400 mg/L after 48 hours. [3]

CRP binds to phosphocholine expressed on the surface of damaged cells, as well as to polysaccharides and peptosaccharides present on bacteria, parasites and fungi. This binding activates the classical complement cascade of the immune system and modulates the activity of phagocyte cells, supporting the role of CRP in the opsonization (i.e. the process by which a pathogen is marked for ingestion and destruction by a phagocyte) of infectious agents and dead or dying cells. When the inflammation or tissue destruction is resolved, CRP levels fall, making it a useful marker for monitoring disease activity. [1]

CRP is not the only inflammatory biomarker that has been shown to predict myocardial infarction and stroke. More sophisticated measures of cytokine activity, cellular adhesion, and immunologic function (such as interleukin-6, intercellular adhesion molecule-1, macrophage inhibitory cytokine. [4]

Other factors that associate with elevated CRP include pulmonary embolism, deep vein thrombosis, myocardial infarction, malignancies, rheumatoid arthritis and autoimmune diseases (including vasculitis). Interleukin 6 (IL-6) is thought to be the main mediator stimulating CRP production, but other cytokines like IL-1 and tumor necrosis factor are also involved Changes of plasma CRP levels have been shown to be useful in the diagnosis of infection and in the follow-up of the clinical course of infection, with a fall in CRP usually accompanying resolution of the disease. [2]

The physiology of inflammatory response is modified by the aging process and is substantially affected by multimorbidity and disability. Infection is the most frequent cause of acute inflammation in both adult and older subjects. C-reactive protein (CRP) is the most used biomarker of inflammation, and a substantial amount of literature has demonstrated its importance and clinical usefulness in adult subjects. However, the clinical significance of serum CRP determination has not been completely clarified in older subjects with acute infection, especially in the light of the age-related rearrangements in immunity and cytokine production. Thus, in the present review, we focus on the existing knowledge about serum CRP level interpretation in geriatric patients hospitalized with acute infection. . [5]

Low-grade inflammation, a minor elevation in the baseline concentration of inflammatory markers such as C-reactive protein (CRP), is nowadays recognized as an important underlying condition in many common diseases. Concentrations of CRP under 10 mg/l are called low-grade inflammation and values above that are considered as clinically significant inflammatory states. Epidemiological studies have revealed demographic and socioeconomic factors that associate with CRP concentration; these include age, sex, birth weight, ethnicity, socioeconomic status, body mass index (BMI), fiber consumption, alcohol intake, and dietary fatty acids. . [7]

Interleukin (IL)-20 and IL-22 belong to the IL-10 family. IL-10 is a well-documented anti-inflammatory cytokine while IL-22 is well known for epithelial protection and its antibacterial function, showing great therapeutic potential for organ damage; however, the function of IL-20 remains largely unknown. [8]

the resolution of pulmonary bacterial infections involves a finely orchestrated balancing act of proinflammatory and antiinflammatory cytokines. On initial encounter with deposited bacteria, resident alveolar macrophages become activated and secrete proinflammatory cytokines and chemokines, resulting in the eventual generation of a proinflammatory amplification loop between resident or recruited macrophages or polymorphonuclear neutrophils and lymphocytes. . [6]

High C-reactive protein (CRP) values are frequently found in patients with bacterial respiratory infection, and CRP testing has been shown to be useful in differentiating pneumonia from other respiratory infections. Raised CRP values may also be found in viral

respiratory infection, and as a result there is a risk that antibiotics may be wrongly prescribed. . [9]

Literature review

1.2.1Bacterial infection

Bacteria are prokaryotic organisms that carry their genetic information in a double-stranded circular molecule of DNA. Some species also contain small circular plasmids of additional DNA. The cell cytoplasm contains ribosomes and there is both a cell membrane and, in all species except Mycoplasma, a complex cell wall. External to the cell wall, some bacteria have capsules, flagella, or pill. Bacteria normally reproduce by binary fission. Under the proper conditions, some bacteria can divide and multiply rapidly. Bacteria are classified as Gram-positive or Gram-negative based on the characteristics of their cell wall, as seen under a microscope after stains have been administered, a procedure called Gram staining that was developed in 1882 by Hans Christian GramClinically, one of the main differences between gram-positive and gram-negative organisms is that gram-negative bacteria tend to produce an endotoxin that can cause tissue destruction, shock, and death. The two classes of bacteria differ in their antibiotic susceptibilities as well. Bacteria can also be classified based on their growth responses in the presence and absence of oxygen. Aerobic bacteria, or aerobes, grow in the presence of oxygen. Anaerobic bacteria such as the Clostridia are able to grow in the absence of oxygen and obligate anaerobes require its absence. Some bacteria are not classified as Gram-positive or Gram-negative. Some bacteria are not classified as Gram-positive or Gram-negativethese include the mycobacteria, of which Mycobacterium tuberculosis is the most well-known, which can be seen under the microscope using a special stain called the acid-fast stain organisms that do not take up Gram stain such as the spirochetes and the Rickettsia [10]Bacterial infections is a common clinical disease that can affect a variety of organs and tissues [11]

C-RP history

C-reactive protein is a homopentameric acute-phase inflammatory protein, a highly conserved plasma protein that was initially discovered in 1930 by Tillet and Francis while investigating the sera of patients suffering from the acute stage of *Pneumococcus* infection and was named for its reaction with the capsular (C)-polysaccharide of *Pneumococcus*[12]C-reactive protein (c-RP) is a positive inflammatory protein easily measurable in

plasmabyhighlysensitiveassaysandsynthesizedbyKupffercellsintheliverinresponseto an acute inflammatory event [13-14]Serum CRP level can be affected by a lot of factors, such as sex, age, and systolic blood pressure[15-16] Functionally, CRP was also the first identified pattern-recognition receptor (PRR) [17-18]

Urinary tract infection

Urinary tract infection is one of the most common bacterial infections, and the incidence in women is much higher than in men. Most UTIs are uncomplicated UTIs, defined as cystitis in a woman who is not pregnant, is not immune compromised has no anatomical and functional abnormalities of the urogenital tract, and does not exhibit signs of tissue invasion and systemic infection. All UTIs that are not uncomplicated are considered to be complicated UTIs. Differentiation between uncomplicated and complicated UTIs has implications for therapy because the risks of complications or treatment failure are increased for patients with a complicated UTI. Febrile UTIs are rue sepsis pyelonephritis, and prostatitis[19]an uncomplicated urinary tract infection UTI is a bacterial infection of the bladder and associated structures [20]*Escherichia coli* (E coli) is the most common cause of uncomplicated UTI [21] other bacteria associated with urinary tract infections includes Proteus spp, Enterococcus, Pseudomonas, Enterobacter, Serratia and Candida spp[22]

Septicemia

Septicemia Septicemia (or sepsis) is a systemic inflammatory response syndrome in response to the presence of pathogenic organisms or their toxins in the blood or tissues.1 Septicemia is often caused by infection by bacteria such as *Staphylococcus auras* (or sepsis) is a systemic inflammatory response syndrome in response to the presence of pathogenic organisms or their toxins in the blood or tissues Septicemia is often caused by infection by bacteria such as *Staphylococcus aurous*Bloodstream infections (BSIs) are one of the most common infections seen in all age groups and in all locations [23]

