TITLE

C-REACTIVE PROTEIN AS A PROBABLE PREDICTOR OF URINARY TRACT INFECTION IN HIV SEROPOSITIVE INDIVIDUALS

A RESEARCH PROJECT REPORT SUBMITTED IN PARTIAL FULFILLMENT OF THE REQUIREMENT FOR THE AWARD OF DEGREE OF MASTER OF SCIENCE (M.Sc.) IN BIOCHEMISTRY (MOLECULAR BIOLOGY), UNIVERSITY OF NIGERIA, NSUKKA

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DEDICATION

This work is dedicated to God almighty and my late father, Late Chief Michael Ogbodo and to all researchers working towards improving the health of humanity.

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ABSTRACT

The aim of the present study is to evaluate the possible use of C-reactive proteins as predictor for urinary tract infection in HIV seropositive individuals. A total of 50 seropositive individuals were selected under purposive sampling during the study period. They consisted of 36 seropositive females and 14 seropositive males. Blood and urine samples were collected from each subject. Isolation of UTI causing species was carried out by standard microbiological methods. Identification of microbial isolates was carried using gram staining techniques, biochemical test and antimicrobial susceptibility techniques. Estimation of Serum C-reactive Protein was carried out by Rapid Quantitative Test using the FineCare CRP rapid quantitative test. Correlation analysis was used to determine and compare whether there is a relationship between the levels of C-reactive protein and the incidence of urinary tract infection in the samples. From the results, of the 50 urine samples of HIV positive individuals collected, only 23 samples recorded microbial growth after culturing for 24 hours in MacConkey agar. Antimicrobial sensitivity test was used to determine the inhibitory activities of different antibiotics on the test bacterial species. A total of 10 antibiotic discs were tested against 23 microbial isolates. Of the 23 organisms tested, 60.87% was resistant to Erythromycin, 34.78% was resistant to Cetraxon, 73.78% was resistant to Ampicillin, 100 % was resistant to Cloxacillin, 8.69% was resistant to LV, 69.56% was resistant to Cephalexin, 17.39 was resistant to Ciprofloxacin, 13.04% was resistant to Gentamycin, 17.40% was resistant to Ofloxacin while 65.21% was resistant to Clindamycin. The result of Gram reaction showed that all microbial isolates were either long or short Gram negative rods. While some of the rods were short and in cluster, some were long and cluster, others were short and single while some were long and single. From the biochemical test, *Klebsiella pneumoniae, E. coli, Staphylococcus aureus and Proteus spp* were confirmed to be present in the urine samples in quantities that show the absence of urinary tract infection. The result of correlation analysis showed a non-significant negative correlation ($p > 0.01$) between the bacteria load and the CRP levels of the samples analyzed. There was also a non-significant negative correlation ($p > 0.01$) between the bacterial load of the samples and hsCRP levels. Although the bacteria load of the urine samples were lower than what could define the presence of UTI, the CRP and hsCRP levels of some samples were very high indicating inflammation or infection in such individuals. The suspected infection could be due to another infection or inflammation but not UTI. Thus, the use of CRP and hsCRP as diagnostic markers for UTI may not be very efficient since some other infections could lead to the increase in the levels of CRP and hsCRP in the absence of UTI.

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LIST OF ABBREVIATIONS

AM	Ampicillin
ASB	Asymptomatic Bacteriuria
CA-UTI	Catheter – Associated Urinary Tract Infection
CD	Clindamycin
CIP	Ciprofloxacin
CL	Cloxacillin
CPE	Cytopathic Effect
CRP	C-reactive Protein
CT	Cetraxon
CX	Cephalexin
DNA	Deoxyribonuceotide acid
DNA	Deoxyribonucleic Acid
E	Erythromycin
GN	Gentamycin
Gp	Glycoprotein
HIV	Human Immune Virus
hsCRP	High Sensitivity C-reactive Protein
IF	Interferone
IL	Interleukine
IP	Intellectual Property
KIA	Kligler Iron Agar
LAV	Lymphadenopathy Associated Virus
LTR	Long Terminal Repeat

MAC	Membrane Attack Complex
MAPK	Mitogen Associated Protein Kinase
mCRP	monomeric C-reactive Protein
MDR	Multidrug Resistance
MHC	Major Histocompatability Complex
nCRP	native C-reactive Protein
OF	Ofloxacin
PCh	Phosphocholine
PCP	Pneumocystis Carinii Pneumonia
Pol	Polymerase
RNA	Ribonucleic Acid
TNF	Tumor Necrosis Factor
UPEC	Uropathogenic *Escherichia coli*
UTI	Urinary Tract Infection
WHO	World Health organization

CHAPTER ONE

INTRODUCTION

Human Immunodeficiency Virus (HIV) is a lentivirus that causes immunodeficiency syndrome, a condition in humans in which progressive failure of the immune system allows life threatening opportunistic infections to thrive. Most untreated people infected with HIV eventually develop AIDS (Miguelles and Connors, 2010). These individuals usually die of opportunistic infections or malignancies associated with progressive failure of the immune system. HIV infects vital cells in the human immune system such as helper T cells (specifically $CD4^+$ T cells), macrophages and dendritic cells. HIV infection leads to low levels of $CD4^+$ T cells through three main mechanisms. First, direct viral killing of the infected cells; second, increased rate of apoptosis in infected cells; and third, killing of infected $CD4^+$ cells by CD8 cytotoxic lymphocytes that recognize infected cells. When $CD4^+$ cells' number decline below a critical level, cell mediated immunity is lost and the body becomes progressively more susceptible to opportunistic infections.

Urinary tract infection (UTI) is one of the significant illnesses that cause burden. It is the most common nosocomial infection and an important source of morbidity (Deokar and Badhankar, 2009). UTI is one of the most common bacterial infection and cause of morbidity and hospitalization in HIV positive individuals (Iweriobor *et al*., 2012). HIV disease is associated with a variety of renal syndromes in patients with low $CD4^+$ cell counts, causing neurologic complications which lead to urinary stasis and ultimately infection (Miguelles and Connors, 2010). Once a patient's $CD4^+$ T cell count falls below 200cells/mm^3, the individual is then at risk of a variety of opportunistic infections. The infectious organisms may include fungi, protozoa, viruses and bacteria. The most predominant causative organisms are encapsulated bacteria notably: *Streptococcus pneumoniae* and *Haemophilus influenzae,* but non-typhoidal Salmonella, *Staphylococcus aureus* and *Pseudomonas aeruginosa* have also been implicated (Miguelles and Connors, 2010). Among opportunistic infections, UTI accounts for 60% of AIDS defining illnesses. Urinary tract infections account for a significant proportion of patient's daily hospital visits in HIV patients. UTI became quite alarming as isolated uropathogens exhibited high percentage resistance to almost all antibiotics (Murugan *et al*., 2012). These multidrug resistant

(MDR) pathogens are relentlessly multiplying in HIV patients and thus, becoming an important circulating source of infection especially in Nigeria.

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* (Tillet and Francis, 1930). In the presence of calcium, CRP binds to polysaccharides such as phosphocholine (PCh) on microorganisms and triggers the classical complement pathway of innate immunity by activating C1q. C-reactive protein exhibits elevated expression during inflam-matory conditions such as rheumatoid arthritis, some cardio-vascular diseases, and infection. As an acute-phase protein, the plasma concentration of CRP deviates by at least 25% during inflammatory disorders. The highest concentrations of CRP are found in serum, with some bacterial infections increasing levels up to 1,000-fold (Thompson *et al*., 1999).

1.2 Overview of Human Immune Virus

The human immunodeficiency viruses (HIV) are two species of *Lentivirus* (a subgroup of retrovirus) that causes HIV infection and acquired immunodeficiency syndrome (AIDS) (Douek, *et al*., 2009). AIDS is a condition in humans in which progressive failure of the immune system allows life-threatening opportunistic infections and cancers to thrive. Without treatment, average survival time after infection with HIV is estimated to be 9 to 11 years, depending on the HIV subtype (UNAIDS, 2007). In most cases, HIV is a sexually transmitted infection and occurs by contact with or transfer of blood, pre-ejaculate semen, and vaginal fluids. Research has shown (for both same-sex and opposite-sex couples) that HIV is untransmissable through condomless sexual intercourse if the HIV-positive partner has a consistently undetectable viral load (Douek, *et al*., 2009). Non-sexual transmission can occur from an infected mother to her infant during pregnancy, during childbirth by exposure to her blood or vaginal fluid, and through breast milk. Within these bodily fluids, HIV is present as both free virus particles and virus within infected immune cells. HIV infects vital cells in the human immune system, such as helper T cells (specifically $CD4^+$ T cells), macrophages, and dendritic cells. HIV infection leads to low levels of $CD4^+$ T cells through a number of mechanisms, including pyroptosis of abortively infected T

cells, apoptosis of uninfected bystander cells, direct viral killing of infected cells, and killing of infected $CD4^+$ T cells by $CD8^+$ cytotoxic lymphocytes that recognize infected cells. When $CD4^+$ T cell numbers decline below a critical level, cell mediated immunity is lost, and the body becomes progressively more susceptible to opportunistic infections, leading to the development of AIDS (Kumar and Vinay, 2012).

1.2.1Classification of Human Immune Virus

HIV is a member of the genus *Lentivirus* part of the family *Retroviridae*. Lentiviruses have many morphologies and biological properties in common. Many species are infected by lentiviruses, which are characteristically responsible for long-duration illnesses with a long incubation period. Two types of HIV have been characterized: HIV-1 and HIV-2. HIV-1 is the virus that was initially discovered and termed both lymphadenopathy associated virus (LAV) and human Tlymphotropic virus 3 (HTLV-III). HIV-1 is more virulent and more infective than HIV-2, and is the cause of the majority of HIV infections globally. The lower infectivity of HIV-2, compared to HIV-1, implies that fewer of those exposed to HIV-2 will be infected per exposure. Due to its relatively poor capacity for transmission, HIV-2 is largely confined to West Africa (Reeves and Doms, 2002)

1.2.2 Structure of Human Immune Virus Genome

HIV is different in structure from other retroviruses. It is roughly spherical with a diameter of about 120 nm, around 60 times smaller than a red blood cell. It is composed of two copies of positive-sense single-stranded RNA that codes for the virus's nine genes enclosed by a conical capsid composed of 2,000 copies of the viral protein p24. The single-stranded RNA is tightly bound to nucleocapsid proteins, p7, and enzymes needed for the development of the virion such as reverse transcriptase, proteases, ribonuclease and integrase. A matrix composed of the viral protein p17 surrounds the capsid ensuring the integrity of the virion particle. This is, in turn, surrounded by the viral envelope, that is composed of the lipid bilayer taken from the membrane of a human host cell when the newly formed virus particle buds from the cell. The viral envelope contains proteins from the host cell and relatively few copies of the HIV Envelope protein, which consists of a cap made of three molecules known as glycoprotein (gp) 120, and a stem consisting

of three gp41 molecules that anchor the structure into the viral envelope (Reeves and Doms, 2002).

The Envelope protein, encoded by the HIV *env* gene, allows the virus to attach to target cells and fuse the viral envelope with the target cell's membrane releasing the viral contents into the cell and initiating the infectious cycle. As the sole viral protein on the surface of the virus, the envelope protein is a major target for HIV vaccine efforts. Over half of the mass of the trimeric envelope spike is N-linked glycans. The density is high as the glycans shield the underlying viral protein from neutralisation by antibodies. This is one of the most densely glycosylated molecules known and the density is sufficiently high to prevent the normal maturation process of glycans during biogenesis in the endoplasmic and Golgi apparatus (Behrens *et al*., 2016). The majority of the glycans are therefore stalled as immature 'high-mannose' glycans not normally present on human glycoproteins that are secreted or present on a cell surface. The unusual processing and high density means that almost all broadly neutralising antibodies that have so far been identified (from a subset of patients that have been infected for many months to years) bind to, or are adapted to cope with, these envelope glycans.. Recombinant trimeric viral spikes are promising vaccine candidates as they display less non-neutralising epitopes than recombinant monomeric gp120, which act to suppress the immune response to target epitopes (Behrens *et al*., 2016). The RNA genome consists of at least seven structural landmarks and nine genes and sometimes a tenth *tev*, which is a fusion of *tat*, *env* and *rev*), encoding 19 proteins. Three of these genes, *gag*, *pol*, and *env*, contain information needed to make the structural proteins for new virus particles. For example, *env* codes for a protein called gp160 that is cut in two by a cellular protease to form gp120 and gp4 (Behrens *et al*., 2016).

The six remaining genes, *tat*, *rev*, *nef*, *vif*, *vpr*, and *vpu* (or *vpx* in the case of HIV-2), are regulatory genes for proteins that control the ability of HIV to infect cells, produce new copies of virus (replicate), or cause disease (Various, 2008). The two *tat* proteins (p16 and p14) are transcriptional transactivators for the LTR promoter acting by binding the TAR RNA element (Various, 2008). The TAR may also be processed into microRNAs that regulate the apoptosis genes *ERCC1* and *IER3*. The *rev* protein (p19) is involved in shuttling RNAs from the nucleus and the cytoplasm by binding to the RRE RNA element. The *vif* protein (p23) prevents the action of APOBEC3G (a cellular protein that deaminates cytidine to uridine in the single-stranded viral

DNA and/or interferes with reverse transcription). The *vpr* protein (p14) arrests cell division at G2/M. The *nef* protein (p27) down-regulates CD4 (the major viral receptor), as well as the MHC class I and class II molecules. *Nef* also interacts with SH3 domains. The *vpu* protein (p16) influences the release of new virus particles from infected cells (Various, 2008). The ends of each strand of HIV RNA contain an RNA sequence called a long terminal repeat (LTR). Regions in the LTR act as switches to control production of new viruses and can be triggered by proteins from either HIV or the host cell. The Psi element is involved in viral genome packaging and recognized by *gag* and *rev* proteins. The SLIP element (TTTTTT) is involved in the frameshift in the *gag-pol* reading frame required to make functional *pol* (Various, 2008).

