CYTOMEGALOVIRUS IgG LEVELS IN CANCER AND NON CANCER PATIENTS ATTENDING AHMADU BELLO UNIVERSITY TEACHING HOSPITAL, ZARIA

BY

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DECLARATION

I declare that the work titled "**Cytomegalovirus IgG levels in Cancer and non Cancer Patients attending Ahmadu Bello University Teaching Hospital, Zaria".** has been done by me under thesupervision of Professor C.M.Z. Whong, Professor V. J. Umoh of the Department of Microbiology, Ahmadu Bello University, Zaria and Dr .A. Abdullahi of the Department of Radiology and Oncology at Ahmadu Bello University Teaching Hospital Zaria. All the information collected from the literature has been duly acknowledged in the text and a list of references is provided. No part of this thesis was presented elsewhere for the award of any certificate. Finally, I take the sole responsibility of all errors therein.

……
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CERTIFICATION

This thesis titled" **Cytomegalovirus IgG levels in cancer and non cancer patients attending Ahmadu Bello University Teaching Hospital, Zaria."** meets the regulations governing the award of the degree of Master of Science in Microbiology of Ahmadu Bello University, Zaria, and is approved for its contribution to knowledge and literary presentation.

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ABSTRACT

Cytomegalo virus is a ubiquitious virus, it presents an asymtomatic infection in the immunocompetent host, but in an immunocompromised patients it causes morbidity and sometimes mortality in these types of patients.This study investigates cytomegalovirus IgG among cancer patients attending Ahmadu Bello University Teaching Hospital, Zaria. A total of 267 blood samples were collected for the study. 178 samples were collected from cancer patients and 89 samples from non cancer patients (controls). Dermographic and other clinic information of the patients were taken in to account using the questionnaire. Samples were collected for a period of three months. The case studies were histopathologically confirmed cancer cases and a control population from non- cancer patients. Blood samples were collected in a sterile plain container,centrifuged, and sera was obtained. These sera were stored in the freezer untill all the 267 samples were collected. Sera samples were screened against CMV IgG, HIV and Total Human IgG with Cytomegalovirus IgG ELISA kits, "Determine" HIV test strip as well as Total human IgG kits respectively. Students T-test and One way ANOVA were used as statistical tools for the analysis of the result. Dermographic information generated in this study reveals that females have higher number of cancer cases than males, adults of age 40years and above have the highest cancer cases. And marital status, educational status, place of residence do not pose as risk factors for cancers. The study further reveals that mean CMV IgG titers of cancer patients varied significantly to that of control population (non-cancer patients). Cervical cancer was to have the highest prevalence followed by breast cancer, then nasopharyngeal cancer. Oropharyngeal cancer was found to have the highest mean CMV IgG. The cancer patients that were diagnosed with HIV had the highest mean Total Human IgG. Based on the results obtained, the study recommends combined treatment of CMV as well as other therapeutic measures for better management of the cancer patients. Patients with immunosupressive diseases should be screened against

CMV. Antenatal patients should also be screened against CMV and if infected the patients should be treated to prevent mother to child transmission.