Wound infection

The skin is the human body's largest organ, colonized by a diverse milieu of microorganisms, most of which are harmless or even beneficial to their host. Colonization is driven by the ecology of the skin surface, which is highly variable depending on topographical location, endogenous host factors and exogenous environmental factors. The cutaneous innate and adaptive immune responses can modulate the skin macrobiotic, but the

macrobiotic also functions in educating the immune system. The development of molecular methods to identify microorganisms has led to an emerging view of the resident skin bacteria as highly diverse and variable[24] wounds all have bacterial contamination, which will not impair healing. Wound contamination must be distinguished from wound colonization and infection. Bacterial infection in wounds depends on the number of organisms present, their virulence, and host resistance [25] The most common bacteria association with Wound infection is *Staphylococcus aurous*, *Pseudomonas aeruginosa Proteus mirabilis*, *Escherichia coli* and *Corynebacterium*spp[26]

Respiratory Tract Infection

Pneumonia

Pneumonia medically it is an inflammation of one or both lungs parenchyma that is more often, but not always, caused by infections. The one causes of pneumonia bacteria(27) Pneumonia is one of the major infectious diseases responsible for significant morbidity and mortality throughout the world(28) Pneumonia can be classified into 2 types based on how the infection is acquired Community-acquired pneumonia Most common type and Nosocomial pneumonia The bacteria which cause pneumonia are S*treptococcus pneumonia*, *Staphylococcus auras*, Group A *Streptococcus*, *Klebsiellapneumoniae*, *Homophilesinfluenza*, *Moraxella catarrhali*sis, anaerobes, and other gram-negative and gram positive organisms[29].

Tonsillitis

Tonsillitis is inflammation of the tonsils, two oval-shaped pads of tissue at the back of the throat one tonsil on each side. Most cases of tonsillitis are caused by infection with a common virus, but bacterial infections also may cause tonsillitis[30] the most common causes of tonsilits is β-hemolytic streptococci[31].

Previous Study

1-A second C-reactive protein (CRP) test to detect inflammatory burst in patients with acute bacterial infections presenting with a first relatively low CRP in the Sour sky Tel-Aviv Medical Center(Israel)

A cross sectional study was conducted between July 2007 and March 2016 The study included 950 patients who were diagnosed as having an infection, assumed to be of bacterial etiology (cellulitis and erysipelas, pneumonia, pyelonephritis, or septicemia) who had a CRP

test during the first 6 hours of hospital admission (baseline CRP), and a successive CRP test up to 12 hours from the first one (recurrent CRP). The control group was of healthy subjects who attended to medical center for a routine annual check-up.Baseline CRP ranged from 0.04 to 454 mg/L. The median CRP velocity was 0.53 mg/L/h. Patients were grouped by baseline CRP into 4 groups (CRP < 10, 10–74.9, 75–199.9, ≥200). There was an increase in median CRP velocity between the first (0.48 mg/L/h) and the second (0.93 mg/L/h) groups, which then was decreased in the next 2 groups (0.46 and −2.58 mg/L/h, respectively). In 45 of 103 (44%) patients of the group of baseline CRP concentration less than 10 mg/dL with bacterial diagnosis, there was a complete overlap with CRP values of apparently healthy individuals during their routine annual checkup**.** a first single low CRP result cannot exclude the presence of a significant bacterial infection. Patients with acute bacterial infection might present with a relatively low CRP value that at times correspond to normal limit CRP concentrations. A second test, obtained within 12 hours of admission, might serve as an important tool to identify patient with an evolving inflammatory burst commonly seen during acute bacterial infection. [32]

2- Study was conducted during the period from May 2022 to August 2022 in Baraah pediatric center, Khartoum, Sudan50 patients were selected as a case group (46% were female and 54% were male.)and healthy donors were selected as control groupProtein C results revealed the mean of protein C in case was (63.7±8.9), and in the control group was(79.8±11.1), when compared protein C mean between case and control groups there was a highly significant decreased with (p value 0.00) Also in the case group there was an insignificant differences between protein C, age, gender, and culture, p value >0.05), . In addition the result showed insignificant correlation between protein C and CRP(p value ≥0.05). The most common types of isolated bacteria in sepsis patients was Klebsiella[33].

3- Study was conducted in (2019) Madras Medical College ChennaiIn UTI differentiation between upper and lower urinary tract infection and compare the levels of CRP in relationship to upper and lower UTI in 100 Patients with urine samples showing positive urine culture and patients showing symptoms of UTI were classified based on symptoms after clinical examinationserum C-Reactive Protein levels were estimated in all the study subjectsthe mean value of CRP in subjects with upper UTI (159.20) was significantly higher than the mean value of lower UTI (16.16). Statistical analysis was done between these two

variables based on chi square test, for which the p value was < 0.001 (< 0.05) which is statistically significant from this study we conclude that Serum C-Reactive Protein level measurements can be done in patients with symptoms of urinary tract infection especially in individuals with risk factors such as Diabetes Mellitus to make an prediction of upper urinary tract involvement [34].

General objectives

Determine C-reactive protein as a biological marker for evaluating the stages of bacterial infection, however, the differences in CRP levels occurring to patient's status are also observing in this study.

Specific objectives

1. To determine the levels of serum CRP (as a biomarker) in patients suffering from diseases related to bacterial infection.
2. To determine the CRP levels across younger and older populations.
3. To determine the CRP levels cross male and female populations.
4. To determine which type of bacteria showed the highest level of CRP, and which one showed the lowest level.
5. To determine which type of disease reveals the highest PRP level, and which one reveals the lowest level.

Justification:

Bacterial infection is considered a worldwide health problem; during the infection the patient undergoes several physiological changes and immunological disturbance. The patient immune system response to the infection releasing too much cytokines including interleukin-6 (IL-6), interleukin-1 (IL-1), and tumor necrosis factor-α (TNF-α) following inflammation, these cytokines tend to stimulate the liver to produce the c-reactive protein (CRP); so we conducted this study to correlate between the type of bacterial infection and the CRP level among known bacterial infected patients. with different age and conditions

CHAPTER TWO

MATERIALS AND METHODS

Study design

Analytical cross-sectional study conducted during the period from July to September 2022.

Study area:

Specimens were collected from Military hospital, Omdurman, Sudan.

Study population

Inclusion criteria

Variant age's patients with different active bacterial infections were admitted to hospitals.

Exclusion criteria

Patients with other known infection beside than bacterial infection {Parasite infection – fungal infection – virus infection etc}.

Sample size:

The sample size was determined according to previous study and literature review 50 samples.