1.2.3 Genomic Structure of HIV Virus

The genomic structure of HIV virus is revealed to be more complex and different from other conventional retroviruses (HTLV-1 and HTLV-II) by molecular cloning and sequencing. It is composed of two copies of single stranded RNA which codes for the nine genes of the virus enclosed by a conical capsid which is made up of about 2000 copies of viral protein p24 (Various, 2008). The genome of RNA is made up of nine genes (gag, pol, env, tat, rev, nef, vif, vpr, vpu) and seven structural landmarks like (LTR, TAR,RRE,PE,SLIP,CRS and INS), where gag, pol and env are classified as structural gene which contains all the information's required to produce the structural proteins for new virus particles (Various, 2008) where rev, tat, nef, vif, vpr and vpu are classified as regulatory genes for proteins that controls the ability of HIV to infect cells, cause disease or ability to replicate new copies of the virus.

Two exons, encodes the tat gene, where the first exons precedes from the gev gene that codes for 72 amino acid sequences, its N-Terminal has the ability for full trans-activation of HIV LTR-specific gene expression and the second exon, precedes within the gev that codes for 12 amino acid sequences, translated from various multiply spliced mRNA. The two tat proteins (p16 and p14) are transcriptional trans-activators for the LTR promote/LTR-directed gene expression which acts by binding the TAR RNA element. MicroRNA that regulates the gene which undergoes apoptosis ERCC1 and IER3 are processed by TAR (Klase *et al*., 2009). It is important for the replication of virus because tat can only make virus when it is provided in Trans. Tat protein was identified to having three important functional domains, the first domain ; is an

acidic amino terminal region which an hydrophobic, polar and acidic residues consistent with an amphipathic alpha-helix. The non-conservative changes in the acidic amino terminal region, inevitably reduces the activity of Tat protein and the conservative changes are well tolerated which does not reduce the Tat activity as proposed by site-directed mutagenesis. The second domain involves a cluster of seven cysteine residues that is highly conserved among divergent isolates of HIV-1, HIV-2 and SIV Tat proteins. Mutation of six out of seven cysteine residues destroys the activity. The third domain involves a stretch of basic amino acids from 49 to 57 amino sequence terminal, required for nuclear localization. A nonfunctional cytoplasmic Tat protein is implicated following a mutation within this region. GRKKR a nuclear signal motif amino an acid 48 to 52, when it is introduced at the amino acid terminus induces a nuclear localization of the normally cytoplasmic B-galactosidase gene product (Mermer *et al*., 1990).

The rev protein (p19) by binding to the RRE RNA element, shuttles the RNAs from the nucleus and the cytoplasm, and its essential for viral replication (Vasudevan *et al*., 2013). Rev protein contained in a variety of multiply spliced mRNAs is expressed from two coding exons. Since Serine substitution mutants are fully involved in transactivation function, phosphorylation of Rev at the serine residue is not important in the transactivation function (Schwartz *et al*., 1990). In the Rev protein, two distinct domain is identified, one; A basic amino acids domain where deletions within it destroys the activity of Rev, it can substitute for nuclear localization of the tat protein and also the motif NRRRRW confers nuclear localization. Nucleolar localization is essential for the activity of Rev in the sense that the removal of the amino terminal portion of the basic region results in the concentration of the Rev in the nucleus and exclusion from the nucleolus thereby resulting in an inactive protein (Vasudevan *et al*., 2013); the second important domain of Rev, involves a leucine-rich sequence, LQLPPLERLTLD (leucine residue). An inactive Rev protein, which acts as transdominant inhibitors of wild-type Rev is yielded upon substitution of the leucine residue. Rev protein has a basic region that acts as a specificity domain responsible for the interaction with RRE-containing transcripts and an activation domain that is responsible for the induction of nuclear export of RRE-containing transcripts (Mermer *et al*., 1990).

The nef protein is a 25-27kDa cytoplasmic protein contained in a single open reading frame at the 3’ end of the genome overlapping the env gene and the 3’ long terminal repeats and codes by a number of spliced mRNA. Nef contains premature termination codons in virus strains

extensively passaged in tissue culture. Nef protein downregulates CD4 and major Histocompatability complex (MHC) class 1 and class two molecules (Stumptmer *et al*., 2001). Nef protein is said to be involved in signal transduction similar to the G-protein because of its location at the inner face of plasma membrane and sequence similar with guanine nucleotide-binding proteins (G protein). Nef protein have homologous sequence with GTP binding sites of G-protein, which includes a P site with a consensus sequence of GXXXXGK (KGGLEG in the case of Nef) and two G sites with consensus sequences NKXD and WRFD (NKGE and RFDS in the case of Nef) (Stumptmer *et al*., 2001).

1.2.4 Tropism in HIV

The cell types a virus infect can be termed viral tropism and one of the clear features of HIV infection is the ability for a selective depletion of CD4+ bearing (helper) T-lymphocytes, macrophages and microglial cells with the base of selective tropism and cytopathic effect of HIV. $CD4^+$ T-lymphocytes encourages the replication of HIV, whereby cell death and syncytia are frequently associated with infection. Studies suggest the blockage of both syncytia and infection by monoclonal antibodies directed against certain epitopes of the CD4 molecules. At the entry of HIV-1 to macrophages mediated via the binding of the HIV-1 envelope glycoprotein (gp120) to the CD4 molecule on the target cells membrane and with chemokine co-receptors as indicated by their co-precipitation by antibodies directed against either of the proteins (Arrildt *et al*., 2012).

Two tropic strain of HIV-1 exist, one; the macrophage-tropic (M-tropic) strains of HIV-1 or non-syncytia-inducing strains (NSI or can be called R5 viruses), uses the B-chemokine receptor (CCR5) for entry to macrophages enabling the replication in both $CD4^+$ T cells and macrophages (Coakley *et al*., 2005) and the second strain is a T-tropic strains of HIV-1, or syncytia-inducing strains(SI; also called X4 viruses). This type of strain replicates in primary $CD4^+$ T cells also in macrophages and uses the alpha-chemokine receptor, CXCR4 for its entry (Coakley *et al*., 2005). Macrophages plays a key role in critical aspects of HIV infection, it is the first infectious HIV cell and thus the source of HIV produced during the depletion of CD4+ cells level in seropositive patient (Coakley *et al*., 2005).

A ligand for CXCR4 (alpha-chemokine SDF-1), causes the suppression of replication of T-tropic HIV-1 isolates by inhibiting the expression of CXCR4 seen on the surface of HIV target cells. Co-receptor alone does not give an explanation to viral tropism, as not all R5 viruses are able to use CCR5 on macrophages for a productive infection (Coakley *et al*., 2005). Addition to helper T-cells and monocyte-macrophages, other cells like follicular dendritic cells, Langerhans cells, glial cells and certain colon tumor cell lines are susceptible to HIV-1 infection or maintains infection when $CD4^+$ T-cell number have depleted drastically to a low level (Knight *et al*., 1990). Knight *et al* (1990) proposed the CD4 molecule is a receptor for HIV, when CD4- cells (like HeLa) which are not target for HIV infection is rendered infectable by transfection of a cloned gene. To confer infectivity to murine cells, expression of human CD4 lacks the ability, introducing a second component present in the human cell to involve in postbinding events in the infection process. Soluble CD4 and monoclonal antibodies against CD4 failed to block HIV-1 infection in the five hepatoma cell lines whereby the cell lines are seen to be infected by HIV-1 even in the absence of detectable CD4 expression

1.2.5 Syncytia Induction and Cytopathic Effect

In cytopathic effect (CPE) of HIV, have various mechanism on T-cell that accommodates the depletion of T- cell even on the infection of few cells. One of the mechanism involves the interaction of HIV-infected cells that expresses viral glycoprotein (gp120 and gp40) on the cell surface and the expression of uninfected cells on CD4 through syncytia formation (Berger *et al*., 1998). Formation of syncytia is unlikely to be the major pathway for cell loss. Syncytia formation could be transcient and by so does not significantly lead to cell death as some cells that do not form syncytia are susceptible to CPE (Clevestig *et al*., 2005). Intracellular complexing of the CD4 and gp120 or the permeability of the membrane with profuse virus budding is involved in playing a role in the single cell killing by HIV. HIV upon infection, accumulates a large amount of unintegrated viral DNA (Clevestig *et al*., 2005).

In clinical manifestation of HIV infection, AIDS patients undergoes an abnormal functioning and depletion of the T-cell caused by a co-receptor switch in the late –stage disease and T-tropic variants that infects a variety of T-cells via CXCRA (Clevestig *et al*., 2005). Tropic variants are replicated in an aggressive manner with an increased virulence that results in a rapid depletion of T-cell, immune system collapse directly or indirectly, leading to the development of

immunodeficiency which in turn allows opportunistic infections of a variety of agents (Clevestig *et al*., 2005). The most common opportunistic infection pneumocystis carinii pneumonia (PCP) represents the activation of the latent infection. *Mycobacterium avium,Cryptococcus neoformans, Candida albicans, Papilloma viruse* and *Histoplasma capsulatum* which causes skin lesion in HIV infected individuals. There are also reactivation of many other types of DNA viruses that can manifest frequently during the infectious phase of HIV like Hepatitis virus, Papova viruses and Herpes viruses. In the brain, a neurological manifestation could as a result of HIV infection or as a results of opportunistic infections like papovavirus infection, mycobacterial infection etc. Some people are resistant to certain strain of HIV like the mutation in CCR5DELTA32 confers resistance to infection caused by R5 virus because it inhibites the binding of HIV to a co-receptor, therefore reducing its ability of infecting target cells (Clevestig *et al*., 2005)

1.3 Overview of C-reactive Protein (CRP)

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* (Tillet and Francis, 1930). In the presence of calcium, CRP binds to polysaccharides such as phosphocholine (PCh) on microorganisms and triggers the classical complement pathway of innate immunity by activating C1q. CRP has many homologs in vertebrates and some invertebrates and is a member of the pentraxin family, which includes other structurally related molecules such as serum amyloid A (Gerwuz *et al*., 1982). Transcriptional induction of the *CRP* gene mainly occurs in hepatocytes of the liver in response to increased levels of inflammatory cytokines, especially interleukin-6 (IL-6). C-reactive protein exhibits elevated expression during inflam-matory conditions such as rheumatoid arthritis, some cardio-vascular diseases, and infection. As an acute-phase protein, the plasma concentration of CRP deviates by at least 25% during inflammatory disorders. The highest concentrations of CRP are found in serum, with some bacterial infections increasing levels up to 1,000-fold (Thompson *et al*., 1999). However, when the stimuli ends, CRP values decrease exponentially over 18–20hours, close to the half-life of CRP. CRP plasma levels increase from around 1μg/mL to over 500μg/mL within 24–72 h of severe tissue damage such as trauma and progressive cancer. IL-6 is reported to be the main inducer of *CRP* gene expression,

with IL-1 enhancing the effect (Szalai *et al*., 1988). However, although IL-6 is necessary for *CRP* gene induction, it is not sufficient to achieve this alone. There are many factors that can alter baseline CRP levels including age, gender, smoking status, weight, lipid levels, and blood pressure. The average levels of CRP in serum in a healthy Caucasian is around 0.8 mg/L, but this baseline can vary greatly in individuals due to other factors, including polymorphisms in the *CRP* gene (Clevestig *et al*., 2005). The human *CRP* gene can be found at 1q23.2 on the long arm of chromosome 1, and to date, there have been no allelic variations or genetic deficiencies discovered for this gene although some polymorphisms have been identified (Clevestig *et al*., 2005). For example, up to 50% of baseline variance in CRP is associated with the number of dinucleotide repeats found in an intronic region of the gene. There is no significant seasonal variation in baseline CRP concentration; however, twin studies show a significant heritable component in baseline CRP values that is independent of age and body mass index. Pankow *et al*. (2001) found evidence that inter-individual variation in blood CRP levels is 35–40% heritable. Increased CRP levels are typically associated with disease, but liver failure is one condition observed to impair CRP production. Very few drugs reduce elevated CRP levels unless they treat the underlying pathology that is causing the acute-phase stimulus (Pankow *et al*. , 2001).

1.3.1 Structure and Synthesis of C-reactive Protein

C-reactive protein is a 206-amino acid member of the short pentraxin family, alongside serum amyloid P component (SAP), with high phylogenetic *conservation (*Mantovani *et al*., 2008*).* Pentraxins share a characteristic structure: five identical nonglycosylated globular subunits - each of which is constituted by two β-pleated sheets, which are noncovalently associated and arranged in a symmetric cyclic pattern around a central pore, determining a pentameric, discoidal, and flattened configuration (Thompson *et al*., 1999).