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LIST OF ABBREVIATIONS

HCMV .....Human cytomegalovirus

CMV.....Cytomegalovirus

IgG.....Immunoglobulin G

MHC.....Major histocompatibility complex

AIDS.....Acquired immunodeficiency virus

HIV.....Human immunodeficiency virus

ELISA.....Enzyme linked immunosorbent assay

ANOVA.....Analysis of variance

ABUTH.....Ahmadu Bello University Teaching Hospital

ICTV.....International Commitee on Taxonomy of Viruses

DNA.....Deoxyribonucleic acid

HHV.....Human herpesvirus

EM.....Electron microscopy

PCR.....Polymerase chain reaction

HSV.....Herpes simplex virus

VZV.....Varicellazoster virus

CG.....Cytosine Guanine

TG.....Thymine Guanine

CA..... Cytosine Adenine

EBV.....Epstein Barr virus

RNA.....Ribonucleic acid

KSHV.....Kaposi's sarcoma associated herpes viruses

LCV.....Lymphocryptovirus

RDV.....Rhadinovirus

NK cells.....Natural killer cells

BMT.....Bone marrow transplant

CNS.....Central nervous system

TNF.....Tumour necrosis factor

DC.....Dendritic cells

STD.....Sexually transmitted diseases

PMN.....Polymorphornuclear

MW.....Molecular weight

APC.....Antigen presenting cells

SC.....Secretory component

MCMV.....Muri cytomegalovirus

CHAPTER ONE

1.0 INTRODUCTION

Cytomegalovirus (CMV) (from the Greek words cyto-, "cell", and megalo "large") is a virus of the family Herpesviridae. The species that infects humans is commonly known as human CMV (HCMV) or human herpesvirus-5 (HHV-5), and is the most studied of all cytomegaloviruses (Ryan and Ray, 2004). Within Herpesviridae, CMV belongs to the Betaherpesvirinae subfamily, which also includes the genera Muromegalovirus and Roseolovirus (HHV-6 and HHV-7) (Moore *et al*., 2007). It is related to other herpesviruses within the subfamilies of Alphaherpesvirinae that includes herpes simplex viruses (HSV)-1 and -2 and varicella-zoster virus (VZV), and the Gammaherpesvirinae subfamily that includes Epstein–Barr virus (Ryan and Ray, 2004). All herpesviruses share a characteristic ability to remain latent within the body over long periods. Although they may be found throughout the body. CMV infections are frequently associated with the salivary glands in humans and other mammals (Moore *et al.*, 2007). Other CMV viruses are found in several mammal species, but species isolated from animals differ from HCMV in terms of genomic structure and have not been reported to cause human disease.

HCMV infects more than 40-60% of the general population and up to 100% within some subpopulations and/or geographic areas (Astegiano *et al.*, 2010). CMV infection most commonly develops between ages 10-35 years and most people are exposed to CMV early in life and do not realize it because they have no symptoms.

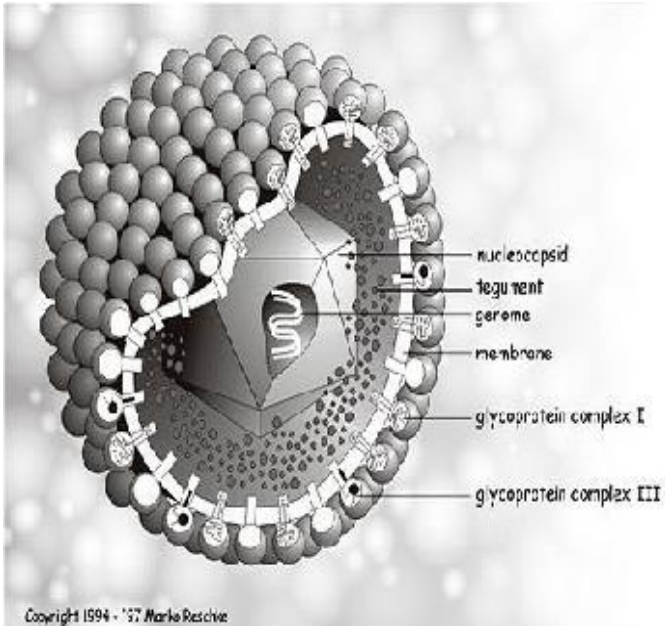

Figure 1: Structure of Cytomegalovirus

HCMV has been reported to induce genetic alterations that can potentially enhance the genotoxicity of environmental factors (radiation, chemicals, etc.) (Albert, 1989). The oncogenic properties may also stem from the ability of HCMV to transactivate proto-oncogenes, which results in mutations in these normal cells that can then lead to cancer development (Boldogh, 1990).

Sonadgol *et al*., (2011) compared CMV prevalence with the total immunoglobulin pattern of some patients with multiple sclerosis, they obtained a prevalence rate of 25%. Most of the researches on CMV have focused on pregnant women due to its resultant congenital defects. A study of seroprevalence of CMV among pregnant women in Finland yielded 77.5% (Mustakangas *et al.,* 2000), and in Japan 70.7% (Nishimura *et al.*, 1999).A similar research in India gave a prevalence rate of 91.05% (Turbadkar *et al.*, 2003) while Egypt has 96.0% prevalence rate (El-nawawy *et al.,* 1996). In Nigeria, a study conducted on pregnant women at

Federal Medical Centre, Bidda, Niger State reported a CMV prevalence rate of 84.2% (Okwori *et al.,* 2009).