List of table

Number	Material
1	cobas c311 Germany
2	Tube Centrifuge
3	50 Syringe 5 ml
4	50 -Plain containers
5	Tourniquet
6	Test tube rack
7	Gloves
8	Ice box
9	Platinum Wire loop
10	Platinum straight loop
11	Calibrated wire loop
12	Incubator
13	Microscope
14	Petri dishes
15	Wooden stick
16	Oil immersion
17	Test tubes
18	Urine container
19	Swab
20	Sputum container
21	Cystine Lactose Electrolyte Deficient media
22	Blood culture media
23	Chocolate blood agar
24	Simmons citrate media for citrate test
25	Christensen's urea media for Urease test
26	Semi solid media for motility
27	Peptone water contain tryptophan for idol test
28	Kilger iron agar media
29	Litmus milk media
30	Bile asciline media

Methods

Specimens (blood — swabs – Urine-sputum) were collected from patients suspected with bacterial infection and cultured ,All samples were collected depend on the availability and possibility of the study time line , Post bacteria growth identified the type of bacteria, followed by collected blood sample for C-RP examination .both of sample were collected at the same time

Collection of Urine

Mid-stream urine (MSU) were Collected the specimen in clean, leak proof, sterile wide mouth container (60 ml capacity container).

Were asked the patient to clean urethra and the external genital area with soap and water. And asked the patient to pass the middle part of urine into the sterile container (Clean catch urine).container labeled and sent to the Lab**.**

Culturing of Urine

The specimens were inoculated on plates of Cystine Lactose Electrolyte Deficient media (CLED), by method of streaking. Cultures were incubated at 35-37°C aerobically for overnight.

Collection of swab

Was Cleaned skin with alcohol before aspirating sample. and Transferred the purulent material from the collection syringe into a sterile tube for submission to the laboratory and Collected specimen on swab and place in transport medium only if volume is insufficient for aspiration. And sent to the Lab**.**

Culturing of Swab

The specimens were inoculated on plates of blood agar Aerobic 35-37°C, Chocolate blood agar CO2 condition 35-37°Cand MacConkey Anaerobic condition 35-37°C, by method of streaking.

Collection of sputum

Were collected in a screw-cap, leak-proof specimen containers, collected deep cough sputum not saliva and scented to the Lab**.**

Culturing of sputum

The specimens were inoculated on plates of blood agar Aerobic 35-37°C, Chocolate blood agar CO2 condition 35-37°Cand MacConkey Anaerobic condition 35-37°C, by method of streaking.

Collection of blood for culture

1. were pressure cuff, and located a suitable vein in the arm. Were Deflated the cuff while disinfecting the vein puncture site.

2. Gloves were Wearing, and disinfected the vein puncture site

As follows:

 were used 2% tincture of iodine followed by 70% ethanol, were cleaned area about 50 mm in diameter. Allow to air-dry. Were Used a circular action, and swabbed the area beginning at the point where the needle will enter the vein. Blood collection

3. Were removed the protective cover from the top of the culture bottle(s). and Wiped the top of the bottle and used an ethanol-ether swab.

4. Were used a sterile syringe and needle, withdraw about 10-20 ml of blood from an adult or about 2-10 ml from a young child.

5. With care, removed the needle from the syringe and replace with another sterile needle of similar size

6. Inserted the needle through the rubber liner of the bottle cap and dispense 5 ml of blood into each culture medium bottle.

Culturing of blood

The specimens were inoculated on Dyphasic medium Incubated at 35–37 ℃ for up to 7 days, examined and sub culture in blood Chocolate and MacConkey

Thioglycollate broth Incubate at35–37 ℃ for up to 2 weeks, examined and sub cultured

Preparation of media

Cysteine lactose electrolyte deficient (CLED)

This medium is best prepared from ready to use dehydrated powder, available from most suppliers of culture media.

Contents: Peptone, Lab-Lemco powder, tryptone, lactose, L-cystine, bromothymol blue, agar.

The medium is usually used at a concentration of18.1 g in every 500 ml distilled water (concentration may vary depending on manufacturer).

- Prepare as instructed by the manufacturer.

1) Sterilize by autoclaving at 121 °C for 15 minutes.

2) Mix well before pouring (avoid air bubbles forming). Dispense aseptically in 15 ml amounts in sterile Petri dishes

3) Date the medium and give it a batch number.

4) Store the plates at 2–8 °C, preferably sealed in plastic bags to prevent loss of moisture.

Shelf-life: Up to 4 weeks or longer provided

MacConkey media

This medium is best prepared from ready to use dehydrated powder, available from most suppliers of culture media.

Contents: Peptone, lactose, bile salts, sodium chloride, neutral red, agar.

1 Prepare as instructed by the manufacturer. Sterilize by autoclaving at 121 °C for 15 minutes.

2- Store the plates at 2–8 °C preferably in plastic bags to prevent loss of moisture.

Blood agar

Nutritious agar ..... 500 ml

Sterile defibrinated blood. .....25 ml

Chocolate blood agar

1- After adding the blood, heat the medium in a 70 °C water bath until it becomes brown in color. This takes about 10–15 minutes during which time the medium should be mixed gently several times.

2-Allow the medium to cool to about 45 °C, remix and dispense in sterile Petri dishes as described for blood agar. Important: Care must be taken not to overheat or prolong the heating of the medium because this will cause it to become granular and unfit for use.

3 -Date the medium and give it a batch number. Store the plates as described for blood agar.

Identification

Colonial morphology

The inoculated media were morphologically examined for size, color, and reaction.

Gram stain

A drop of normal saline was placed on slide. The suspected colonies were emulsified and smeared. The smears should be fixed by dry heat and then cover with crystal violet stain for 30-60seconds. The stain rapidly washed by tap water and tipped the side. Stained smear then cover with lugoś s iodine for 30-60 seconds. Iodine immediately washed off and the smear was decolorized with than olfor few seconds. Suffranin was added to the smear for 2 minutes. The red stain then washed off with tap water and smear preparation subsequently air dried and microscopically examined using higher solution objective power.

Identification of gram negative rods

Kligler iron agar(KIA)

A tested organism inculcated by sterile straight loop by stapling on the but tthen blocked the pore and streaked the slop of the media and incubated at 37***C*** for 24 hour. Glucose fermentation indicated by yellow butt, yellow slop indicated the lactose fermentation, gas produce in the end of the tube as cracking and H_2S produce blacking in the media .

Citrate utilization test

In this test organism has ability to use citrate as only source of carbon. By straight loop apart of tested colonies was emulsified in kosers citrate media and incubated at 37°C for24hours. A blue color with growth indicated positive, no change in color indicated the negative result

Indole test

In this test the tested organism produced tryptophanaseenzyme which break down tryptophan and produce indole, which react with kovac´s reagent and give pink ring. The tested organism was inoculated into peptone water and incubated at 37 ***°C*** for over night, the kovac' s reagent was added in next day. If there is pink ring the result was indicated as positive. If there is no pink ring in the surface the result was indicated as negative.

Urease test

In this test organism produce urease enzyme which breakdown urea and produce ammonia, which make the Ph of media alkaline, the tested organism inoculated in Christensen's urea agar.

Positive: pink color. Negative :no change in color.

Motility test

The motile organism can be detected by it is diffusion in semi solid medium

Diffusion in the medium……+ve test.

No diffusion in the medium.....… -ve test.

Identification of Gram positive cocci

Catalase test

The differentiation between staphylococci (which produce catalase) from streptococci (non catalase production) was made by catalase test. Catalase acts as catalyst in the breakdown of hydrogen peroxide to oxygen and water. Using sterile wooden stick, suspected colonies were immersed in tube containing 2ml of 3% hydrogen peroxide.

A Positive result was indicated by production of air Bubble. Negative result indicated by no change in tube.