C-reactive protein is predominantly synthesized in the liver (1q23.2), typically within the transcriptional phase of the response to pro-inflammatory cytokines. IL-6 appears to be the main regulator, by promoting *de novo* synthesis of CRP via upregulation of C/EBPβ and C/EBPδ, key transcription factors in this process (Thompson *et al*., 1999). In addition, IL- 6 signaling may be reinforced by IL-1β and TNF, both of which increase transcription rate of *CRP* (Thompson *et al*., 1999). Serum CRP levels have also been closely linked to signaling by pro-inflammatory

cytokines released by visceral adipose tissue (Black *et al*., 2004). Indeed, both hypo-adiponectinemia and hyperleptinemia, two adipokine disturbances common in subjects with obesity and insulin resistance, have been linked to increased hepatic production of CRP, as well as augmented *in situ* synthesis of CRP in vascular endothelial cells in hyperleptinemia. Following synthesis and release into circulation, serum CRP levels tend to increase significantly 6–8 hours after initial stimulation, peaking at 24–48 hours, with a half-life of approximately 19 hours. CRP concentration in circulation is primarily determined by its synthesis rate. Although the liver is the main site for production and release of CRP, its mRNA has been found in a myriad of extrahepatic sites, including adipose tissue, lungs, epithelial cells of renal cortical tubules, lymphocytes, and atherosclerotic lesions, in both macrophages and smooth muscle cells (Taube *et al*., 2012). Numerous studies have focused on identifying other extrahepatic sources of CRP production that may underlie the lower and more sustained CRP concentrations which appear to predict cardiovascular risk; these include findings of CRP synthesis in coronary smooth muscle cells in response to inflammatory cytokines. Locally produced CRP may play an important role on endothelial cell activation (Calabro *et al*., 2003)

1.3.2 Isoforms of C-reactive Protein

The pentameric protein, termed native CRP (nCRP), is characterized by a discoid configuration of five identical non-covalently bound subunits, each 206 amino acids long with a molecular mass of about 23kDa. These five subunits lie in the same orientation around a central pore and arranged in a characteristic “lectin fold” with a two-layered beta sheet (Eisenhardt *et al*., 2009). Each subunit lies with the phosphocholine binding site facing the “recognition” face of the nCRP molecule. The molecule has a ligand-binding face that has a characteristic feature of having two calcium ions per protomer. The calcium ions are important for the stability and binding of ligands. The “opposite” face interacts with the C1q aspect of the complement pathway as well as interacting with Fc receptors. The pentameric protein is synthesized primarily in liver hepa tocytes but has also been reported to be synthesized in other cell types such as smooth muscle cells, macrophages, endothelial cells, lymphocytes, and adipocytes (Calabró *et al*., 2005). CRP is first synthesized as monomers and then assembled into the pentamer in the endoplasmic reticulum of the source cell. In hepatocytes, the pentameric protein is retained in the endo-

plasmic reticulum by binding to two carboxylesterases, gp60a and gp50b. While in a resting (non-inflammatory) state, CRP is released slowly from the endoplasmic reticulum, but following an increase in inflammatory cytokine levels, the binding CRP to the carboxylesterases decreases and CRP is secreted rapidly (Thompson *et al*., 1999).

The stimulation of CRP synthesis mainly occurs in response to pro-inflammatory cytokines, most notably IL-6 and to a lesser degree IL-1 and tumor necrosis alpha (TNF-α) (Zhang *et al*., 1996). Pentameric CRP can be irreversibly dissociated, with the resultant free subunits termed monomeric (or modified) CRP (mCRP). The dissociation of nCRP into free subunits has been observed at either high concentrations of urea or high temperatures in the absence of calcium. The mCRP molecules are distinguished from nCRP by their different antigenic, biological, and electrophoretic activities and by the fact that they express different neoepitopes (Khreiss, *et al*., 2004). The two isoforms of CRP have been shown to have distinct biological functions in the inflammatory process. For example, Khreiss *et al*. (2004) provided evidence that nCRP suppresses the adherence of platelets to neutrophils, whereas mCRP enhances these interactions. This difference in function can be explained by the two isoforms binding to differing types of Fcgamma (Fcγ)-receptor involved in the signaling process. The mCRP isoform utilizes the low-affinity immune complex binding immunoglobulin G (IgG) receptor called FcγRIIIb (CD16b) on neutrophils and FcγRIIIa (CD16a) on monocytes, while nCRP binds to the low-affinity IgG receptor FcγRIIa (CD32) (Du Clos, 2013). Evidence is emerging of new structural intermediates of CRP with biological function. Ji *et al*. (2007) found that the native protein first dissociates into subunits while retaining some of the native conformation before fully dissociating into mCRP. This intermediate, termed $mCRP_m$, is formed when the nCRP is bound to cell membranes and then dissociates, allowing the subunits to retain some of the conformation before fully dissociating into mCRP subunits on detachment from the membrane. It is suggested that this transitional process allows for more effective regulation of CRP function, with mCRPm allowing for the enhanced activation of the classical complement pathway (Ji *et al*. 2007). Further work needs to be conducted to determine the biological functions of the $mCRP_m$ intermediate, but initial findings suggest that it behaves in a similar manner to mCRP, typically promoting pro-inflammatory activity (Thompson *et al*., 1999).

1.3.3 The Role of C-reactive Protein in Disease and Pathology

The majority of CRP research has focused on the role of CRP and its isoforms on cardiovascular disease and stroke. CRP is used as a clinical marker of inflammation, with elevated serum levels being a strong independent predictor of cardiovascular disease in asymptomatic individuals (Ridker *et al*., 2002). CRP levels have been linked to prognosis in patients with atherosclerotic disease, congestive heart failure, atrial fibrillation, myocarditis, aortic valve disease, and heart transplantation, suggesting that it has an active role in the pathophysiology of cardiovascular disease (Albu *et al*., 1994). High- sensitivity assays, such as nephelometric assays, are used to detect baseline levels of CRP and patients who are at risk of cardiovascular disease. An individual with a CRP level higher than 3 mg/L has an increased risk of coronary heart disease, and this risk increases in those with type 2 diabetes. Increased levels of CRP have been found in patients with appendicitis, cholecystitis, pancreatitis, and meningitis. In patients suffering possible symptoms of appendicitis, acute appendicitis can be excluded in those with CRP levels lower than 25 mg/L in blood taken 12 h after the onset of symptoms (Albu *et al*., 1994). When clinical symptoms of cholecystitis occur concurrently with CRP levels of over 30 mg/L, an accurate diagnosis of cholecystitis can be obtained with 78% sensitivity, suggesting that CRP is a more sensitive marker than erythrocyte sedimentation rate and white cell count in supporting cholecystitis diagnosis (Albu *et al*., 1994).

In terms of acute pancreatitis, CRP levels of more than 210 mg/L were able to discriminate between mild and severe cases, with 83% sensitivity and 85% specificity. Serum CRP is elevated in bacterial meningitis, and resolution of symptoms following treatment with antibiotics is slow in those with the highest CRP levels. Measurement of CRP in cerebrospinal fluid has a sensitivity of 100% and a specificity of 94% for differentiating between patients with bacterial meningitis, viral meningitis, and no infection. Although studies have shown that CRP levels increase during infections and inflammatory diseases, the precise role of CRP isoforms in their development and progression remains largely unknown (Albu *et al*., 1994). Thus, urgent investigations are required to determine the effects of each CRP isoform on specific cellular processes during disease development. Evidence shows that in general nCRP tends to exhibit more anti-inflammatory activities relative to the mCRP isoform, possibly because nCRP limits

the generation of the membrane attack complex (MAC) and C5a, thus inhibiting the alternative complement activation (Albu *et al*., 1994). In contrast, mCRP can have marked pro-inflammatory properties both *in vitro* and *in vivo* by promoting monocyte chemotaxis and the recruitment of circulating leukocytes to areas of inflammation *via* Fcy-RI and Fcy-RIIa signaling (Thiele, 2014). Thus, in addition to therapeutic strategies to inhibit CRP activity, more targeted therapies have been proposed for the treatment of CRP-mediated pathologies, including inhibiting mCRP activity or preventing the dis-sociation of nCRP into mCRP (Albu *et al*., 1994).

1.3.4 C-reactive Protein and Inflammation

C-reactive protein levels are known to increase dramatically in response to injury, infection, and inflammation (Figure 1). CRP is mainly classed as an acute marker of inflammation, but research is starting to indicate important roles that CRP plays in inflammation. CRP is the principal downstream mediator of the acute-phase response following an inflammatory event and is primarily synthesized by IL-6-dependent hepatic biosynthesis (Pradhan *et al*., 2001). The main role of CRP in inflammation tends to focus around the activation of the C1q molecule in the complement pathway leading to the opsonization of pathogens (Pradhan *et al*., 2001). Although CRP can initiate the fluid phase pathways of the host defense by activating the complement pathway, it can also initiate cell-mediated pathways by activating complement as well as to binding to Fc receptors of IgG. CRP binds to Fc receptors with the resulting interaction leading to the release of pro-inflammatory cytokines. CRP also has the ability to recognize self and foreign molecules based on the pattern recognition, something that other activators of complement such as IgG cannot achieve because these molecules only recognize distinct antigenic epitopes (Pradhan *et al*., 2001).

Evidence suggests that CRP is not only just a marker of inflammation but also plays an active role in the inflammatory process. However, most early research in the literature only refers to CRP and does not distinguish between the two isoforms. Thus, unlike more recent publications, the findings of early work on CRP can seem somewhat unclear and at times conflicting since it was often not specified which CRP isoform was measured or utilized in experiments, whether responses attributed to nCRP were in fact possibly due to partial/full dissociation into mCRP or if lipopolysaccharide (LPS) contamination could be present (Pradhan *et al*., 2001). More recent studies generally distinguish between the differential effects of each CRP isoform on

inflammatory processes, but since antibodies for mCRP are not commercially available to date, few laboratories are able to conduct studies investigating the mCRP isoform. There is increasing evidence that CRP has a functional role in the inflammatory process. It is well established that CRP is an acute marker of inflammation and that its concentration incre-ases in circulation during inflammatory events. CRP is deposited at sites of inflammation and tissue damage in both naturally occurring and experimental conditions (Braig *et al*., 2017). However, there is a raft of published data investigating CRP that does not consider its two different isoforms. Understandably, when some of these studies were conducted, the existence of two CRP isoforms was not well established and available antibodies would have been raised against the pentameric nCRP alone. Another issue with published data is that CRP localization is often investigated in only a narrow range of inflammatory conditions and tissue types. Although the mCRP isoform has been shown to be insoluble in plasma, it becomes localized in inflamed tissues and amplifies a pro-inflammatory response by a positive feedback loop. The literature suggests that CRP binds to damaged cell membranes and contributes to the inflammatory response, with CRP molecules becoming associated with terminal complement complexes, especially in atherosclerotic lesions (Braig *et al*., 2017).

Lagrand *et al*. (1997) provided evidence that CRP localizes to infarcted heart tissue and promotes local complement activation, triggering further damage to the heart tissue. Gitlin *et al*. (1977) concluded that CRP was localized to the nuclei of cells within the synovium of rheumatoid arthritis patients, but the cell type was not identified at the time. However, other studies indicate no significant localization of CRP in a number of pathologies, suggesting that CRP is found predominantly in the fluid phase rather than becoming deposited in tissues at sites of inflammation or injury. There has been little research conducted on the localization of CRP in inflammatory cells to date. There is a correlation between the localization of CRP in neutrophil infiltrates, especially in lesions of vasculitis and allergic encephalomyelitis (Du Clos *et al*., 2013).

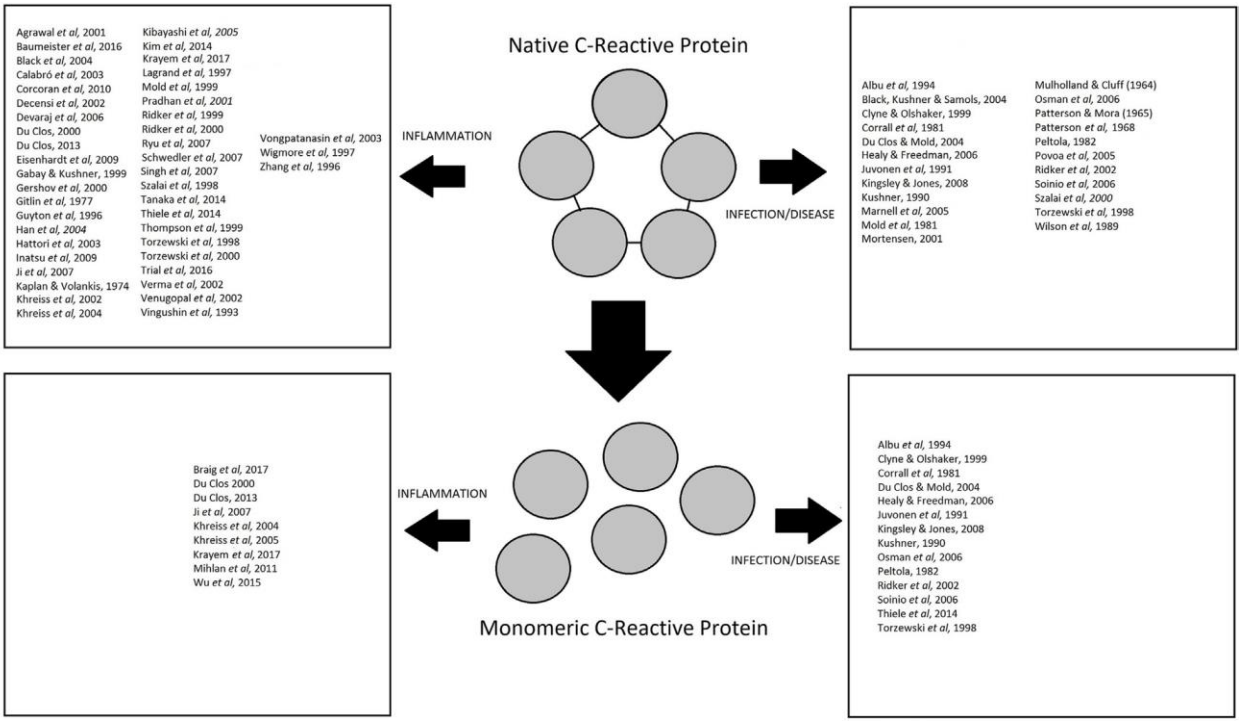

Fig 1: The role of native C-reactive protein (CRP) and monomeric CRP in inflammation, infection, and disease (Sproston and Ashworth, 2018).