Total immunoglobulin G (IgG) levels of these cancer patients will help provide information about their IgG levels and possibly deduce the effect of the viral infection, cancer, and the immune status of these patients.The major clinical indication for measuring IgG subclasses is the occurence of abnormally frequent and/ or prolonged or severe infections (Aucouturier *et al.*, 1992).

An antibody response may result in changes in the distribution of IgG subclasses in plasma, depending upon the nature of the antigen (e.g. protein or polysaccharide) and the frequency and duration of the antigenic stimulation. This may result in increased or diminished levels of one or more IgG subclasses (Morella, 1994).

1.1 Statement of the research problem

Cancer cells do not experience programmatic death and instead continue to grow and divide. This leads to a mass of abnormal cells that grow out of control (Peter, 2012). Cytomegalovirus infection is extremely wide-spread, but usually harmless infection in humans. It is passed along at birth, in breast milk, or sexually. Blood tests for antibodies, which provide evidence of a past immune response to a specific micro organism, show that more than half of any population sampled has been infected with Cytomegalovirus. Like other members of the herpesviridae family, CMV will often lie dormant inside certain cells long after the initial mild infection. The immune system can keep the virus in check for a lifetime (Cobbs*etal.*,2002).

However, just as the herpes varicella zoster (chicken pox) virus can reawaken in older or immunocompromised patients to cause painful shingles, CMV is now suspected of reactivating

in certain patients to contribute to malignancy. Researchers had connected persistent viral infections with cancers of the blood, liver, and cervix but no one had focused on CMV and brain tumours. Evidence has been found of active CMV in colon and prostate cancer cells (Cobbs *et al.*, 2002). An important new study from the laboratory of developmental genetics has confirmed cytomegalovirus as a cause of the most common salivary gland cancers. It may also be connected to other cancers beside the salivary gland cancers. Cytomegalovirus joins a group of fewer than 10 identified oncoviruses (Melnick, 2011).

Cytomegalovirus uses a variety of strategies that target host defenses,from the disruption of antigen-processing pathways to the modulation of cytokines (Tortorella *et al.*, 2000), all of which may contribute to the success of the virus in establishing coexistence with other cells of the body. The figure below is an illustration of a cancerous growth in a human body.

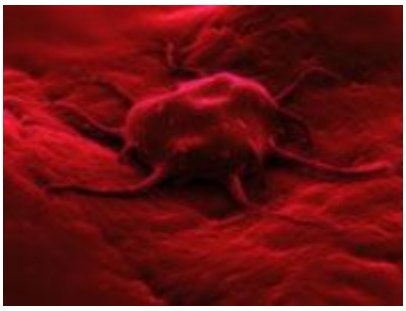

Figure 2: Illustration of cancereous growth

1.2 Justification

Cancer is a class of disease characterized by out-of-control cell growth. There are over 100 different types of cancer, and each is classified by the type of cell that is initially affected. Cancer harms the body when damaged cells divide uncontrollably to form lumps or masses of tissue called tumours (Peter, 2012).

The International Agency for Research on Cancer (IARC), the specialized cancer agency of the World Health Organization published a report in 2013 predicting millions of cancer cases have occured in 2012.

GLOBOCAN 2012 reveals striking patterns of cancer in women and highlights that priority should be given to cancer prevention and control measures for breast and cervical cancers globally. According to GLOBOCAN 2012, an estimated 14.1 million new cancer cases and 8.2 million cancer-related deaths occurred in 2012, compared with 12.7 million and 7.6 million, respectively, in 2008. Prevalence estimates for 2012 show that there were 32.6 million people (over the age of 15 years) alive who had had a cancer diagnosis in the previous five years. The global burden of cancer continues to increase largely because of the aging and growth of the world population alongside an increasing adoption of cancer-causing behaviours, particularly smoking, in economically developing countries (WHO)

Although HCMV infection of tumour cells was initially reported 30 years ago in patients with carcinomas such as prostate or colon cancer, later pathologic investigations provided conflicting results (Cinatl *et al.*, 2004). A renewed interest in the role of HCMV in cancer diseases was promoted by recent studies using highly sensitive techniques for virus detection which indicated the presence of genome and antigens of HCMV in tumour cells (but not in adjacent normal tissue) of more than 90% of patients with certain malignancies, such as colon cancer, malignant glioma, prostate carcinoma, and breast cancer (Harkin e*t al.*, 2002).