Coagulate test

Coagulate causes plasma to clot by converting fibrinogen to fibrin.

Clumping within 10 secs .....S. aurous

No clumping within 10 secs .....No bound coagulate

DNase test

Using sterile loop to inoculate the suspected colonies under a septic condition into DNA media, after overnight, aerobic incubation at 37◦c

Hydrochloric acid (1% HCL) was to the spots of an organism. Clear zone around the colonies mean positive result.

Mannitol salt agar(MSA)

It is a useful media for identifying staphylococci species, which area belt grow on agar containing 70-100 g/l sodium chloride. Some species of staphylococci area belt ferment mannitol and other can not ferment mannitol.

The test done by inoculating the organism under test in MSA media which contain phenol red indicator, and then incubated the plate at 37℃ for 24hours, and then change in color is observed

Litmus milk

Heavy inoculums of the test organism are incubated for up to 4 hours in a tube containing litmus milk. Reduction of the litmus milk is indicated by a change in color of the medium from mauve to white or pale yellow.

Result Move color Positive.

Bile esculin test

Bile-esculin test is based on the ability of certain bacteria, notably the group D streptococci and *Enterococcus species*, to hydrolyze esculin in the presence of bile (4% bile salts or 40% bile). Note: Many bacteria can hydrolyze esculin, but few can do so in the presence of bile.

Result Change color to black.

Collection of specimens

Fifty blood specimens were collected from patients in sterile plain containers. These specimens were collected from Military hospital patient.

The specimen immediately spread by tube centrifuge then exanimate serum by cobas c311S.

Identification of blood for C-RP test

Fifty blood specimens were collected from patients in sterile plain containers.

These specimens were collected from Military hospital patient.

1- Were checked patient arms for an easily accessible vein. This is usually in the inner part of patient arm on the other side of the elbow.Once they've located a vein, were cleaned and disinfect the area.

2- Then inserted a small needle into patient vein to take a blood sample. This may feel like a small pinch.

3- After they insert the needle, a small amount of blood was collected in a test tube.

4- Once they have enough blood to test, were removed the needle and holed a cotton ball or gauze on the site to stop the bleeding.

5- Were placed a bandage over the site, and you'll be finished. The entire procedure usually takes less than five minutes.

6- Then were separated the blood to get the plasma, If the examination is delayed, the samples will be stored for weak at 4-8 Then were measured by C-RP device (cobas c311 (Roche - Germany).

CHAPTER THREE

RESULTS

3.1 Demographic data

During the period between July to September 2022, total of 50 samples were collected from bacterial infection patients in Military hospital. To demonstrate elevated C-RP levels. (23) Samples were female, which represents (46%) of total sample number and (27) were male, represents(54%) of total number, (Table3.1). (Figure3.1). We demonstrate the average ages of patients correlated with the bacterial infection, we found that, the range less than 20 years old show(6%),and in 20-40 years (28%), Range 41-60 years show (22%) , More than 60 years Show (44%), (Table3.2),(Figure 3.2).

A total of 50 samples were collected from patients suffering of bacterial infection, the samples were collected are 20(40%) urine samples, 10(20%) blood samples, 8(16%) wound swab, 9(18%) sputum samples,3(6%) throat swab, these samples revealed by (Figure3.3).

We also assessed thetype of bacteria, and our results reveal that, in 14 samples we found the predominant isolated bacteria,14S.aureus(28%) , 11 E,coli(22%) , 9 K.pneumoniae (18%) ,8 Ps.aeruginosa(16%) , 4 E.feacalis(8%) , 4 Proteus mirabilis (8%)as illustrated in (Figure 3.4). Post demonstrate the type of bacteria, we diagnosing the type of infection, the previous type of Bactria initiate the following diseases, 3 Tonsillitis 6% caused by (S.aureus 6%) 8 wound infection 16% caused by (2K.pneumoniae 4% , 2 S.aureues 4%, 3 Ps.aeruginosa6% , 1 Proteus mirabilis (2%) ,9 Respiratory tract infection 18%(3 K. pneumoniae6% , 3 Ps.aeruginosa 6% , 3 E.coli 9% 10 Septicemia 20% caused by (7 S.aureues 14% , 2 E.Coli 4%, 1 Proteus mirabilis 2%) 20 Urinary tract infection 40% (6 E .coli 12% , 2 Ps.aeruginosa4%, 4 E.feacelis 8% , 4 K.pneumoniea 8% , 2 S.aureues 4%,2 Proteus mirabilis 4%) (Figure 3.5).

We classified the result of C-RP level according to severity of inflammation, (Mild inflammation {10-40mg/L} – the Moderate {40-100mg/L} – Marked {100-200mg/L} – sever bacterial infection according to international reference {<200mg/L}).

The most common group among these groups is moderate 23 (46%) patients followed by severe11 (22%), mild9(18%), marked7(14%). (Table 3.3)(Figure 3.6).

In this study there is no association between result of C-RP and demographic data cross the younger and older population (age groups) and across male and female (gender group) because the P-value was less than 0.05 (table 3.4)

Table 3:1 shows Percentage of infected in male and Female

Gender	Percentage of infected
Female	46%
Male	54%

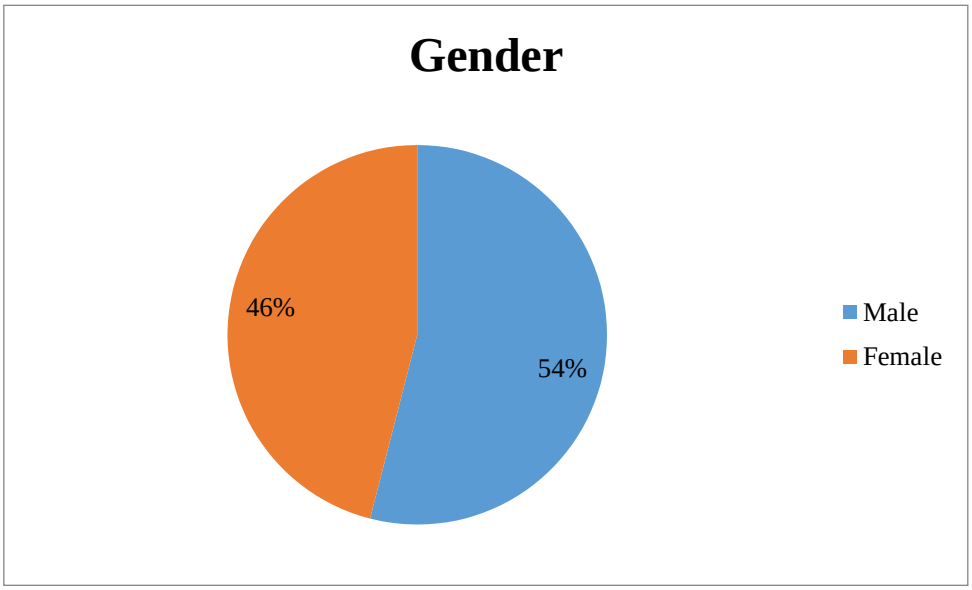


Figure 3:1 shows Percentage of infected in male and Female

Table 3:2 shows different age of patients

Age	Percentage of infected
Less than 20 years	6%
20-40 years	28%
41-60 years	22%
More than 60 years	44%