1.3.5 The Role of C-reactive Protein in Development and Progression of Infection

C-reactive protein is a marker for inflammation, and its levels increase during bacterial infection. Kingsley and Jones (2008) stated that CRP increases during infection in response to monocytic mediators such as IL-1 and IL-6 and that it has a stable decay rate. It is thought that most of the interaction between CRP and the immune response to pathogens involves the binding of CRP to phosphorylcholine and the activation of the classical complement pathway. Mold *et al*. (1981) showed that CRP provides mice with protection against infection by the gram-positive pathogen *Streptococcus pneumoniae* by binding to a phosphorylcholine determinant of the pathogen cell wall and activating the complement pathway. Mice pretreated with 200μg CRP before being infected showed an increase in percentage survival across all pathogen doses tested. The study concluded that the ability of CRP to protect against infection lies in its ability to bind to pneumococcal polysaccharide C in the bacterial cell wall (Mold *et al*., 1981). Szalai *et al*. (2000) showed that CRP can confer protective benefits against *Salmonella enterica* serovar

Typhimurium, a gram-negative pathogen that provides a model of typhoid fever in mice. By using transgenic mice expressing human CRP, the study found that CRP offered protection against a low dose of Typhimurium and increased resistance to a fatal infection with a low dose of Typhimurium. Szalai *et al*. (2000) concluded that CRP increases the early clearance of intravenously injected bacteria from the blood and reduces dissemination of bacteria to the liver and spleen during the initial stages of infection, thus allowing the mice to survive infection. Marnell *et al*. (2005) reviewed the protective role CRP against *Haemophilus influenza* infection in both transgenic and wild- type mice treated by passive inoculation. CRP was shown to bind the pneumococcal C-polysaccharide of bacteria and opsonize them for phagocytosis. This process did not require the use of the Fcγ receptors, suggesting that CRP is not primarily protective by direct opsonization but more likely through activation of complement and subsequent opsono-phagocytosis. Kingsley and Jones (2008) tested whether CRP could be used to distinguish different types of infections. They discovered that mean CRP levels in a spreading infection were higher than those in other colonized, critically colonized, and locally infected groups. All cases of infection showed an increase in CRP levels compared to non-infected controls, but CRP levels could not distinguish between the infection types, showing that it is infection in general that causes CRP levels to increase, rather than the type of infection. This was also noted by Healy and Freedman (2006) who showed that CRP levels can be used only as a method of detecting infection, rather than distinguishing it. C-reactive protein can mediate host responses to *Staphylococcus aureus* including some protective function against infection and an increase in phagocytosis of this pathogen. Povoa *et al*. (2005) stated that the normal CRP level for the healthy population is about 0.08 mg/dL, and this increases to more than 8.7mg/dL during chronic *S. aureus* infection. Thus, CRP can be used as an indicator of infection, alongside a body temperature of more than 38.2°C. Patterson and Mora (1965) observed that enhanced resistance to intra-articular infection with *S. aureus* in chickens was associated with an increase in serum CRP and that isolated preparations of the protein produced antibacterial activity. Mulholland and Cluff (1964) discovered that endotoxin-induced changes in resistance to local infection with *S. aureus* in rabbits were correlated with the circulating levels of leukocytes in the blood. The study showed that induced resistance was paralleled by an increase in CRP and leukocytes. This was collaborated by Patterson et al. (1968) who found an association between CRP and non-specific resistance to infection, including *S. aureus* and showed that CRP was acting upon the

polysaccharide bacterial cell wall. Black *et al*. (2004) stated that CRP enhances the *in vitro* phagocytosis of many microorganisms (including *S. aureus*) by leukocytes. Their work confirmed this finding even in the absence of complement, suggesting that the enhancement of phagocytosis by CRP is due to the interactions with Fcγ receptors.

1.3.6 C-reactive Protein and the Complement System

Complement is one of the major defenses of the human immune system that is involved in the clearance of foreign particles and organisms after recognition by antibody. The complement pathway is made up of 35 plasma or membrane proteins that are important in immunity and the defense of the host against microbial infection. The components of the complement system can be activated in three different pathways to trigger a cascade of proteins, which are used to help bind microbial surfaces for the immune system to recognize and activate phagocytosis (Li *et al*., 2007). The classical pathway is triggered by a target bound antibody, whereas the lectin pathway is triggered by microbial repetitive polysaccharide structures and the alternative pathway is triggered by recognition of other foreign surface structures. Even though the triggers are different, the three pathways merge at a pivotal activation of the C3 and C5 convertases. A majority of the components are synthesized in the liver, C1 in the intestinal epithelium, and factor D in the adipose tissue (Li *et al*., 2007).

The role of CRP in activating the complement pathway has been extensively investigated. In 1974, Kaplan and Volanakis first described the ability of CRP to activate the classical comple ment pathway using C-polysaccharide and phospholipid ligands. The activation of complement by CRP is considered a crucial step since when complement was depleted, and the effects of CRP were abrogated. The opposite face of the CRP molecule, which is typically complexed with polyvalent ligand or chemically cross-linked, binds to C1q and activates the classical complement pathway. C1q is a large 460kDa molecule made up of six identical subunits, each made up of three structurally similar but distinct polypeptide chains. This process requires the use of calcium ions for the stable formation of the C1 complex. CRP is most effective during the early classical pathway activation of C1, C4, and C2. This is because the ligand-bound interaction with C1q leads to the formation of C3 convertase, triggering the complement

activation of C1–C4 but with little activation of the late complement proteins C5–C9. Activation of complement by CRP varies from activation by antibody in that CRP has selective activation of early components without the need to form the MAC. In addition to activating the classical complement pathway, CRP can inhibit the alternative complement pathway by decreasing C3 and C5 convertase activities and inhibiting the complement amplification loop. This is achieved by the recruitment of factor H to the cell surface and by preventing C5 convertase cleaving C5 to recruit neutrophils and prevent the formation of the MAC (Mihlan *et al*., 2011).

As the levels of CRP increase, this causes decreased binding of C3b and C5b-9 to liposomes, possibly also explaining the lack of C5–C9 consumption by CRP during classical pathway activation. Both the initiator (C1q) and the inhibitor (C4bp) of the classic complement pathway compete for mCRP binding, with the competition controlling the local balance of activation and inhibition of the pathway in tissues. Interestingly, mCRP but not nCRP binds the C4bp inhibitor, suggesting that mCRP rather than nCRP is able to provide a high degree of control over the classic complement pathway (Mihlan *et al*., 2011).

1.3.7 C-reactive Protein in Nitric Oxide Production and Regulation

C-reactive protein has the ability to attenuate NO production with a marked reduction in *in vitro* angiogenesis, cell migration, and capillary-like tube formation by CRP at concentrations known to cause cardiovascular risk. Eisenhardt *et al*. (2009) showed that CRP upregulated the expression of adhesion molecules and inhibited endothelial nitric oxide synthase (eNOS) expression, indicating a role for CRP in the production of NO. Several studies have revealed that CRP inhibits NO production *via* down-regulation of eNOS in cardiovascular endothelial cells, thereby inhibiting angiogenesis *in vitro* and promoting the pathogenesis of atherosclerotic vascular disease through vasoconstriction, leukocyte adherence, and inflammation (Mihlan *et al*., 2011).. Another study found that it was in fact the nCRP isoform that downregulated eNOS and thus impaired endothelial function in ApoE knockout mice, *via* a mechanism thought to involve iNOS (Mihlan *et al*., 2011).

Eisenhardt *et al*. (2009) provided evidence that nCRP suppresses endothelium- dependent NO-mediated dilation by activating the p38 mitogen- activated protein kinase (MAP kinase) pathway

and NADPH oxidase, suggesting that multiple pathways could be interacting with this process. In contrast, mCRP has the opposite effect, enhancing NO production in neutrophils *via* upregulation of eNOS with reverse transcription polymerase chain reaction showing an amplification of eNOS mRNA, but not iNOS or nNOS mRNA. This study highlighted that mCRP initiates calcium (Ca^{2+}) mobiliza-tion and activation of calmodulin and PI_3 kinase to induce NO formation in neutrophils (Mihlan *et al*., 2011). The effect of CRP isoforms on other inflammatory cells, such as monocytes or macrophages, has not been investigated to date.

1.3.8 The Role of C-reactive Protein in the Production of Inflammatory Cytokines

Interleukin-6 is a pro-inflammatory cytokine secreted by various cells including inflammatory cells, keratinocytes, fibroblasts, and endothelial cells. It regulates the acute-phase response, and its main role involves the host response to infection. Even though it is predominantly a pro-inflammatory cytokine, in some cells, IL-6 can have regenerative and anti-inflammatory effects through the activation of membrane-bound IL-6 receptor signaling. Interleukin-6 is synthesized in the initial stages of inflammation and induces a number of acute-phase proteins, including CRP. IL-6 can also reduce the production of fibronectin, albumin, and transferrin as well as the promotion of CD4+ T helper cells, which initiates the linking of innate and acquired immunity. There is a correlation between increasing levels of IL-6 during inflammation and increasing levels of CRP, with IL-6 inducing the *CRP* gene (Mihlan *et al*., 2011).

However, most investiga-tions of CRP production by IL-6 generally fail to indicate which isoforms of CRP are generated. In some cases, the antibodies used suggest that nCRP is present, but given IL-6 occurs at the sites of inflammation, the pentameric CRP may be dissociating into mCRP. When CRP levels become elevated in atheroma, this leads to the induction of IL-6 by macrophages indicating that CRP may have a direct effect on IL-6 release. Krayem *et al*. (2017) found that a combination of mCRP, nCRP, and oxLDL decreases IL-6 production in a model of atherosclerosis. This triple combination suggests that nCRP might downregulate the IL-6 release by mac-rophages that have been stimulated by both mCRP and oxLDL (Krayem *et al*., 2017).

Interleukin-8 is a cytokine produced by numerous cell types including inflammatory cells, keratinocytes, fibroblasts, and endothelial cells. IL-8 acts as a potent chemo-attractant of

neutrophils and is overexpressed in chronic inflammatory diseases and during septic shock. IL-8 stimulates the release of granules from neutrophils by a process called degranulation. These granules contain a range of antimicrobial effectors that can help combat infection. Neutrophils are the first inflammatory cells to arrive at the site of inflammation, and they carry out the phagocytosis of bacteria and release chemotactic mediators that recruit other leukocytes to the affected tissue. Kibayashi *et al*. (2005) indicated that CRP plays a role in atherosclerosis *via* enhanced IL-8 production and increased expression of IL-8 mRNA in a CRP dose-dependent manner. They showed that CRP promotes IL-8 production *via* the activation of the ERK, p38 MAPK, and JNK pathways. Conversely, Wigmore *et al*. (1997) indicated that IL-8 induces CRP production in hepatocytes, providing a potential feedback loop. The effect of the different CRP isoforms on IL-8 production has been investigated. Khreiss *et al*. (2004) showed that nCRP had no detectable effect on the production of IL-8, whereas mCRP increased IL-8 production and IL-8 gene expression, promoting pro-inflammatory activity through a p38 MAPK-dependent mechanism. When treated with anti-CD16, there was inhibition of mCRP-stimulated NO formation and IL-8 release.

Tumor necrosis factor-α is a component of the acute-phase response and is mainly produced by monocytes and macrophages but can be produced by numerous other immune cells such as neutrophils, natural killer cells, and eosinophils. TNF-α is not usually detectable in a healthy host, but levels become elevated in a number of inflammatory and infectious conditions. The main stimulant of TNF-α production is LPS, but many other pathological conditions such as trauma infection, impaired wound healing, and heart failure also induce its production (Krayem *et al*., 2017). TNF-α mediates various processes such as cell proliferation, differentiation, and apoptosis. Studies have shown a correlation between TNF-α production and the concentration of CRP. TNF-α induces a dose-dependent secretion of CRP in hepatocytes, which corresponds to an increase in CRP mRNA . Conversely, elevated CRP levels in atheroma leads to the induction of IL-1β, IL-6, and TNF-α production by macrophages. Research shows a close relationship between TNF-α and IL-6 levels in inflammation (Popa *et al*., 2007), with both TNF-α and IL-6 inducing the transcription of CRP. However, there is some contradictory evidence showing a potential inhibitory effect of CRP on TNF-α production, suggesting that there could be a negative feedback mechanism whereby elevated levels of CRP inhibit further stimulation of CRP by reducing the TNF-α production. A combination of mCRP, nCRP, and oxLDL also causes a

decrease in both TNF-α and IL-6 production in a macrophage model of atherosclerosis (Krayem *et al*., 2017). This triple com-bination suggests that nCRP might downregulate TNF-α and IL-6 production by macrophages stimulated by both mCRP and oxLDL.

1.4 Overview of Urinary Tract Infection

According to the CDC, UTIs are the most common bacterial infection requiring medical care, resulting in 8.6 million ambulatory care visits in 2007, 23% of which occurred in the emergency department (CDC, 2017). Over 10.8 million patients in the United States visited the emergency department for the treatment of UTIs between 2006 and 2009 and 1.8 million patients (16.7%) were admitted to acute care hospitals (CDC, 2017). The economic burden of using the emergency departments for the treatment of UTIs is estimated at $2 billion annually. In addition, UTIs rank as the number one infection that leads to an antibiotic prescription after a physician's visit. Catheter-associated UTIs (CA-UTIs) are the most common type of health care–associated infections reported to the National Healthcare Safety Network, making up two-thirds of hospital-acquired UTIs (CDC, 2017). The symptoms of UTIs are generally mild, and inappropriate use of antibiotics can lead to antibiotic resistance; therefore, it is important to establish the appropriate criteria for treatment using narrow-spectrum antibiotics for the optimal duration.