Chemotherapy is generally used to treat cancer that has spread or metastasized because the medicines travel throughout the entire body, this procedure in turn lowers the immune status of the patients and exposes them to opportunistic infections (Stewart, 2001).

An idea about the total IgG level of cancer patients will help provide information on the immune status (IgG is the most abundant immunoglobulin in blood), and possibly deduce immune deficiencies in such patients and the effect of the immunosupressive drugs.

The Clinical Oncology Journal featured a new study that showed that CMV chemotherapy together with local therapy improves survival outcomes as first line adjunctive treatment in invasive bladder cancer. The recent results show a statistically significant 16% reduction in the risk for death, which corresponds to an increase in 10-year survival from 30 to 36%, among Cancer patients treated with CMV (Daniel, 2011).

1.3 Aim and Objectives

The aim of this study Cytomegalovirus IgG among cancer patients attending Ahmadu Bello University Teaching Hospital, Zaria.

Specific objectives

1. To determine the CMV IgG titer among the study participants.
2. To determine the total Human IgG profile for all the study participants.
3. To determine the gender, age group and other risk factors associated with CMV infection as well as the type of cancer that has the highest prevalence.

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CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Herpesviruses

Historically, herpesvirus taxonomy has been addressed since 1971 by the International Committee on Taxonomy of Viruses (ICTV) (Wildy, 1971). A provisional approach to endowing herpesviruses with formal names (Roizman *et al*., 1973) was followed by classification into subfamilies largely on the basis of biological criteria (Roizman *et al*., 1981). This effort was rather successful, but not free from what turned out in hindsight to be a few misclassifications (Roizman *et al*., 1992). Further division of the subfamilies into genera utilized molecular data to a greater extent than before, primarily in relation to genome characteristics such as size and structure (Roizman *et al*., 1992). In the latest report of the ICTV Herpesvirus study Group (Davison *et al*., 2005), the family *Herpesviridae* consists of three subfamilies: *Alphaherpesvirinae* (containing the *Simplexvirus*, *Varicellovirus*, *Mardivirus* and *Iltovirus* genera), *Betaherpesvirinae* (containing the *Cytomegalovirus*, *Muromegalovirus* and *Roseolovirus* genera) and *Gammaherpesvirinae* (containing the *Lymphocryptovirus*and *Rhadinovirus* genera). In addition, genus *Ictalurivirus* is unattached to any subfamily and a large number of species not assigned to genera. All but one of the viruses assigned to taxa infect mammals or birds, although a substantial number of unassigned herpesviruses have lower vertebrate (reptilian, amphibian and fish) or invertebrate (bivalve) hosts.

The primary criterion for inclusion of an agent in the family Herpesviridae is that of virion morphology. The virion is spherical, and comprises of four major components: the core, the capsid, the tegument and the envelope The diameter of the virion depends on the viral species, but is approximately 200nm. The core consists of a single copy of a linear, double-

stranded DNA molecule packaged at high density into the capsid. The capsid is an icosahedron, and has an external diameter of 125–130nm. It consists of 162 capsomeres, 12 of which are pentons and 150 hexons, each containing five and six copies, respectively, of the major capsid protein. The capsomeres are joined via the triplexes, each of which contains two copies of one protein and one copy of another. The tegument, which surrounds the capsid, contains perhaps 30 or more viral protein species and is poorly defined structurally. In the tegument, structures positioned with symmetry corresponding to that of the capsid are detectable only in the region close to the capsid. The lipid envelope surrounds the exterior of the tegument, and is studded with at least ten viral membrane glycoproteins, in addition to some cellular proteins. The protein composition of the tegument and envelope varies widely across the family.(Cheng *et al*,).