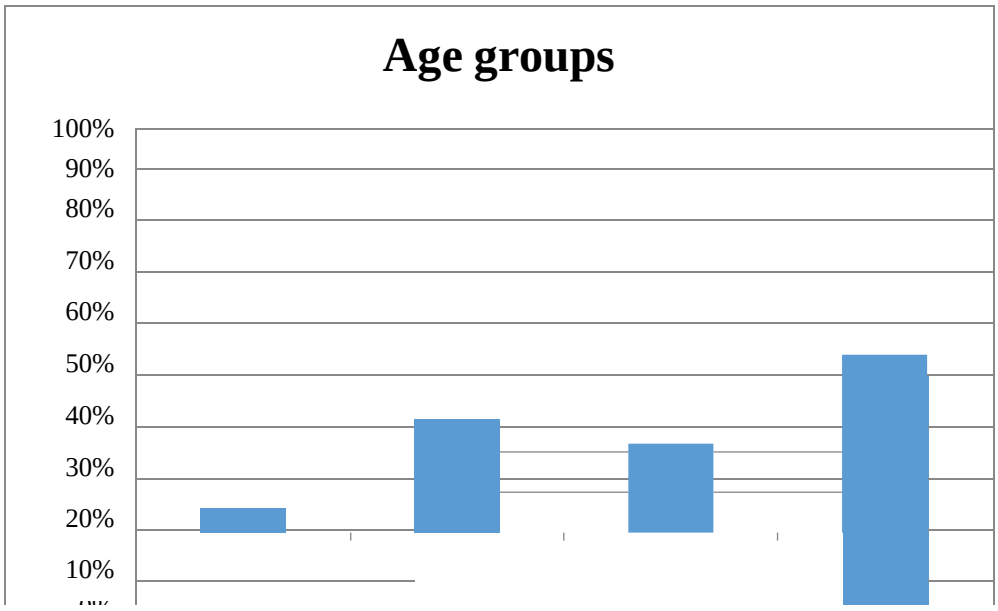


Figure 3:2 shows different age of patients

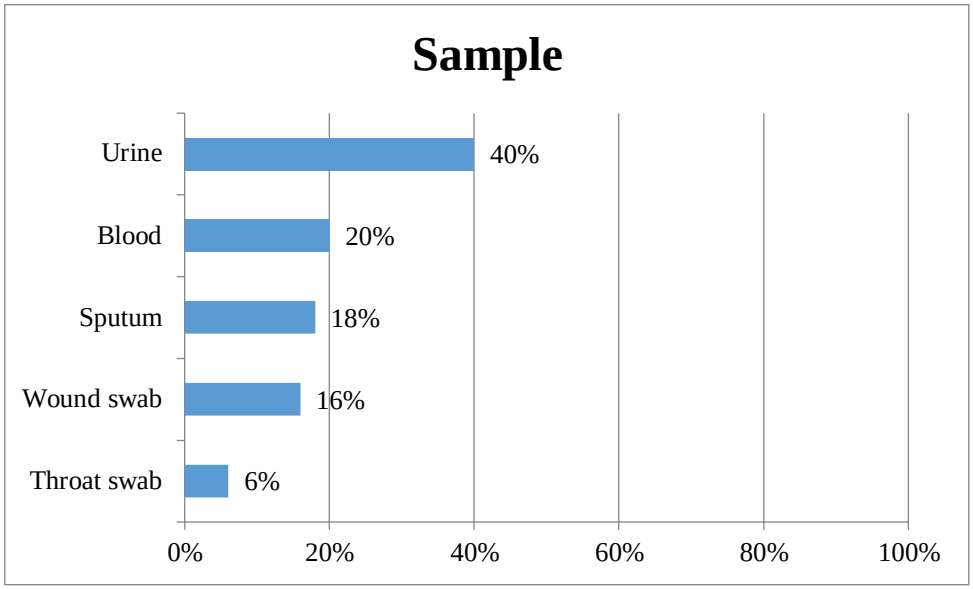


Figure 3:3 shows types of samples were collected

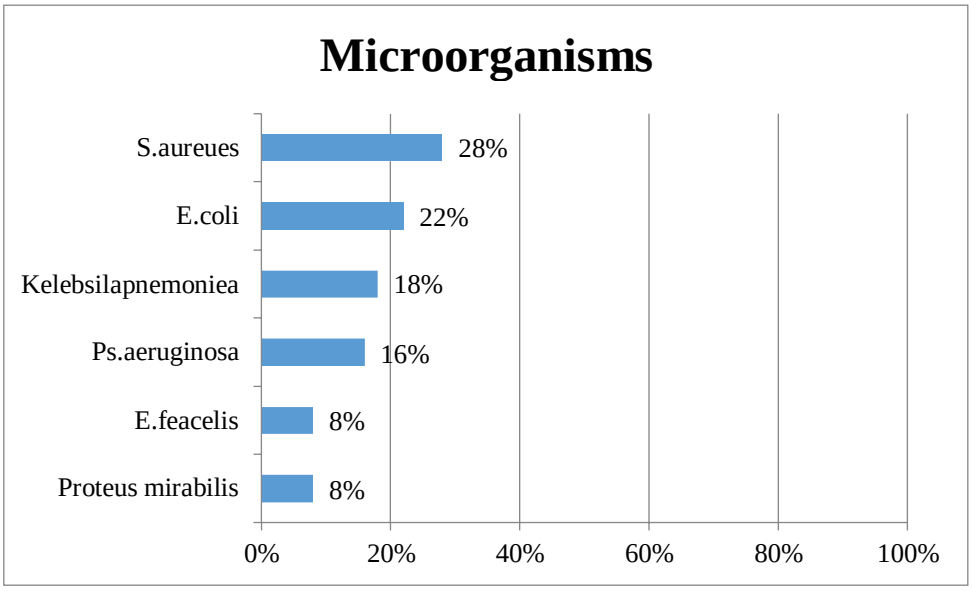


Figure 3:4 shows different types of isolated Microorganisms

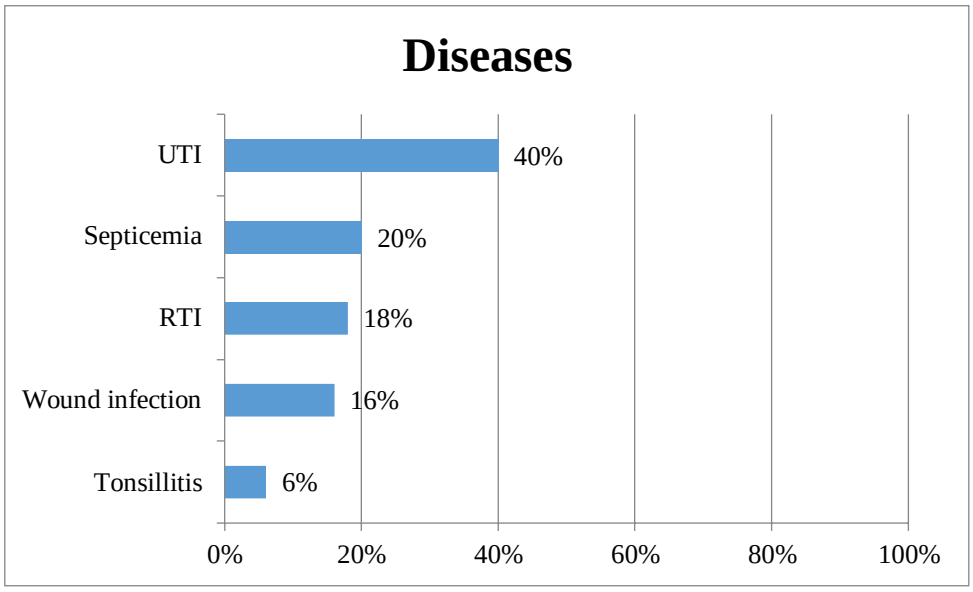


Figure 3:5 shows different types of diseases

Table (3.3) show the number of patients infected according to Severity of inflammation