Clinically, UTIs are categorized as uncomplicated or complicated. Uncomplicated UTIs typically affect individuals who are otherwise healthy and have no structural or neurological urinary tract abnormalities, these infections are differentiated into lower UTIs (cystitis) and upper UTIs (pyelonephritis). Several risk factors are associated with cystitis, including female gender, a prior UTI, sexual activity, vaginal infection, diabetes, obesity and genetic susceptibility. Complicated UTIs are defined as UTIs associated with factors that compromise the urinary tract or host defense, including urinary obstruction, urinary retention caused by neurological disease, immunosuppression, renal failure, renal transplantation, pregnancy and the presence of foreign bodies such as calculi, indwelling catheters or other drainage devices. In the United States, 70–80% of complicated UTIs are attributable to indwelling catheters10, accounting for 1 million cases per year (CDC, 2017). Catheter-associated UTIs (CAUTIs) are associated with increased morbidity and mortality, and are collectively the most common cause of secondary bloodstream infections. Risk factors for developing a CAUTI include prolonged catheterization, female

gender, older age and diabetes. UTIs are caused by both Gram-negative and Gram-positive bacteria, as well as by certain fungi. The most common causative agent for both uncomplicated and complicated UTIs is uropathogenic *Escherichia coli* (UPEC) (CDC, 2017).

For the agents involved in uncomplicated UTIs, UPEC is followed in prevalence by *Klebsiella pneumoniae*, *Staphylococcus saprophyticus*, *Enterococcus faecalis*, group B *Streptococcus* (GBS), *Proteus mirabilis*, *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Candida spp*. For complicated UTIs, the order of prevalence for causative agents, following UPEC as most common, is *Enterococcus* spp., *K. pneumoniae*, *Candida* spp., *S. aureus*, *P. mirabilis*, *P. aeruginosa* and GBS9. Patients suffering from a symptomatic UTI are commonly treated with antibiotics; these treatments can result in long-term alteration of the normal microbiota of the vagina and gastrointestinal tract and in the development of multidrug-resistant microorganisms (CDC, 2017).

1.4.1 Epidemiology and Pathophysiology of UTI

Up to 60% of women have at least one symptomatic UTI during their lifetime. Around 10% of women in the United States have one or more episodes of symptomatic UTIs each year. Young, sexually active women 18–24 years of age have the highest incidence of UTIs. About 25% of these women have spontaneous resolution of symptoms, and an equal number become infected (CDC, 2017). The prevalence of UTIs in men is significantly lower than in women, occurring primarily in men with urologic structural abnormalities and in older adult men. Lower UTIs, also known as cystitis, are significantly more prevalent in women than in men. This is primarily because of anatomic differences, including shorter urethral length and moist periurethral environment in women. Urinary tract infections typically start with periurethral contamination by an uropathogen residing in the gut, followed by colonization of the urethra and, finally, migration by the flagella and pili of the pathogen to the bladder or kidney.

Bacterial adherence to the uroepithelium is a key in the pathogenesis of UTI. Infections occur when bacterial virulence mechanisms overcome efficient host defense mechanisms. Upper UTIs, also known as pyelonephritis, develop when uropathogens ascend to the kidneys by the ureters. Infections can occur when bacteria bind to a urinary catheter, a kidney, or a bladder stone or

when they are retained in the urinary tract by a physical obstruction. In severe cases of pyelonephritis, the affected kidney may be enlarged, with raised abscesses on the surface (as revealed in imaging studies). *Staphylococcus aureus* bacteremia or endocarditis can lead to hematogenous seeding of the bacteria to the kidneys, causing suppurative necrosis or abscess formation within the renal parenchyma. In contrast, gram-negative bacilli rarely cause kidney infection by the hematogenous route. According to an experimental model of pyelonephritis, the main renal abnormality reported is the inability to maximally concentrate the urine. This concentration defect occurs early in the infection and is rapidly reversible with antibiotic therapy. An obstruction can lead to progressive destruction of the affected kidney and subsequent renal insufficiency.

1.4.2 Predisposing Factors

In the non-pregnant adult woman with a normal urinary tract, bacteriuria infrequently progresses to symptomatic cystitis or pyelonephritis. The urethra is usually colonized with bacteria, and sexual intercourse can force bacteria into the female bladder. Furthermore, spermicides increase colonization of the vagina with uropathogens and adherence of *Escherichia coli* to vaginal epithelial cells. Patients with structural abnormalities develop UTIs largely from obstruction of the urine flow. Urinary stasis increases susceptibility to infection. Men of any age and pregnant women are susceptible to lesions that result in obstruction (CDC, 2017).

1.4.3 Typical Causative Organisms and Antibiotic Resistance

Urinary tract infections are primarily caused by gram-negative bacteria, but gram-positive pathogens may also be involved. More than 95% of uncomplicated UTIs are monobacterial. The most common pathogen for uncomplicated UTIs is *E. coli* (75%–95%) followed by *Klebsiella pneumoniae*, *Staphylococcus saprophyticus*, *Enterococcus faecalis*, group B streptococci, and *Proteus mirabilis*. Distribution of uropathogens may differ by type of infection or patient population. *E. coli* can cause both uncomplicated and complicated UTIs. *P. mirabilis*, *Pseudomonas aeruginosa*, and *Enterococcus* spp. predominantly cause complicated infections and are more commonly isolated in hospitals and long-term care facilities. *Corynebacterium urealyticum* is an important nosocomial uropathogen associated with indwelling catheters. *S.*

saprophyticus tends to cause infection in young women who are sexually active, accounting for 5%–15% of acute cystitis in the United States. Coagulase positive staphylococci can invade the kidney from hematogenous spread, resulting in renal abscesses. Fungi, particularly *Candida* spp., may cause UTIs in patients with indwelling catheters who are receiving antibiotic therapy.

Antibiotic resistance to *E. coli* has steadily been increasing; thus, incorporating the local antibiotic susceptibility patterns of *E. coli* into clinical decision processes is critical to optimal antibiotic selection. According to the Surveillance Network of urine isolates from female outpatients in the United States, *E. coli* resistance rates to nitrofurantoin, ciprofloxacin, and trimethoprim/ sulfamethoxazole in 2012 were 0.9%, 11.8%, and 22.2%, respectively (Sanchez, 2016). Susceptibility rates with cephalosporins and fluoroquinolones among 2013–2014 isolates were significantly lower in hospital- than in community acquired UTIs, and *E. coli* resistance to ciprofloxacin was 29% in patients 65 and older (CDC, 2017). The Study for Monitoring Antimicrobial Resistance Trends reported that among 3498 *E. coli* isolates from hospitals in Canada and the United States, extended-spectrum β-lactamase (ESBL) rates increased from 7.8% in 2010 to 18.3% in 2014 (CDC, 2017). Of note, percent susceptibilities of *E. coli* isolates collected in 2014 in the United States to ceftriaxone, cefepime, ciprofloxacin, levofloxacin, piperacillin/ tazobactam, and amikacin were 80.5%, 83.4%, 64,7%, 65.3%, 96.2%, and 99.4%, respectively (Lob, 2016). In recent years, worldwide spread of ESBL-producing *E. coli* such as CTX-M-15 has emerged as a significant cause of community-associated UTIs (CDC, 2017).

Highly antibiotic resistant uropathogens, including AmpC β-lactamase or carbapenemase-producing Enterobacteriaceae (e.g., New Delhi metallo-β-lactamase [NDM]) and *Acinetobacter* spp., are increasingly being reported among health care–associated complicated UTIs. Carbapenem-resistant Enterobacteriaceae (CRE) is a growing concern worldwide.

According to the CDC, an isolate is considered a CRE if it is resistant to imipenem, meropenem, doripenem, or ertapenem by susceptibility testing or if it is identified to have a carbapenemase by genotype testing (CDC, 2017). The CDC is tracking CRE types such as *K. pneumoniae* carbapenemase (KPC), NDM, IMP-1, and OXA β-lactamases. Among these, KPC is the most prevalent type in the United States, and NDM is the most antibiotic resistant type, often resistant to new cephalosporin/β-lactamase inhibitor combinations (CDC, 2017).

1.4.4 Diagnosis

A urinalysis is often used to detect UTIs, and a clean-catch dipstick leukocyte esterase test is a rapid screening test for detecting pyuria, with a high sensitivity and specificity for detecting more than 10 WBC/mm3 in urine (CDC, 2017). Of note, the presence of pyuria is nonspecific and does not always indicate clinical UTI. Furthermore, bacteriuria alone is not a disease and usually does not necessitate treatment. For symptomatic UTIs, most patients have more than 10 leukocytes/ mm3; however, negative tests for bacteriuria may occur because of low bacterial burden. Organisms like *E. coli*, *Klebsiella* spp., *Enterobacter* spp., *Proteus* spp., *Staphylococcus* spp., and *Pseudomonas* spp. reduce nitrate to nitrite in the urine, and the presence of nitrite on a urinalysis is another marker of UTIs. Urine culture is not recommended for managing acute uncomplicated cystitis. However, for acute pyelonephritis and any type of complicated UTIs, a urine culture should be obtained before empiric therapy to optimize the subsequent definitive antibiotic regimen once the susceptibility results are available. Most symptomatic UTIs have 105 CFU/mL or greater, indicating a 95% probability of infection. One study of 226 healthy premenopausal women with acute cystitis showed that the detection of 10–102 CFU/mL of *E. coli* in voided clean-catch midstream urine was highly predictive of bladder infection (CDC, 2017). However, detection of *Enterococcus spp.* and group B streptococci at any colony count in this population was not predictive of cystitis but suggested urethral contamination. Urine in the bladder is normally sterile.

In contrast, the urethra and periurethral areas are not sterile, and contamination can occur during urine collection. Therefore, proper cleansing before urine collection is critical, especially in women, to avoid contamination with bacteria from the urethral areas. Of note, gram-positive organisms and fungi may not reach 105 CFU/mL in patients with infection. Specimens with 104 CFU/mL or less may contain skin organisms, such as diphtheroids, *Neisseria spp*., and staphylococci. Screening for ASB is necessary for select patients (pregnant women, individuals undergoing invasive genitourinary procedures, and renal transplant recipients) (CDC, 2017). If screening is indicated, urine should be collected by clean-catch midstream, catheterization, or suprapubic aspiration.

1.4.5 Treatment of UTI

The first step in treating UTIs is to classify the type of infection, such as acute uncomplicated cystitis or pyelonephritis, acute complicated cystitis or pyelonephritis, CA-UTI, asymptomatic Bacteriuria (ASB), or prostatitis (CDC, 2017). The Infectious Diseases Society of America (IDSA) recommends that empiric regimens for uncomplicated UTIs be guided by the local susceptibility, particularly to *E. coli*. They recommend considering trimethoprim/sulfamethoxazole if the local resistance rate is less than 20% and fluoroquinolones if the resistance rate is less than 10% (Gupta, 2011). The empiric regimen for complicated UTIs should also be guided by local susceptibility trends of uropathogens, and definitive regimens should be tailored according to susceptibility results, when available (CDC, 2017). Collateral damage should be considered when deciding on treatment for uncomplicated UTIs (Paterson, 2004). Collateral damage refers to ecological adverse effects, including the selection of drug-resistant organisms from antibiotic use, particularly when broad-spectrum cephalosporins and fluoroquinolones are used to treat UTIs. Broad-spectrum cephalosporins have been associated with subsequent infections caused by vancomycin-resistant enterococci, ESBL-producing *K. pneumoniae*, β-lactam–resistant *A. baumannii*, and *Clostridium difficile* infection. Prior use of fl uoroquinolones has been linked to subsequent colonization or infections with methicillin-resistant *S. aureus* or fluoroquinolone-resistant *P. aeruginosa* (Paterson, 2004). The preserved in vitro susceptibility of *E. coli* to nitrofurantoin and fosfomycin suggests that they cause limited collateral damage, perhaps because of their minimal effects on bowel flora. Antibiotics with a lower potential for collateral damage are preferred for uncomplicated cystitis because the infection is often self-limiting, even without treatment, and the risk of progression to tissue invasion or sepsis is minimal. In fact, studies have shown that 25%–42% of women with uncomplicated cystitis achieved clinical cure even though they did not receive antibiotic treatment or received an inactive antibiotic (Paterson, 2004)

1.4.6 Overview of Selected Antibiotics to Treat UTIs

Most uncomplicated UTIs are treated in the outpatient setting. However, patients who present with fevers or systemic symptoms of infection (e.g., systemic inflammatory response with a

suspected urinary source) should be hospitalized and treated with parenteral antibiotics. Initial therapy is based on the local susceptibility patterns of *E. coli* and other uropathogens. For the treatment of cystitis, an adequate urinary antibiotic concentration is important to ensure response to therapy (Paterson, 2004). For all oral antibiotics commonly used in UTIs, adequate urinary concentrations are usually achieved. Further research is necessary to help clinicians determine effective treatment of UTIs in patients with renal insufficiency, including anuric patients.

1.4.6.1 Nitrofurantoin

Nitrofurantoin is recommended for the treatment of cystitis. It is highly active against *E. coli*, with 0.9% resistance among female outpatients (Sanchez, 2016). Nitrofurantoin achieves high urinary concentration but does not penetrate well into the renal parenchyma; therefore, it should not be used for the treatment of pyelonephritis. According to the package insert, nitrofurantoin should be avoided in individuals with a CrCl of 60 mL/minute/1.73 m^2 or less because of lack of efficacy and the potential for peripheral neuropathy and pulmonary adverse effects. Before 2015, nitrofurantoin was listed on the American Geriatrics Society's Beers Criteria of medications potentially inappropriate for use in older adults. In the 2015 Beers Criteria update, the threshold for CrCl was decreased to 30mL/minute/1.73 m2 because of the results of a large cohort study, which found similar effectiveness and low rates of serious adverse effects associated with nitrofurantoin. Serious adverse effects, including renal failure in infected women 18 and older with an estimated glomerular filtration rate of 30–50 mL/minute/1.73 m^2, were comparable with those with an estimated glomerular filtration rate greater than 80 mL/minute/ 1.73 m^2 (Geerts, 2013). However, use of nitrofurantoin for long-term suppression of UTIs remains potentially inappropriate in older adult patients because of the risk of adverse effects.