2.2 Phenotypic structure of herpesviruses

The phenotypic architecture of the *Herpesviridae* family viruses characterizes these viruses. Customarily, herpesviruses have a central viral core that contains a linear double stranded DNA. This DNA is exemplified by a hole through the middle and the DNA is embedded in a proteinaceous spindle (Furlong *et al.,* 1972). The capsid is icosadeltahedral (16 surfaces) with 2-fold symmetry and a diameter of 100–120nm that is partially dependent upon the thickness of the tegument. The capsid has 162 capsomeres. The three dimensional structure of the HHV-8 capsid was determined by cryo-electron microscopy (EM) and was found to be composed of 12 pentons, 150 hexons, and 320 triplexes arranged as expected in the icosadeltahedral lattice with 20 faces; the capsids are 125nm in diameter (Wu *et al.*, 2000) Transmission EM showed a bulls-eye appearance in the virions with electron dense cores and amorphous teguments surrounding the viral core (Renne *et al.,* 1996) Interestingly, these

structural characteristics were seen in endemic Kaposi Sarcoma lesions as early as 1984, but were not recognized at that time as the possible etiology of the disease (Walter *et al.*, 1984).

The herpesvirus tegument, an amorphorous proteinaceous material that under EM lacks distinctive features, is found between the capsid and the envelope; it can be asymmetric in distribution. Thickness of the tegument is variable dependent upon its location in the cell and varies between different herpesviruses (McCombs *et al.*, 1971).

The herpesvirus envelope contains viral glycoprotein protrusions on the surface of the virus (Knipe and Howley, 2001). As shown by EM there is a trilaminar appearance (Epstein, 1962) derived from the cellular membranes (Morgan, 1968) and contains some lipid (Asher *et al.*, 1969). Glycoproteins protrude from the envelope and are more numerous and shorter than those found on other viruses. The presence of the envelope can influence the size measurement of the virus under EM conditions (Knipe and Howley, 2001).

2.3 Genetic properties of herpes viruses

A virus species is defined as a polythetic class of viruses constituting a replicating lineage and occupying a particular ecological niche (Van Regenmortel, 1989; 1990). Members of a polythetic class share a subset of properties, with each property possessed by several members but no property possessed by all. Herpesviruses are defined as separate species if their nucleotide sequences differ in a readily assayable and distinctive manner across the entire genome and if they occupy different ecological niches by virtue of their distinct epidemiology and pathogenesis or their distinct natural hosts (Roizman *et al*., 1992; Roizman and Pellett, 2001; Davison *et al*., 2005) but no property possessed by all. However, genomic data have come to dominate

biological properties, with taxa corresponding to genetic lineages defined by sequence comparisons and identification of genes unique to certain lineages. An increasing number of herpesviruses in the tissues of various animals are being inferred from short PCR-derived sequences, usually from a single locus in the genome and often in the absence of any other information. These "virtual viruses" cannot readily be classified under the current species definition. However, their incorporation (perhaps in a special category) could be facilitated. Members of the family *Herpesviridae* replicate their tegument, capsid and core (Davison, 2005).

Electronmicroscopy of negatively stained capsids gives the impression that the core consists of the viral DNA molecule wrapped toroidally around a protein spindle (Furlong *et al*., 1972). Images reconstructed from electron micrographs of virions frozen in ice in the absence of stain, a technique by which morphology is better preserved, show that the core consists of the DNA packed at high density in liquid crystalline form, probably as a spool lacking a spindle (Booy *et al*., 1991; Zhou *et al*., 1999). Herpesvirus genomes consist of linear, double-stranded DNA molecules that range in size from about 125 to 240 kbp and in nucleotide composition from 32 to 75% G+C depending on the virus species (Honess, 1984). The genome termini are not covalently closed as in the *Poxviridae;* (Moss, 2001) or covalently linked to a protein as in the *Adenoviridae* (Shenk, 2001). In those herpesvirus genomes that have been examined in sufficient detail, unpaired nucleotides are present at the termini; for example, Herpessimplexvirus-1, Varicellazoster and HCMV have a single 3 overhanging nucleotide at each terminus (Mocarski and Tan, 2001). Herpesvirus genomes are accommodated in larger capsids, but the relationship is not proportional, as the packing density of the DNA varies somewhat between species (Trus *et al*., 1999; Bhella *et al*., 2000). The reasons for the striking range in nucleotide composition of

herpesvirus genomes are not clear, but a similar phenomenon is found in other virus families and in cellular organisms.