Severity of inflammation	Number	Percent
Mild	9	18%
Moderate	23	46%
Marked	7	14%
Severe	11	22%
Total	50	100%

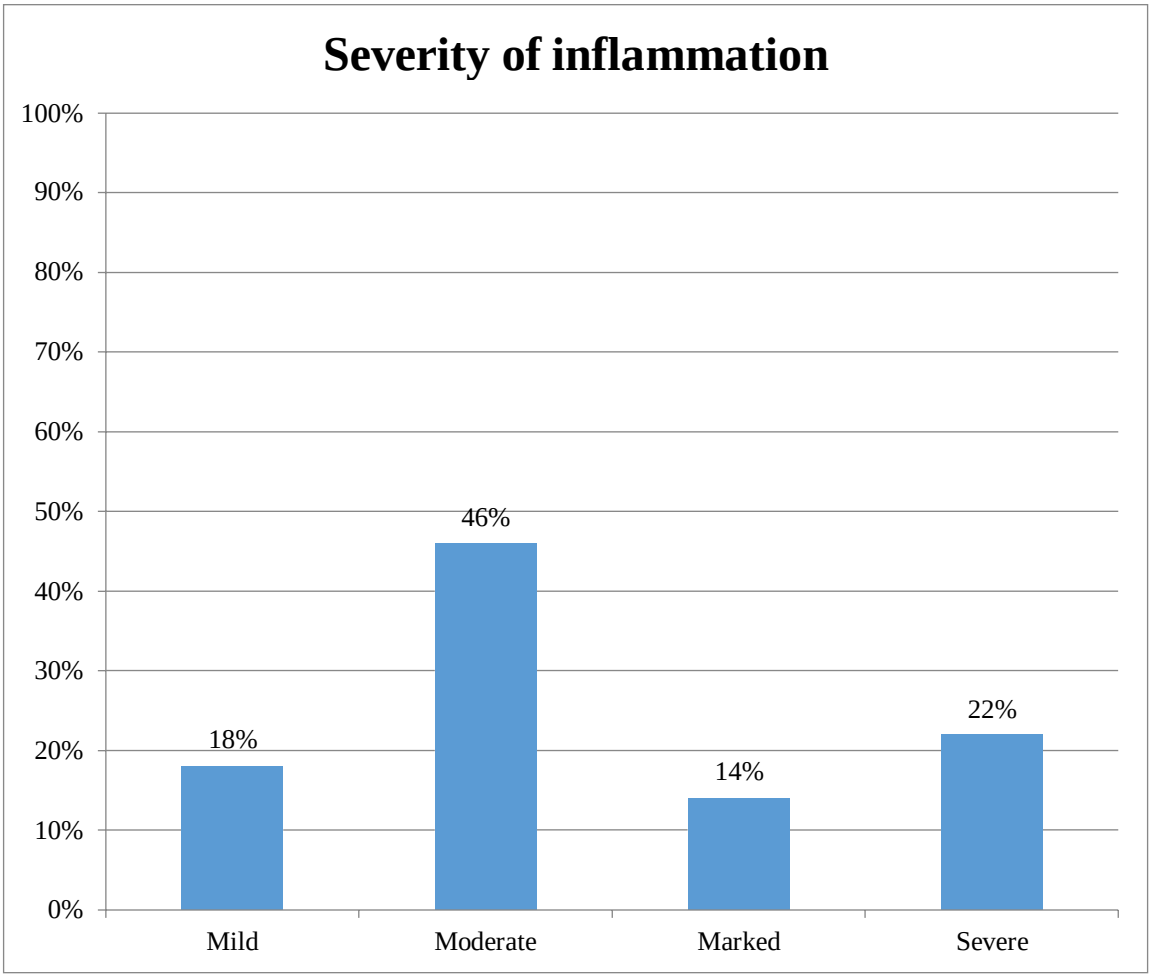


Figure (3.6) show the number of patient infected according to Severity of inflammation

Table (3.4) Show association between CRP level to age or gender, or type of bacteria and P Value result

Variables		Severity of inflammation				Fisher's Exact Test P value
		Mild	Moderate	Marked	Severe	
		5	12	4	6	
	Male	18.50%	44.40%	14.80%	22.20%	
		4	11	3	5	
Gender	Female	17.40%	47.80%	13.00%	21.70%	0.999*
		2	0	1	0	
	Less than 20 years	66.70%	0.00%	33.30%	0.00%	
		2	8	3	1	
	20-40 years	14.30%	57.10%	21.40%	7.10%	
		1	6	1	3	
	41-60 years	9.10%	54.50%	9.10%	27.30%	
		4	9	2	7	
Age groups	More than 60 years	18.20%	40.90%	9.10%	31.80%	0.265*
		1	5	2	3	
	E.coli	9.10%	45.50%	18.20%	27.30%	
		0	4	0	0	
	E.feacelis	0.00%	100.00%	0.00%	0.00%	
		4	6	1	3	
	S.aureues	28.60%	42.90%	7.10%	21.40%	
		0	4	3	1	
	Ps.aeruginosa	0.00%	50.00%	37.50%	12.50%	
		4	2	1	2	
	Kelebsilapnemoniea	44.40%	22.20%	11.10%	22.20%	
		0	2	0	2	
Microorganisms	Proteus mirabilis	0.00%	50.00%	0.00%	50.00%	0.374*
		5	11	1	3	
	UTI	25.00%	55.00%	5.00%	15.00%	
		2	2	2	4	
	Septicemia	20.00%	20.00%	20.00%	40.00%	
		0	4	3	1	
	Wound infection	0.00%	50.00%	37.50%	12.50%	
		1	4	1	3	
	RTI	11.10%	44.40%	11.10%	33.30%	
		1	2	0	0	
Diseases	Tonsillitis	33.30%	66.70%	0.00%	0.00%	0.343*

CHAPTER FOUR

DISCUSTION, CONCLUTION, AND RECOMMENDATIONS

Discussion:

C-reactive protein (CRP) is widely used in younger populations, but its value for diagnosing bacterial infection in the older population is not well established. So far, there is no study focusing on the vital role of CRP for illustration of bacterial infection, and applicable use of this protein in severity of inflammation, type of bacteria, in addition to ages of patients. Moreover, this study examined the usefulness of serum CRP levels in the early detection of bacterial infection in older and younger patient, this we hypothesized that the CRP levels could be converted according to the age group of patients on the other hand, the main objective of the study is to how to use the C-RP level as a biomarker for the detection of severity of inflammation among the older and younger population. 50specimens were collected for patients suffering from varied bacterial infection with different ages and sex ,all samples prepared, and adopted different standardized tools and methods for the realization of the problem through isolation and identification of bacterial strains which cause bacterial infection.