1.4.6.2 Trimethoprim/Sulfamethoxazole

Trimethoprim/sulfamethoxazole remains a highly effective agent for the treatment of uncomplicated cystitis, with cure rates of 90%–100%. It is also effective in the treatment of UTIs in men. Trimethoprim/sulfamethoxazole was noninferior to ciprofloxacin for early clinical and bacterial cure rates. A 20% resistance rate has been recommended as the threshold to avoid treatment with trimethoprim/ sulfamethoxazole (Gupta, 2011). However, trimethoprim/

sulfamethoxazole may remain effective at a clinical cure rate of 85%, even when the resistance rate is 30% (Gupta, 2001).

1.4.6.3 Fluoroquinolones

Fluoroquinolones (e.g., levofloxacin or ciprofloxacin) are recommended for the treatment of uncomplicated pyelonephritis and complicated UTIs, including urosepsis when the local resistance is less than 10% (Gupta 2011). In addition to collateral damage, the FDA's drug safety communication issued in 2016 states that the serious adverse events (e.g., tendinitis, peripheral neuropathy, and CNS effects) outweigh the benefits in patients with uncomplicated cystitis when other treatment options are available. Alternatives to fluoroquinolones such as nitrofurantoin or amoxicillin/clavulanate are recommended for uncomplicated UTIs (Alternatives to fluoroquinolones). Of note, according to the manufacturer's package insert, about 20% of moxifloxacin is excreted unchanged in the urine, and moxifloxacin is currently not recommended for the treatment of UTIs (Paterson, 2004).

1.4.6.4 Fosfomycin Trometamol

Fosfomycin trometamol has in vitro activity against most Enterobacteriaceae *spp*. including ESBL-producing isolates and *Enterococcus spp*. (regardless of vancomycin susceptibility). Given the high *E. coli* susceptibility rates and its low potential for collateral damage, fosfomycin is one of the agents recommended by the IDSA for uncomplicated UTIs. However, increased use of fosfomycin has been associated with increased resistance; thus, routine use of fosfomycin for uncomplicated cystitis remains unclear. A study evaluating 17,602 UTI cases caused by *E. coli* showed that fosfomycin resistance among ESBL-producing *E. coli* increased from 2.2% in 2003 to 21.7% in 2008 with the increased use of fosfomycin by 50% (Oteo, 2009). The price of fosfomycin remains relatively high. The average wholesale price for each 3-g sachet is #40,000.99, and insurance coverage is variable, which may limit its routine use for uncomplicated cystitis.

1.4.6.5 Oral β-Lactam Agents

Studies of β-lactam antibiotics (e.g., amoxicillin/clavulanate, cefaclor, cefdinir, cefpodoxime, and ceftriaxone) report lower efficacy than with fluoroquinolones and trimethoprim/sulfamethoxazole. β-Lactam antibiotics are considered alternative agents in managing uncomplicated UTIs. Although cephalexin is not recommended by the IDSA for the treatment of uncomplicated UTI, it is commonly used in the outpatient setting and is an acceptable alternative for treating uncomplicated cystitis and ASB (Gupta 2011). Amoxicillin and ampicillin are currently not recommended for empiric therapy because of the increased prevalence of resistance, but they may be prescribed for the treatment of ASB or UTIs when culture data show susceptibility, especially to *E. faecalis*.

1.5 Rationale for Study

The increasing incidence and susceptibility of urinary tract infection in HIV seropositive individuals has led to the more recent research attention attracted to the area of diagnosis and understanding of possible management approaches to urinary tract infection in individuals of such category. Although a body of knowledge exists both at the literature and clinical levels about the diagnosis of urinary tract infection, however, the development of a more rapid and specific test for UTI in HIV seropositive individuals will not only improve clinical diagnosis but may also serve as a lead in the understanding of the infectious process and possible development of better therapeutic regimen for the treatment of the infection. It is towards this goal that the current study was designed and justified.

1.6 Aim and Objectives of the Study

1.6.1 Aim of the Study

The aim of the present study is to evaluate C-reactive protein as a probable predictor of urinary tract infection (UTI) in HIV seropositive subjects.

1.6.2 Specific Objectives of the Study

The specific objectives of the present study include:

1. To isolate UTI - causing organisms from urine samples of HIV - seropositive subjects.
2. To identify and quantify UTI - causing organisms from urine samples of HIV - seropositive subjects.
3. To determine the serum levels of C-reactive protein in HIV - seropositive subjects.
4. To investigate, through correlational analysis, the relationship between UTI incidence in HIV- seropositive subjects and the serum levels of C-reactive protein.

CHAPTER TWO

MATERIALS AND METHODS

2.0 Materials

2.1 Study Area

This study was conducted at General Hospital, Enugu Ezike in Igbo-Eze North Local Government Area of Enugu State. This hospital is a major Intellectual Property (IP) for CARITAS a non-governmental organization that has actively supported international HIV program for almost a decade. The program provides prevention of mother to child transmission, care and treatment, stigma reduction and antiretroviral therapy.

2.2 Equipment, Blood Samples and Reagents used

The equipment used for the study include C-Reactive Protein reagent kit, MacConkey agar, Nutrient agar, Antibiotic disk, 10 ml valcutainer, 10 ml syringes, hand gloves, universal bottles and EDTA bottles.

2.3 Methods

2.3.1 Collection of Samples

A total of 5000 HIV - seropositive subjects attend General Hospital, Enugu Ezike for HIV counseling, viral load testing and ARV collection. 50 seropositive individuals were selected under purposive sampling during the period of November 2019 to December 2019 for the study. They consisted of 36 seropositive females and 14 seropositive males. About 5 ml blood samples of the HIV - seropositive subjects were collected and transferred into a valcutainer using a 10 ml syringe labeled accordingly with a tracking number for easy identification. After blood sample collection, the samples were packaged in cooler containing ice bags. The cooler was placed in a clear and impervious plastic bag and transported to the laboratory for analyses. Ethical clearance was obtained from the Ethics Committee of the Faculty of Biological Sciences, University of Nigeria, Nsukka.

Mid stream urine samples of HIV - seropositive individuals (both male and female) were collected in sterile universal bottles and labeled by taking all necessary precautions to avoid contamination. The urine samples were immediately packaged after collection in an iced container, transported to the laboratory and cultured within 3 hours of collection.

2.3.2 Preparation of Whole Blood Samples for Biochemical Analysis.

About 5ml whole blood samples of HIV seropositive patients were collected using 10ml valcutainer following standard procedures. **T**he blood samples were allowed to stand for 30 minutes at room temperature to clot before spinning and separation and equal volume of whole blood samples were balanced in a bucket centrifuge and spinned for 5 minutes. **A**fter spinning, the blood samples are arranged accordingly in a stand**.** Using a clean disposable pastuer pipette, plasma/serum was separated from whole blood samples into an EDTA bottles (which contains an anticoagulants) labeled with a tracking number**.** Immediately after separation, the serum was stored in a refrigerator in order to preserve coagulation factors and other plasma proteins.

2.3.3 Estimation of Serum C-reactive Protein by Rapid Quantitative Test

The FineCare CRP rapid quantitative test is a fluorescence immunoassay used alongside FineCare fluorescence immunoassay machine (Model NO: FS-112/FS-113/FS-205) for quantitative determination of C-reactive protein in human serum/plasma. It is used as a marker to detect infection and inflammation (UTI in HIV whole blood samples).

2.3.3.1 Principle

C-reactive protein Rapid quantitative test is based on the fluorescence immunoassay technique. The fineCare CRP Rapid quantitative test uses a sandwich immunodetection method. When the blood sample is added into the sample well of the test cartridge, the fluorescence-labeled detector CRP antibodies on the sample pad binds to the CRP antigens in blood specimen, forming immunocomplexes. As the complexes migrates on the nitrocellulose matrix of the test strip by capillary action, the complexes of detector antibodies and CRP are captured to the CRP antibodies that have been immobilized on the test strip. The more CRP antigens in blood specimen, the more complexes accumulated on test strip-signal intensity of fluorescence of detector antibodies reflect the amount of captured CRP.

2.3.3.2 Test Procedure

Test was conducted at room temperature

- **PREPARATION:** Before testing, activate "use" in the setting and then SAVE. Insert the ID chip into FineCare fluorescence immunoassay system, and ensure that the ID chip matches with the lot number of the test cartridges as well as the detection buffer.
- **SAMPLING:** Draw 5µl of serum with a transfer pipette and add into the detection buffer tube
- **MIXING:** Close the lid of the detection buffer and mix the sample thoroughly by shaking it for about 10 times
- **LOADING:** Pipette 75µl of the sample mixture and load it into the sample well of the test cartridge
- **TESTING:** Standard test mode was used for testing. Test cartridge was inserted onto the test cartridge holder of FineCare fluorescence immunoassay system, right after adding sample mixture to the sample well. "'TEST" was pressed to start testing. After testing, results was displayed on the main screen, results was saved and printed out by clicking on the "PRINT" button.
- Followed by discarding of used test cartridge, transfer pipette, pipette tip according to local regulation and procedures.

2.3.4 Evaluation of UTI in Urine Samples

2.3.4.1 Procedures for Urine Culture

About 5.4g of MacConkey Agar (Enterobacteriacea family) was dissolved in 105ml distilled water. The medium was heated gently to dissolve. The medium was sterilized by autoclaving at 15psi (120ºC) for 15 minutes and allow to cool to 45-50ºC. After autoclaving, the medium was poured into a petri dish and allowed to gel and properly labeled with a tracking number. About 10µl of the urine sample was pipetted into culture plates containing the medium. Followed by streaking of the plates using a sterile loop. Wire loop was flamed in a Bunsen burner to sterilize. The loop was dragged across the surface of the agar back and forth in a zigzag motion until about

90% of the plate has been covered. The inoculants were incubated inverted at 37ºC for 24 hours to allow growth and reproduction of micro-organism. After incubation, the total heterotrophic aerobic bacterial counts were carried out, then the plates were sub-cultured for further identification.

2.3.4.2 Isolation and Characterization of the Microbial Isolates

2.3.4.2.1 Media Used

Isolation and identification of the bacterial organisms were carried out with the following media; nutrient broth, nutrient agar, Mac Conkey agar, deoxycholate citrate agar (Biotec), selenite F broth, peptone water, sulphide indole motility agar (Oxoid), Kligler iron agar (Lab M) urea medium, Simomn's citrate agar, Urea agar base (Oxoid), Muller Hinton broth and Muller Hinton agar (Lab M). all culture media except where otherwise indicated, were products of Fluka laboratories.

About 26g of MacConkey agar was dissolved in 500ml of distilled water. Dissolved agar was autoclaved for 45 minutes. After autoclaving, the medium was poured evenly in a mix culture plate and allowed to solidify by using a pour plate method. Microbial growths on the mix cultured plate was picked out with a wire loop to sub-culture, create a well with the microbe. Four corners of the culture plate was streaked in a zigzag motion starting from the point of well creation until about 90% of the plate was covered with the microbial isolates. The sub cultured isolates was incubated for 24 hours at 37ºC to allow growth and reproduction.

2.3.4.2.2 Growth on Kligler iron agar

In most situations, presumptive identification was based on the reaction of the isolate on Kligler iron agar (KIA). Colonies were carefully picked from agar media by selecting one discrete colony. Extreme care was taken to avoid picking up contaminants that might be present on the surface of the agar by making sure that the inoculating needle did not go through the colony to touch the surface of the plate. Tubes of KIA were inoculated by stabbing the butt and streaking the surface of the slant. The caps of the tubes were loosened before incubation. After incubation for 2hours at 370C, the KIA slants were observed for reactions and the result recorded. Yellowing of the butt indicated glucose fermentation; yellowing of the slant indicated lactose

fermentation; while reddening of the slant indicated inability of the organism to ferment lactose. Gas production was indicted by air bubbles, cracks or displacement of the medium. Hydrogen sulphide production was indicated by blackening of the medium.

2.3.5 Antimicrobial Susceptibily Profile of the Isolates

2.3.5.1 Preparation

Nutrient agar was prepared by dissolving 14g in 500ml of distilled water. Allowed to solidify after pouring into culture plate. Colonies were selected and transferred to the nutrient medium with inoculation loop.

2.3.5.2 Antibiotic sensitivity testing

The disc diffusion method was used to carry out the antibiotic sensitivity testing. A sterile loop was dipped into the sub cultured plate, pick colonies of microbes and transfer into the nutrient agar. Entire agar plate surface was streaked 3 times turning the plate 60 degrees between streaking to obtain even inoculation. The lid was left opened for 3 minutes to allow any surface moisture to be absorbed before applying the Gram +VE antibiotic sensitivity disc. Appropriate sensitivity test disks was selected. Antibiotic sensitivity disc was placed with a sterile forceps on the surface of the medium. It is best to place disks that give predictably small zones in an effort to avoid overlapping zones. It is also important to pay attention to how close the disks are to the edge of the plate, because if the disk is placed too close to the edge of the plate, the zones may not be fully round. A disk should not be relocated once it has come into contact with the agar surface. The plate agar was placed in a 37ºC incubator for 24 hours. After 24 hours of incubation the cultured plate was examined. The zone of inhibition diameters were measured to the nearest millimeter using meter rule and recorded.

2.3.6 Biochemical Test (Kliger Iron Agar)

This is a culture media contining peptone (15g/l), HM peptone B(3g/l), Yeast extract (3g/l), proteose peptone (5g/l), lactose (10g/l), Dextrose (1g/l), ferrous sulphate (0.02g/l), sodium chloride (5g/l), sodium thiosulphate (0.03g/l) and phenol red (0.0024g/l), Agar (15g/l). The

Kligler’s Iron Agar test employes a medium for the identification of enterobacteriacieae, based on double sugar fermentation and hydrogen sulphide production. It is used to differentiate organisms by demonstrating hydrogen sulphide production and the fermentation of dextrose and lactose.