In contrast to the Alpha and Beta*herpesvirinae*, the genomes of most Gamma*herpesvirinae* are generally deficient in the CG dinucleotide In vertebrate genomes, this phenomenon is thought to be due to spontaneous deamination of 5-methylcytosine residues in DNA to thymidine residues, followed by fixation through DNA replication. CG depletion in herpesviruses, and concomitant enrichment in TG and CA, has been taken as indicative of latency in dividing cell populations, in which the latent genome is obliged to replicate as host cells divide (Honess *et al*., 1989). Thus, HSV-1, which is resident in non-dividing neurons, has a CG content consistent with its nucleotide composition, where as EBV, which latently infects dividing B cell populations, is depleted. Local CG suppression of the major immediate early gene locus of HCMV has also been noted (Honess *et al*., 1989).

The herpesvirus family consists of a group of viruses distinguished by the large size of their linear doublestranded DNA genomes (130–250kbp) and a common architecture of infectious particles (Gibson, 1996; Chiu and Rixon, 2002). Indeed, before the birth of molecular biology and the availability of genomic sequencing, the common hallmark structural features shared by these viruses were the most important criteria for the classification of a herpesvirus (Roizman and Pellett, 2001). All herpes viruses identified to date, which include eight different types that are known to infect human, and more than 170 other viruses that are found in animals as well as in fish and amphibians (Roizman and Pellett, 2001), exhibit identical structural design. Thus, These viruses have a highly ordered icosahedral-shape nucleocapsid of about 125–130nm in diameter, which encases the viral DNA genome. The nucleo capsid is surrounded by a partially ordered proteinaceous layer called the tegument, which in turn is enclosed within the

envelope, a polymorphic lipid bilayer containing multiple copies of more than 10 different kinds of viral glycoproteins that are responsible for viral attachment and entry to host cells (El zein *et al,* 2008).

2.4 Genomic structure and genes of herpesviruses

There are six defined DNA genomic sequence arrangements for viruses in the *Herpesviridae* family. Of the human herpesviruses, Epstein Barr virus and Human herpesvirus-8 are in class C. In this grouping, the number of direct terminal repeats are smaller than for other herpesviruses and there are other repeats found within the genome itself that subdivide the genome into unique stretches (Knipe and Howley, 2001).

All known herpesviruses have capsid packaging signals at their termini (Deiss *et al.,* 1986) The majority of herpes genes contain upstream promoter and regulatory sequences, an initiation site followed by a 5' nontranslated sequence, the open reading frame (Orf) itself, some 3' nontranslated sequences, and finally, a polyadenylation signal. There are exceptions to this format because initiation from an internal in-frame methionine has been reported (Markovitz *et al.*, 1999).

Gene overlaps are common, whereby the promoter sequences of antisense strand (3') genes are located in the coding region of sense strand (5') genes; Orfs can be antisense to one another. Proteins can be embedded within larger coding sequences and yet have different functions. Most genes are not spliced and therefore are without introns and sequences for noncoding RNAs are present (Knipe and Howley, 2001).

Herpesviruses code for genes that code for proteins involved in establishment of latency, production of DNA, and structural proteins for viral replication, nucleic acid packaging, viral entry, capsid envelopment, for the blocking or modifying host immune defenses, and transitions from latency to lytic growth. Although all herpesviruses establish latency, some (e.g., HSV) do not absolutely require latent protein expression to remain in latency, unlike others (e.g., EBV and HHV-8). Herpesviruses can alter their environment by affecting host cell protein synthesis, host cell DNA replication, immortalizing the host cell, and the host's immune responses (e.g., blocking apoptosis, cell surface MHC I expression, modulation of the interferon pathway) (Knipe and Howley, 2001).