Peter stenvinkel et al [(35)] determined the CRP levels between inflamed male and female, and his result reveals that the elevated CRP is a strong predictor of inflammation outcome, moreover, the inflamed males had a significantly higher mortality rate than inflamed females. Contrarily, in this study we found that the CRP increase significantly in female group (46%) than male group (54%).

Mancinella and his group [(36)] add study to analyze the interplay among markers of inflammation, such as fibrinogen and high CRP levels among older age group, A. Mancinella performed a cross-sectional study comparing markers of inflammation between 99 patients affected by dementia (mean age: 83 ± 6 years) and 99 controls (mean age: 83 ± 7 years), and he found that Patients affected by dementia had higher CRP levels than controls. In the same context, our data showed CRP levels vitiated according to Age groups. The CRP level increase significantly in range more than 60 years, and lower secreted Less than 20 years.

(John et al. 2015) [37] reveals that, (*Hemophiles influenza*) was found to predominant bacterial isolated in stable Respiratory tract infected patients. and our study showed that the Predominate isolated Bacterial was (E. coli). The justification of this result could be due to different geographical distribution. Additionally, (John et al. 2015) reported CRP is an important biomarker in Respiratory tract infection and indirect evidence of infection. Similarly, our results reflected that the CRP could be used as indicator for infection and early marker to assessing the efficacy of the treatment.

Conclusions

- Of the 50 positive sample the highest level of C-RP show 288.9 mg/L and lowest level of C-RP 7.7mg/L.
- There is no association between result of C-RP and demographic data across younger and older group (Age group).
- There is no association between result of C-RP and male and female (Sex group).
- Bacteria show highest level of C-RP *Ps.aeruginose* , Bacteria show lowest level of C-RP *E.coli.*
- Study show highest level of C-RP in Respiratory tract infection , followed by septicemia followed by urinary tract infection followed by wound infection finally Tonsillitis.

Recommendation

For a more accurate study to assess serum CRP as a predictive marker for bacterial infection, and its relationship with the age population, we recommended increasing the sample size, besides performing further advanced techniques, to obtain more viable result. Additionally we recommended determine the C-RP levels compared with different bacteria and viral infection

APPENDIX I

Questionnaire

Program: Medical laboratory science

Department: Microbiology

Semester: 8

Batches: 3

Academic year: 2022 – 2023

Age : ---------- _____

Gender : Male ☐ Female ☐

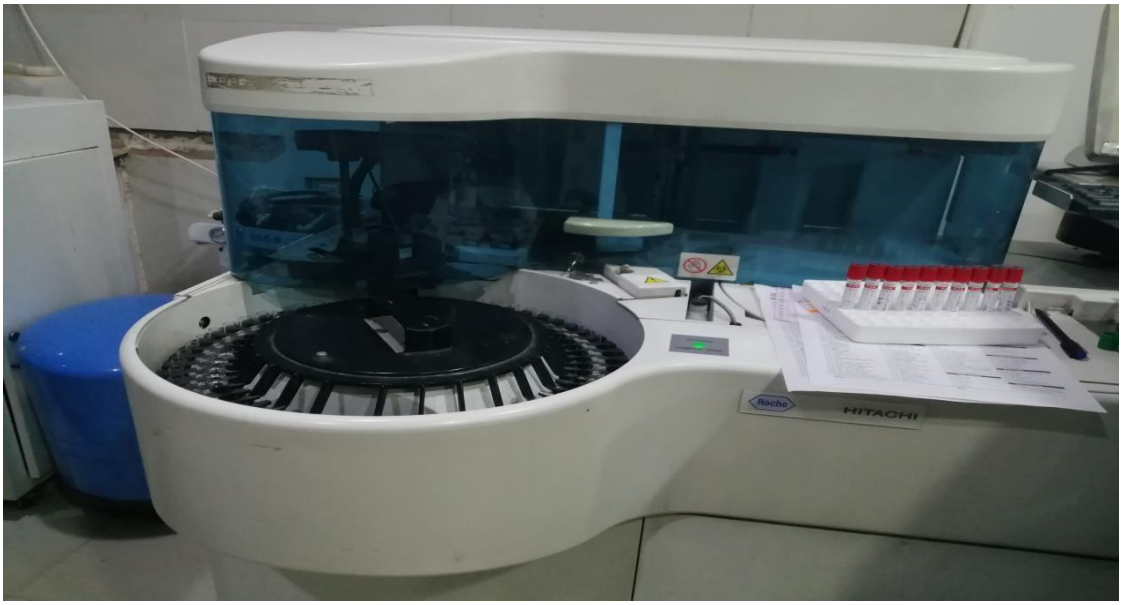
Antibiotic Administration Yes ☐ No ☐

Type of sample-_____

APPENDIX II

Centerfuge to seprate Serum samples

Serum sample Saparated in Plain Container



Cobas C311 analyzer – Roche diagnostic for measure C-RP leaves

REFERANCESs

1. World Health Organization. C-reactive protein concentrations as a marker of inflammation or infection for interpreting biomarkers of micronutrient status. World Health Organization; 2014.
2. Liu A, Bui T, Van Nguyen H, Ong B, Shen Q, Kamalasena D. Serum C-reactive protein as a biomarker for early detection of bacterial infection in the older patient. Age and ageing. 2010 Sep 1;39(5):559-65.
3. World Health Organization. C-reactive protein concentrations as a marker of inflammation or infection for interpreting biomarkers of micronutrient status. World Health Organization; 2014.
4. Ridker PM. Clinical application of C-reactive protein for cardiovascular disease detection and prevention. Circulation. 2003 Jan 28;107(3):363-9. doi: 10.1161/01.cir.0000053730.47739.3c. PMID: 12551853.
5. Ticinesi A, Lauretani F, Nouvenne A, Porro E, Fanelli G, Maggio M, Meschi T. C-reactive protein (CRP) measurement in geriatric patients hospitalized for acute infection. European Journal of Internal Medicine. 2017 Jan 1;37:7-12.
6. Moore TA, Standiford TJ. The role of cytokines in bacterial pneumonia: an inflammatory balancing act. Proceedings of the Association of American Physicians. 1998 Jul 1;110(4):297-305.
7. Eklund CM. Proinflammatory cytokines in CRP baseline regulation. Advances in clinical chemistry. 2009 Jan 1;48:111-36.
8. He Y, Feng D, Hwang S, Mackowiak B, Wang X, Xiang X, Rodrigues RM, Fu Y, Ma J, Ren T, Ait-Ahmed Y, Xu M, Liangpunsakul S, Gao B. Interleukin-20 exacerbates acute hepatitis and bacterial infection by downregulating IκBζ target genes in hepatocytes. J Hepatol. 2021 Jul;75(1):163-176. doi: 10.1016/j.jhep.2021.02.004. Epub 2021 Feb 18. PMID: 33610678; PMCID: PMC8323118.
9. Melbye H, Hvidsten D, Holm A, Nordbø SA, Brox J. The course of C-reactive protein response in untreated upper respiratory tract infection. British Journal of General Practice. 2004 Sep 1;54(506):653-8.Krzemień G, Roszkowska-Blaim M, Kostro I, Szmigielska A, Karpińska M, Sieniawsk

10. Doron, S., &Gorbach, S. L. (2008). Bacterial Infections: Overview. International Encyclopedia of Public Health, 273–282. https://doi.org/10.1016/B978-012373960-5.00596-7.