CHAPTER THREE

RESULTS

3.1 Urine culture of HIV Seropositive Individuals

Table 1 shows the result of the urine culture. From the results, of the 50 urine samples of HIV positive individuals collected, only 23 samples recorded microbial growth after culturing for 24hours in MacConkey agar. Samples (15, 18, 30, 32 and 47) were co-infectors due to the presence of fermenters and non-fermenters in same urine sample. About 11 samples recorded the growth of fermenters alone while 7 samples recorded the growth of non-fermenters alone.

Table 1 Urine culture of HIV Seropositive Individuals on MacConkey agar

Sample number	Lactose Fermenters ($X10^2$cfu/ml)	Non-fermenters ($X10^2$cfu/ml)
S1		1
S2		1
S3		3
S5	15	
S6	2	
S12	2	
S14	6	
S15	1	1
S16	2	
S17	5	
S18	100	144
S20	8	
S30	120	52
S32	64	192
S35	1	
S36		11
S37	4	
S40	2	
S45		5
S46		171
S47	240	198
S48		3
S49	392	

3.2 Antibiotic Susceptibility Profiles

Table 2 shows the result of antimicrobial susceptibility profiles. Each of the bacterial isolate was challenged with a panel of 10 antibiotics. Of the 23 bacterial isolates tested, the highest resistance (100%) was recorded against CL. This was followed by (73.91%) against AM, while the least resistance (8.69%) was recorded against LV. 34.78% was resistant to CT, 69.56% was resistant to CX, 17.39 was resistant to CIP, 13.04% was resistant to GN, 17.40% was resistant to OF while 65.21% was resistant to CD.

Table 2 Antibiotic sensitivity

S/N	Antibiotics	Total number of organisms	Number of resistant organisms	Number of susceptible organisms	Percentage resistance	Percentage susceptible
1	E	23	14	9	60.87	39.13
2	CT	23	8	15	34.78	65.22
3	AM	23	17	6	73.91	26.09
4	CL	23	23	0	100	0.00
5	LV	23	2	21	8.69	91.31
6	CX	23	16	7	69.56	30.44
7	CIP	23	4	19	17.39	82.61
8	GN	23	3	20	13.04	86.96
9	OF	23	4	19	17.40	82.60
10	CD	23	15	8	65.21	34.79

Key:

E = Erythromycin

CT = Cetraxon

AM =Ampicillin

CL = Cloxacillin

CX = Cephalexin

CIP = Ciprofloxacin

GN = Gentamycin

OF = Ofloxacin

CD = Clindamycin

3.3: Antibiotic sensitivity on *E. coli*

Table 3 shows the result of antibiotics sensitivity of *E. coli.* A total of ten (10) antibiotics were tested against 10 *E coli* isolates. 80% were resistant to E, 20% was resistant to CT, 80% was resistant to AM, 90% was resistant to CL, 20% was resistant LV, 80% was resistant to CX, 20% was resistant to CIP, 30% was resistant to GN, 20% was resistant to OF while 60% was resistant to CD.

Table 3 Antibiotic sensitivity on *E. coli*

S/N	Antibiotics	Total number of organisms	Number of resistant organisms	Number of susceptible organisms	Percentage resistance	Percentage susceptible
1	E	10	8	2	80	20
2	CT	10	2	8	20	80
3	AM	10	8	2	80	20
4	CL	10	9	1	90	10
5	LV	10	2	8	20	80
6	CX	10	8	2	80	20
7	CIP	10	2	8	20	80
8	GN	10	3	7	30	70
9	OF	10	2	8	20	80
10	CD	10	6	4	60	40

Key:

E = Erythromycin

CT = Cetraxon

AM =Ampicillin

CL = Cloxacillin

CX = Cephalexin

CIP = Ciprofloxacin

GN = Gentamycin

OF = Ofloxacin

CD = Clindamycin

3.4 Antibiotic sensitivity on *Staphylococcus aureus*

Table 4 shows the result of antibiotics sensitivity of *Staphylococcus aureus.* A total of ten (10) antibiotics were tested against 6 *Staphylococcus aureus* isolates. 66.7% were resistant to E, 33.3% was resistant to CT, 83.3% was resistant to AM, 100% was resistant to CL, 0% was resistant LV, 66.7% was resistant to CX, 16.7% was resistant to CIP, 16.7% was resistant to GN, 16.7% was resistant to OF while 50% was resistant to CD.

Table 4 Antibiotic sensitivity on *Staphylococcus aureus*

S/N	Antibiotics	Total number of organisms	Number of resistant organisms	Number of susceptible organisms	Percentage resistance	Percentage susceptible
1	E	6	4	2	66.7	33.3
2	CT	6	2	4	33.3	66.7
3	AM	6	5	1	83.3	16.7
4	CL	6	6	0	100	0
5	LV	6	0	6	0	100
6	CX	6	4	2	66.7	33.3
7	CIP	6	1	5	16.7	83.3
8	GN	6	1	5	16.7	83.3
9	OF	6	1	5	16.7	83.3
10	CD	6	3	3	50	50

Key:

E = Erythromycin

CT = Cetraxon

AM =Ampicillin

CL = Cloxacillin

CX = Cephalexin

CIP = Ciprofloxacin

GN = Gentamycin

OF = Ofloxacin

CD = Clindamycin

3.5 Antibiotic sensitivity on *Klebsiella pneumoniae*

Table 5 shows the result of antibiotics sensitivity of *Klebsiella pneumoniae.* A total of ten (10) antibiotics were tested against 5 *Klebsiella pneumoniae* isolates. 40% were resistant to E, 40% was resistant to CT, 80% was resistant to AM, 100% was resistant to CL, 0% was resistant LV, 80% was resistant to CX, 0% was resistant to CIP, 0% was resistant to GN, 0% was resistant to OF while 80% was resistant to CD.

Table 5 Antibiotic sensitivity on *Klebsiella pneumoniae*

S/N	Antibiotics	Total number of organisms	Number of resistant organisms	Number of susceptible organisms	Percentage resistance	Percentage susceptible
1	E	5	2	3	40	60
2	CT	5	2	3	40	60
3	AM	5	4	1	80	20
4	CL	5	5	0	100	0
5	LV	5	0	5	0	100
6	CX	5	4	1	80	20
7	CIP	5	0	5	0	100
8	GN	5	0	5	0	100
9	OF	5	0	5	0	100
10	CD	5	4	1	80	20

Key:

E = Erythromycin

CT = Cetraxon

AM =Ampicillin

CL = Cloxacillin

CX = Cephalexin

CIP = Ciprofloxacin

GN = Gentamycin

OF = Ofloxacin

CD = Clindamycin

3.6 Antibiotic sensitivity on *Proteus spp.*

Table 6 shows the result of antibiotics sensitivity of *Proteus spp.* A total of ten (10) antibiotics were tested against 5 *Proteus spp.* isolates. 100% were resistant to E, 33.3% was resistant to CT, 100% was resistant to AM, 100% was resistant to CL, 16.7% was resistant LV, 50% was resistant to CX, 16.7% was resistant to CIP, 0% was resistant to GN, 16.7% was resistant to OF while 100% was resistant to CD.

Table 6 Antibiotic sensitivity on *Proteus spp.*

S/N	Antibiotics	Total number of organisms	Number of resistant organisms	Number of susceptible organisms	Percentage resistance	Percentage susceptible
1	E	6	6	0	100	0
2	CT	6	2	4	33.3	66.7
3	AM	6	6	0	100	0
4	CL	6	6	0	100	0
5	LV	6	1	5	16.7	83.3
6	CX	6	3	3	50	50
7	CIP	6	1	5	16.7	83.3
8	GN	6	0	6	0	100
9	OF	6	1	5	16.7	83.3
10	CD	6	6	0	100	0

Key:

E = Erythromycin

CT = Cetraxon

AM =Ampicillin

CL = Cloxacillin

CX = Cephalexin

CIP = Ciprofloxacin

GN = Gentamycin

OF = Ofloxacin

CD = Clindamycin

3.7 CRP Levels of HIV Seropositive Subjects

Table 7 shows the result of the C-reactive proteins concentration of the HIV seropositive individuals sampled. From the results, sample 1, 3 – 12, 16, 18 and 36 – 49 recorded CRP levels less than 5. Sample 2 had a CRP level of 27.8, sample 14 had a CRP level of 40, sample 15 had a CRP level of 10.7, sample 17 had a CRP level of 26.2, sample 20 had a CRP level of 33.2, sample 30 had a CRP level of 6.7 while samples 32 and 35 had CRP levels of 5.6 and 5.5 respectively. It can be inferred from the table that samples 2, 14, 15, 17 and 20 run a very high risk of cardiovascular disease due to the high plasma levels of CRP. The high sensitivity C-reactive protein (hsCRP) result showed that sample 45 has hsCRP level of 3.5mg/l followed by sample 5 with 3.1mg/l. Samples 2, 14, 15, 17, 20, 30, 32 and 35 has hsCRP levels greater than 5mg/l which is considered to be very high and indicative of the presence of inflammation due to viral infection. Low levels of hsCRP were seen in samples 1, 16, 36, 37, 48 and 49 with hsCRP of less than 0.0mg/l.

Table 7 CRP Level of HIV Seropositive Subjects

Sample number	CRP(mg/l)	hsCRP(mg/l)
S1	<5	<0.5
S2	27.8	>5
S3	<5	<0.5
S5	<5	3.1
S6	<5	2.3
S12	<5	2.3
S14	40.0	>5
S15	10.7	>5
S16	<5	<0.5
S17	26.2	>5
S18	<5	1.4
S20	33.2	>5
S30	6.7	>5
S32	5.6	>5
S35	5.5	>5
S36	<5	<0.5
S37	<5	<0.5
S40	<5	3.0
S45	<5	3.5
S46	<5	2.0
S47	<5	1.2
S48	<5	<0.5
S49	<5	<0.5

3.8 Gram Staining Reaction

Table 8 shows the result of the Gram staining reaction of the microbial isolates of the urine samples collected. The results show that majority of the microbial isolates were Gram negative while some were gram positive. While gram negative bacteria's have a rod like shape, gram positive bacteria's have a cocci shape. Some of the rods were short and in cluster and some were long and clustered, others were short and single while some were long and single.

Table 8 Gram Staining Reaction of Bacterial Isolates

Sample number	Gram reaction	Morphology
S1	-ve	Rod
S2	+ve	Cocci
S3	-ve	Rod
S5	-ve	Rod
S6	-ve	Rod
S12	-ve	Rod
S14	-ve	Rod
S15^{F}	-ve	Rod
S15^{NF}	+ve	Cocci
S16	-ve	Rod
S17	-ve	Rod
S18	+ve	Cocci
S20	-ve	Rod
S30	-ve	Rod
S32	+ve	Cocci
S34	-ve	Rod
S35^{F}	-ve	Rod
S35^{NF}	+ve	Cocci
S36	-ve	Rod
S37	-ve	Rod
S40^{F}	-ve	Rod
S40^{NF}	-ve	Rod
S45	+ve	Cocci
S46	-ve	Rod
S47^{F}	-ve	Rod
S47^{NF}	-ve	Rod
S49	-ve	Rod

3.9 Biochemical Test

Table 9 Show the result of the biochemical test. From the results, of all the microbial isolates identified, *E. coli* were identified as lactose fermenters with gas ($CO_{2)}$ production while acid (H_2S) was not produced in 10 samples. *Klebsiella pneumoniae* was also identified as lactose fermenters with strong reaction with gas production and no acid (H_2S) production. *Proteus Spp* was identified as glucose fermenters with acid (H_2S) production and gas ($CO_{2)}$ production. Some produced acid only while some produced only gas without acid production. From the result *E. coli* was identified as the most dominant bacterial specie associated with urinary tract infection in the studied samples. *Staphylococcus aureus* was identified as a lactose fermentor. Although the majority produced neither gas nor acid, some produced acid only while some produced only gas without acid production.

Table 9 Biochemical Test of Bacterial Isolates

Sample number	Organism	Glucose fermenter	Lactose fermenter	H_2S production	CO_2 production
S1	*E. coli*	-	Z	-	+
S2	*Staphylococcus aureus*	-	Z	-	-
S3	*E. coli*	-	Z	-	+
S5	*E. coli*	-	Z	-	+
S6	*Klebsiella spp.*	-	Z	-	++
S12	*E. coli*	-	Z	-	+
S14	*E. coli*	-	Z	-	+
S15^{F}	*E. coli*	-	Z	-	+
S15^{NF}	*S aureus*	-	Z	-	+
S16	*E. coli*	-	Z	-	+
S17	*K. spp.*	-	Z	-	++
S18	*S aureus*	-	Z	-	-
S20	*E. coli*	-	Z	-	+
S30	*E. coli*	-	Z	-	+
S32	*S aureus*	X	-	+	-
S34	*K. spp.*	-	Z	-	++
S35^{F}	*K. spp.*	-	Z	-	++
S35^{NF}	*S aureus*	-	Z	-	-
S36	*Proteus Spp.*	Y	-	+	-
S37	*E. coli*	-	Z	-	+
S40^{F}	*Proteus Spp.*	Y	-	+	-
S40^{NF}	*Proteus Spp*	Y	-	+	-
S45	*S aureus*		Z	-	-
S46	*Proteus Sp*	Y	-	+	+
S47^{F}	*Proteus Sp.*	Y	-	-	+
S47^{NF}	*K. spp.*	-	Z	-	++
S49	*Proteus Spp.*	Y	-	+	+

Key:

X = Alkaline / Alkaline

Y = Alkaline / Acid

Z = Acid / Acid

A total of 23 bacterial isolates belonging to the 4 genera were isolated from the urine specimens of the subject.