2.5 Classification of herpes viruses

More than 100 herpesviruses have been discovered, of which all are double-stranded DNA viruses that can establish latent infections in their respective vertebrate hosts; however, only eight regularly infect humans. The *Herpesviridea* family is subdivided into three subfamilies: the Alpha-, Beta-, or Gamma *herpesvirinea*. This classification was created by the Herpesvirus Study Group of the International Committee on Taxonomy of Viruses using biological properties and it does not rely upon DNA sequence homology. However, researchers have been able to identify and appropriately characterize the viral subfamilies using DNA sequence analysis of the DNA polymerase gene; other investigators have been successful using the glycoprotein B gene (Knipe and Howley, 2001).

Based on their biological properties such as growth characteristics and tissue tropism, herpesviruses can be further divided into three subfamilies. Among the eight human herpesviruses, the alpha subfamily includes neurotropic viruses and contains the herpes simplex

virus (HSV) 1 and 2, and Varicella zoster virus (VZV). The members of the gamma subfamily are lymphotropic viruses and include Epstein–Barr virus (EBV) and Kaposi's sarcoma associated herpesvirus (KSHV). The viruses of the beta subfamily appear to be able to establish infections in many different types of cells and tissues, and include human cytomegalovirus (HCMV), and human herpesvirus 6 and 7. This sub family classification system is largely consistent with the extensive genomic information that is now available (McGeoch *et al*., 2000).

While studies have been attempted to investigate the structure and architecture of each of the eight human herpesviruses, virion and virus related particles of herpes simplex virus 1 (HSV-1), the prototype of all herpesviruses, have been subjected to the most extensive structural studies (Schrag *et al*., 1989; Booy *et al*., 1991; Newcomb *et al*., 1993; Zhou *et al*., 1999, 2000). During the last several years, significant progress has also been made in understanding the structure of cytomegaloviruses (Bhella *et al*., 2000; Chen *et al*., 1999; Trus *et al*., 1999), the prototype of the beta herpesvirus family, and KSHV, a representative of the gamma herpesvirus family (Wu *et al*.2000; Nealon *et al*., 2001; Trus *et al*., 2001; Lo *et al*., 2003;)

2.5.1 The Alphaherpesviridea

The Alpha*herpesviridea* are defined by variable cellular host range, shorter viral reproductive cycle, rapid growth in culture, high cytotoxic effects, and the ability to establish latency in sensory ganglia. In humans, these are termed herpes simplex viruses 1 and 2 (HSV-1 and HSV-2) and varicella zoster virus (VZV), and represent human herpesviruses 1, 2, and 3 (Knipe and Howley, 2001).

Human herpesviruses 1, 2, and *3* have been classified as alphaherpesviruses based originally upon their biological properties, and subsequently on the sequences of their respective

genomes (Minson *et al*., 2000). All of these viruses maintain latent infections in sensory ganglia and can productively infect a variety of human cells, including the living cells of mucous membranes and skin. These epithelial sites also provide exit points for the virus to infect other individuals.

2.5.2 The Betaherpesviridea

The Beta*herpesviridea* have a more restricted host range with a longer reproductive viral cycle and slower growth in culture. Infected cells show cytomegalia (enlargement of the infected cells). Latency is established in secretory glands, lymphoreticular cells, and in tissues such as the kidneys among others. In humans, these are termed human cytomegalovirus (HCMV or herpesvirus 5), human herpesviruses 6A and 6B (HHV-6A and -6B), and human herpesvirus 7 (HHV-7). HHV-7 has also been called the roseolavirus, after the disease roseola infantum it causes in children (Knipe and Howley, 2001).

The two major lineages in the *Betaherpesvirinae* are the cytomegaloviruses (the *Cytomegalovirus* and *Muromegalovirus* genera, plus a number of other viruses whose taxonomy is only partially defined) and the *Roseolovirus* genus.The best characterized members of these lineages are HCMV (the prototype of the subfamily) and HHV-6. Cytomegaloviruses are present in a wide range of mammalian species, and have been termed “salivary gland viruses” because of their ease of isolation from explanted tissue. An earlier divergence of the *Betaherpesvirinae* may be represented by a herpesvirus of elephants (Richman *et al*., 1999; Ehlers *et al*., 2001).