11- Xiao, Y., &Cai, W. (2020). Autophagy and Bacterial Infection.Advances in experimental medicine and biology, 1207, 413–423. https://doi.org/10.1007/978-981-15-4272-5_29.

12. Tillet WS, Francis T. Serological reactions in pneumonia with a non-protein somatic fraction of Pneumococcus. J Exp Med (1930) 52(4):561–71. 10.1084/jem.52.4.561 [PMC free article] [PubMed] [CrossRef] [Google Scholar] [Ref list]

13- Moutachakkir, M.; LamraniHanchi, A.; Baraou, A.; Boukhira, A.; Chellak, S. Immunoanalytical characteristics of C-reactive protein and high sensitivity C-reactive protein. Ann. Biol. Clin. 2017, 75, 225–229. [CrossRef] [PubMed]

14- Sproston, N.R.; Ashworth, J.J. Role of C-Reactive Protein at Sites of Inflammation and Infection. Front. Immunol.2018, 9, 754. [CrossRef] [PubMed]

15- Labonté ME, Dewailly E, Chateau-Degat ML, Couture P, Lamarche B. Population-based study of high plasma C-reactive protein concentrations among the Inuit of Nunavik. Int. J. Circumpolar Health.2012 doi: 10.3402/ijch.v71i0.19066. [PMC free article] [PubMed] [CrossRef] [Google Scholar] [Ref list]

16-Neyestani TR, et al. Predictors of serum levels of high sensitivity C-reactive protein and systolic blood pressure in overweight and obese nondiabetic women in Tehran: A cross-sectional study. Metab.Syndr.Relat.Disord.2011;9:41–47. doi: 10.1089/met.2010.0075. [PubMed] [CrossRef] [Google Scholar] [Ref list]

17- Mantovani A., Garlanda C., Doni A., Bottazzi B. Pentraxins in innate immunity: From C-reactive protein to the long pentraxin PTX3. J. Clin. Immunol.2008;28:1–13. doi: 10.1007/s10875-007-9126-7. [PubMed] [CrossRef] [Google Scholar] [Ref list]

18-Du Clos T.W. Function of C-reactive protein.Ann. Med. 2000;32:274–278. doi: 10.3109/07853890009011772. [PubMed] [CrossRef] [Google Scholar] [Ref list]

19- Geerlings S. E. (2016). Clinical Presentations and Epidemiology of Urinary Tract Infections.Microbiology spectrum, 4(5), 10.1128/microbiolspec.UTI-0002-2012. https://doi.org/10.1128/microbiolspec.UTI-0002-2012.

20- Bono, M. J., Leslie, S. W., &Reygaert, W. C. (2022). Urinary Tract Infection.InStatPearls.StatPearls Publishing.

21- Barnett, B. J., & Stephens, D. S. (1997). Urinary tract infection: an overview. The American journal of the medical sciences, 314(4), 245-249.

22- Orenstein, R., & Wong, E. S. (1999). Urinary tract infections in adults.American family physician, 59(5), 1225.

23- Abayneh, M., HaileMariam, S., &Asnake, M. (2021). Bacterial profile and multi-drug resistance pattern of bacterial isolates among septicemia suspected cases: A meta-analysis report in Ethiopia. Journal of Laboratory Medicine, 45(3), 167-178.

24- Grice, E. A., & Segre, J. A. (2011). The skin microbiome.Nature reviews. Microbiology, 9(4), 244–253. https://doi.org/10.1038/nrmicro2537

25- Dow, G., Browne, A., &Sibbald, R. G. (1999). Infection in chronic wounds: controversies in diagnosis and treatment. Ostomy/wound management, 45(8), 23-7.

26- Bessa, L. J., Fazii, P., Di Giulio, M., & Cellini, L. (2015). Bacterial isolates from infected wounds and their antibiotic susceptibility pattern: some remarks about wound infection. International wound journal, 12(1), 47-52.

27- Sattar, S. B. A., & Sharma, S. (2018). Bacterial pneumonia.

28- Sharma, S., Maycher, B., &Eschun, G. (2007). Radiological imaging in pneumonia: recent innovations. Current opinion in pulmonary medicine, 13(3), 159-169.

29- Pahal, P., Rajasurya, V., & Sharma, S. (2018). Typical bacterial pneumonia.

30- Bartlett, A., Bola, S., & Williams, R. (2015). Acute tonsillitis and its complications: an overview. Journal of the Royal Naval Medical Service, 101(1).

31- Windfuhr, J. P., Toepfner, N., Steffen, G., Waldfahrer, F., &Berner, R. (2016). Clinical practice guideline: tonsillitis I. Diagnostics and nonsurgical management. European archives of oto-rhino-laryngology : official journal of the European Federation of Oto-Rhino-Laryngological Societies (EUFOS) : affiliated with the German Society for Oto-Rhino-Laryngology - Head and Neck Surgery, 273(4), 973–987. https://doi.org/10.1007/s00405-015-3872-6

32- Goldberg, I., Shalmon, D., Shteinvil, R., Berliner, S., Paran, Y., Zeltser, D., Shapira, I., Shenhar-Tsarfaty, S., Meilik, A., Wasserman, A., Goldiner, I., Ziv-Baran, T., Sprecher, E., Levinson, T., &Rogowski, O. (2020). A second C-reactive protein (CRP) test to detect

inflammatory burst in patients with acute bacterial infections presenting with a first relatively low CRP.Medicine, 99(42), e22551.

33- Mohamod, Z. M., Merghani, M. M., &Babiker, N. E. (2022). Measurement of Protein C Levels among Sudanese Children and Infants with Sepsis at Khartoum state, 2022. Journal of Drug Delivery and Therapeutics, 12(5-S), 54-58.

34- Krishna, R. (2019). A Study to Differentiate Upper and Lower Urinary Tract Infection with Blood CRP Levels (Doctoral dissertation, Madras Medical College, Chennai).

35- Stenvinkel, P., Wanner, C., Metzger, T., Heimbürger, O., Mallamaci, F., Tripepi, G., ... & Zoccali, C. (2002). Inflammation and outcome in end-stage renal failure: does female gender constitute a survival advantage?. Kidney international, 62(5),

36- Mancinella, A., Mancinella, M., Carpinteri, G., Bellomo, A., Fossati, C., Gianturco, V., ... &Marigliano, V. (2009). Is there a relationship between high C-reactive protein (CRP) levels and dementia?. Archives of gerontology and geriatrics, 49, 185-194.

37- John, S. A., & Srinivasan, S. V. (2015).. investigation of relationship between serum C-Reactive Protein level and the rate of microbial infection patient