3.10 Correlation and Regression analyses

Correlation analysis was used to determine the existence of a linear relationship between the bacteria load of the samples and the levels of CRP and hsCRP. The result of correlation analysis showed a non-significant negative correlation ($p > 0.01$) between the bacteria load and the CRP levels of the samples analyzed. There was also a non-significant negative correlation ($p > 0.01$) between the bacterial load of the samples and hsCRP levels. The scatter/Dot plot of bacteria load versus CRP and hsCRP showed a negative slope with R^2 values of 0.062 and 0.031 respectively (Appendix I).

The regression analysis was used to investigate the possibility of predicting bacterial load from CRP levels and vice versa. The R square value obtained from the model summary table (Appendix II) was 0.062 which is the same value obtained from the scatter / Dot plot during the correlation analysis. The R square value indicates how much of the total variation in the dependent variable (CRP) can be explained by the independent variable (Bacterial load). This implies that the changes in CRP levels in the studied samples can only be accounted for by only 6.2% of change in Bacterial load of the urine samples studied. this is a very low degree of correlation and thus explains further, the negative non-significant relationship between the CRP levels and the incidence of UTI with respect to bacterial load.

The ANOVA table (Appendix III) shows that, overall, the regression model statistically does not predict the outcome variable. Thus, the CRP level may not be effectively predicted from the bacterial load, even on an inverse basis with respect to the negative nature of the relationship, since the relationship is very low. From the coefficient table (Appendix IV), the prediction of CRP levels and hsCRP levels was derived to have the equation:

CRP = 10.91 + 0.00(Bacterial load).

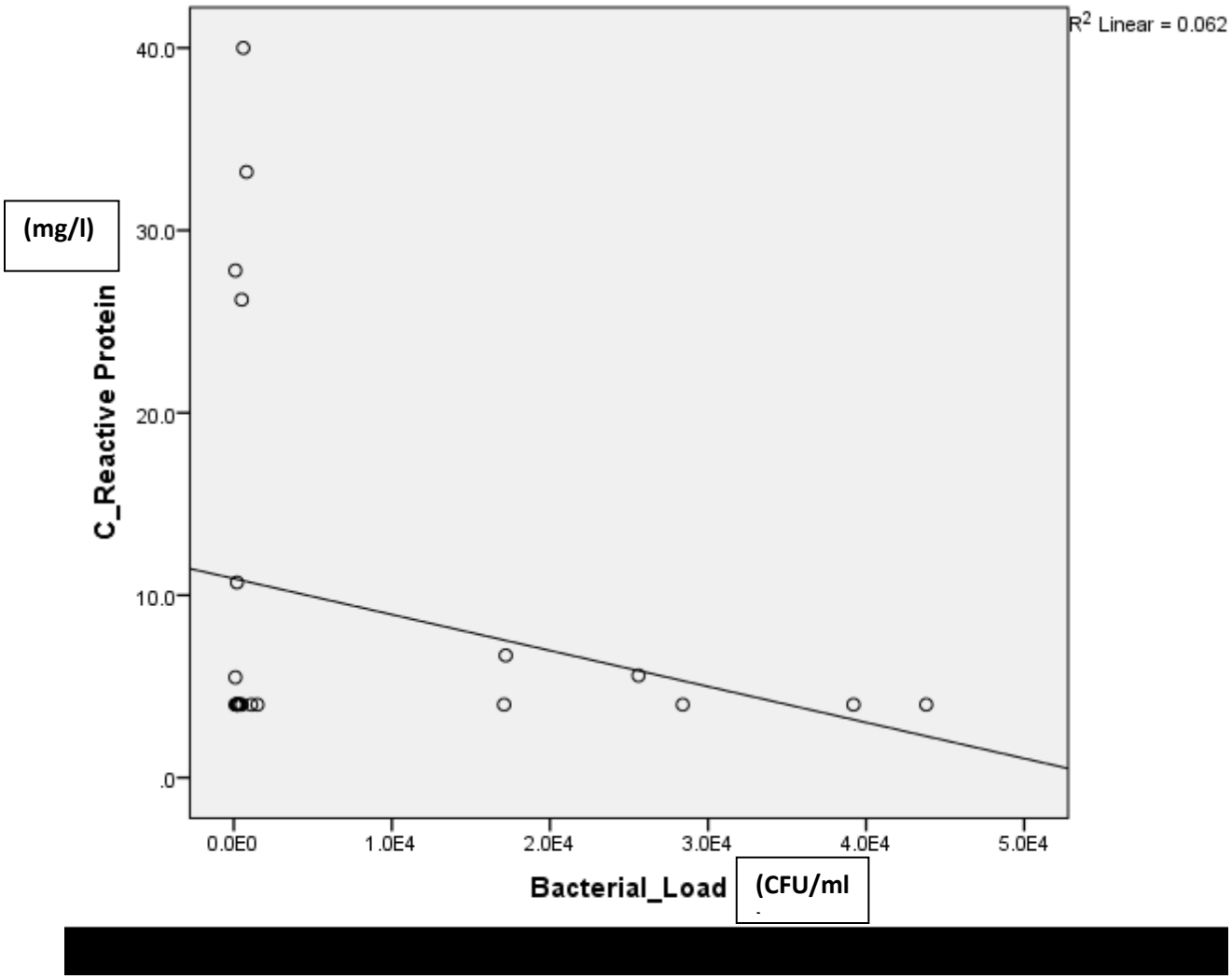

Fig 3.1: Scatter/Dot plot of Creactive protein and Bacterial Load

CHAPTER FOUR

DISCUSSION

Infections of the lower urinary tract (acute cystitis) are one of the most frequent diseases in primary medical care which are treated with antibiotics. Currently, the diagnosis of UTI is carried out through urinalysis and a clean-catch dipstick leukocyte esterase test which is a rapid screening test for detecting pyuria with a high sensitivity and specificity for detecting more than 10 WBC/mm^3 in urine (Sobel, 2014). It is important to note that the presence of pyuria is nonspecific and does not always indicate clinical urinary tract infection. Organisms like *E. coli*, *Klebsiella* spp., *Enterobacter* spp., *Proteus* spp., *Staphylococcus* spp., and *Pseudomonas* spp. reduce nitrate to nitrite in the urine, and the presence of nitrite on a urinalysis is another marker of UTIs. The immunological state of HIV seropositive individuals predispose them to several secondary infections including UTI. Sepsis is a clinical syndrome with high mortality rates demonstrating a directly proportionate relationship to disease severity. Therefore, the study of biomarkers for sepsis has been an area of interest and research. The most important feature of a biomarker is its potential to influence clinical decision-making. Procalcitonin and C-reactive protein are among such markers that have shown great promise in identifying sepsis, assessing severity of illness and guiding antibiotic management. The current study was therefore conducted to evaluate the possible application of procalcitonin and C-reactive protein as predictors of urinary tract infection in HIV seropositive individuals.

From the results, of the 50 urine samples of HIV positive individuals collected, only 23 samples recorded microbial growth after culturing for 24hours in MacConkey agar. Samples (15, 18, 30, 32 and 47) were co-infectors due to the presence of lactose fermenters and non-fermenters in same urine sample. About 11 samples recorded the growth of fermenters alone while 7 samples recorded the growth of non-fermenters alone. This result is in agreement with the report of Sammon (2014) who reported the growth of fermenting and non-fermenting microbes in the urine culture of individuals diagnosed with UTI. The presence of co-infectors, indicate the severity of the infection in some of the samples collected. The isolates were stocked in nutrient agar for subsequent analyses.

Antimicrobial sensitivity test was used to determine the inhibitory activities of different antibiotics on the test bacterial species. A total of 10 antibiotic discs were tested against 23 microbial isolates. Of the 23 organisms tested, 60.87% was resistant to E, 34.78% was resistant to CT, 73.78% was resistant to AM, 100 % was resistant to CL, 8.69% was resistant to LV, 69.56% was resistant to CX, 17.39 was resistant to CIP, 13.04% was resistant to GN, 17.40% was resistant to OF while 65.21% was resistant to CD. The above result suggests that GN is the most effective antibiotics in the treatment of the microbial species. The high resistance of the microbial species to CL, CD and AM may be due to the generation of the antibiotics and the associated evolution of resistant bacteria species as supported by literature.

The result of Gram reaction show that all microbial isolates were either long or short Gram negative rods. While some of the rods were short and in cluster, some were long and cluster, others were short and single while some were long and single. According to Ana and Jenifer, (2015), urinary tract infection are severe public health condition usually caused by a arrange of gram positive and gram negative bacteria especially E. coli and Klebsiella species. Thses organisms reduce nitrate to nitrite in the urine, and the presence of nitrite in urinalysis is another marker of UTIs.

From the results of the biochemical tests, of all the microbial isolates identified, *E. coli* were identified as lactose fermenters with gas ($CO_{2)}$ production while acid (H_2S) was not produced in 10 samples. *Klebsiella pneumonia* was also identified as lactose fermenters with strong gas production and no acid production. *S. paratyphi A* was identified as glucose fermenter with acid production and no gas production. From the result, five bacteria species were identified to from the urine samples and these include *E. coli, Salmonella paratyohi A, Salmonella paratyphi B, Shigella specie* and *Klebsiella pneumonia*. *E. coli* was identified as the most dominant bacterial specie associated with urinary tract infection in the studied samples. UTIs are caused by both Gram-negative and Gram-positive bacteria, as well as by certain fungi. The most common causative agent for both uncomplicated and complicated UTIs is uropathogenic *Escherichia coli* (UPEC). For the agents involved in uncomplicated UTIs, UPEC is followed in prevalence by *Klebsiella pneumoniae*, *Staphylococcus saprophyticus*, *Enterococcus faecalis*, group B *Streptococcus* (GBS), *Proteus mirabilis*, *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Candida* spp (Foxman, 2014).

Correlation analysis was used to determine the existence of a linear relationship between the bacteria load of the samples and the levels of CRP and hsCRP. The result of correlation analysis showed a non-significant negative correlation ($p > 0.01$) between the bacteria load and the CRP levels of the samples analyzed. There was also a non-significant negative correlation ($p > 0.01$) between the bacterial load of the samples and hsCRP levels. The scatter/Dot plot of bacteria load versus CRP and hsCRP showed a negative slope with R^2 values of 0.062 and 0.031 respectively.

The regression analysis was used to investigate the possibility of predicting bacterial load from CRP levels and vice versa. The R square value obtained from the model summary table was 0.062 which is the same value obtained from the scatter / Dot plot during the correlation analysis. The R square value indicates how much of the total variation in the dependent variable (CRP) can be explained by the independent variable (Bacterial load). This implies that the changes in CRP levels in the studied samples can only be accounted for by only 6.2% of change in Bacterial load of the urine samples studied. This is a very low degree of correlation and thus explains further, the negative non-significant relationship between the CRP levels and the incidence of UTI with respect to bacterial load. The ANOVA table shows that, overall, the regression model statistically does not predict the outcome variable. Thus, the CRP level may not be effectively predicted from the bacterial load, even on an inverse basis with respect to the negative nature of the relationship, since the relationship is very low. From the coefficient table, the prediction of CRP levels and hsCRP levels was derived to have the equation:

CRP = 10.91 + 0.00(Bacterial load).

From this equation, the CRP levels cannot be predicted from the bacterial load by changing the bacterial load due to the presence of 0.00 factor in the equation. This result also point to the fact that predicting UTI from CRP levels will be misleading.

This result showed that the CRP levels of the HIV seropositive individuals studied in this work may not serve as a predictor of urinary tract infection since the linear relationship between the UTI-causing bacteria load of the individuals do not correlate positively nor significantly at the tested level of significance. Another implication of this result is that a negative correlation between a dependent and independent variable indicates that as one variable increases, the other decreases and thus the negative slope obtained from the scatter/Dot plots. Increase in the serum

levels of CRP is mediated by acute systemic inflammation due to injury, cardiovascular diseases and pathogenic infection (Braig *et al*., 2017). The presence of urinary tract infection is expected to correlate positively with the serum levels of CRP. However, our data showed that there is negative correlation between the observed CRP levels and the levels of UTI in the samples with respect to the bacteria load. This observation is explainable. First of all, a UTI is defined as a pure culture of $\geq 10^5$ colony forming units (CFU) / ml in mid-stream urine from a patient with symptoms attributable to urinary tract infection. Our results show that the bacterial load of the samples studied where between 1.0 x 10^2 to 3.9 x 10^4 CFU/ml. as a result, the samples could be said to not have UTI. Although the UTI-causing microbial loads of the samples were lower than 10^5 CFU/ml, very high CRP levels and hsCRP levels were observed in most of the samples. For example, sample 14 and sample 2 recorded CRP levels of 40 and 27mg/l respectively, while their bacterial loads were 6 x 10^5 and 1.0 x 10^5 CFU/ml respectively. These bacteria loads are very small when compared to those of samples 49 and 47 (3.9 x 10^4 and 2.4 x 10^4) CFU/ml with CRP levels of < 5 each. It is possible that the increase in CRP levels observed in these samples is a response to inflammation due to another infection that is caused by microbial species that are either resistant to the antibiotics administered, or a fungal specie. Since the bacteria load from the urine samples is less than the value that defines a UTI, the claim that the inflammation is due to another infection is supported. From the above, it is evident that the use of CRP levels to predict urinary tract infection in HIV seropositive subjects could be misleading.

4.2 Conclusion

The results of this study have established an inverse non-significant relationship between the microbial load of mid-stream urine of HIV seropositive individuals and the levels of CRP and hsCRP. Although the bacteria load of the urine samples were lower than what could define the presence of UTI, the CRP and hsCRP levels of some samples were very high indicating inflammation or infection in such individuals. It was suggested that suspected infection could be due to another form of infection or inflammation but not UTI. It was therefore, concluded that the use of CRP and hsCRP as diagnostic markers for UTI may not be very efficient since some other infection could lead to the increase in the levels of CRP and hsCRP in the absence of UTI.

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