2.5.3 The Gammaharpesviridea

The Gamma*herpesviridea* have a host range that is found within organisms that are part of the Family or Order of the natural host. In vitro replication of the viruses occurs in lymphoblastoid cells, but some lytic infections occur in epithelial and fibroblasts for some viral species in this subfamily. Gammaherpesviruses are specific for either B or T cells with latent virus found in lymphoid tissues. Only two human Gammaherpesviruses are known, human herpesvirus 4, referred to as Epstein-Barr virus (EBV), and human herpesvirus 8, referred to as HHV-8 or Kaposi's sarcoma-associated herpesvirus (KSHV) (Knipe and Howley, 2001).

The gammaherpesviruses subfamily contains two genera (a classification of closely related viruses) that includes both the gamma-1 or *Lymphocryptovirus* (LCV) and the gamma-2 or *Rhadinovirus* (RDV) virus genera. EBV is the only LCV and HHV-8 is the only RDV discovered in humans. LCV is found only in primates but RDV can be found in both primates and subprimate mammals. RDV DNAs are more diverse across species and are found in a broader range of mammalian species. It is thought that RDVs evolved before LCVs (Knipe and Howley, 2001).

HHV-8 has sequence homology and genetic structure that is close to another RDV, *Herpesvirus saimiri* (HVS)(Moore and Chang 2001) HVS can cause fulminant T-cell lymphoma in its primate host and can immortalize infected T-cells (Mesri *et al.*, 1996) Rhadinaviruses can infect ungulates, mice, and rabbits and all share a particular genomic organization characterized by large flanking, highly repetitive DNA repeats of high G/C content (Neipel *et al.*, 1998).

2.6 Human cytomegalovirus

Human cytomegalovirus is a widely distributed human herpesvirus with a highly restricted host range. It is the prototypic betaherpesvirus with a life cycle that includes the ability to establish a life-long latent infection within the host. During latency, viral gene expression is highly restricted, and infectious virions are not produced (Morcaski and Tan, 2001). Periodically, HCMV is able to reactivate from latency, resulting in the production of new infectious virus, a process which often leads to life-threatening disease in immunosuppressed individuals. While HCMV infection causes mild or clinically non apparent disease in the immunocompetent host, it is a major cause of morbidity and mortality in immunocompromised individuals such as patients with Acquired Immunodeficiency Syndrome, allograft recipients (bone marrow and solid organ), and neonates (Pass, 2001). Despite the major clinical impact of this virus on selected populations, many aspects of HCMV biology and pathogenesis remain poorly understood. In particular, little is known about the molecular mechanisms that underlie the ability of the virus to persist in a latent form.(Boeckh et al,2001).

Human cytomegalovirus is the main cause of congenital viral infection (Ljungman *et al*., 2002; Sissons and Carmichael, 2002). The viral genome is one of the largest and most complex genomes known, and many regions of variation have been identified, more so in clinical isolates than in laboratory-adapted strains (Cha *et al*., 1996; Prichard *et al*., 2001). Human cytomegalovirus can replicate in several cell types, including endothelial cells, which is important in intrauterine infections as well as in associations with cardiovascular disease (Jarvis and Nelson, 2002; Maidji *et al*., 2002).

Cytomegalovirus is able to establish a life-long relationship with its host via a latent infection. Patients with compromised immunity, including those with human immunodeficiency virus infection, malignancies and organ transplants, or individuals

receiving immunosuppressive therapy, are at high risk for reactivation of latent CMV. Most knowledge regarding CMV reactivation has come from studies of transplant

recipients and AIDS patients (; Limaye *at al.,* 2001; Alberola *et al.,* 2001. Chemaly *et al.*, 2004). However, increasing numbers of patients receiving chemotherapy who experience symptomatic CMV reactivation have been observed, although there are few published data concerning this phenomenon (Han, 2007).

2.6.1 Structure of human cytomegalovirus

Human cytomegalovirus is a ubiquitous beta humanherpesvirus type 5. Compared to other human herpesviruses, HCMV is the largest, with a genome of 235kb encoding 165 genes (Davison *et al.,* 2003). The virion consists of a double-stranded linear DNA core in an icosahedral nucleocapsid, enveloped by a proteinaceous matrix (the tegument) (Chen *et al.,* 1999). These components are enclosed in a lipid bilayer envelope that contains a number of viral glycoproteins (Morcaski *et al.,* 2007). Mature virions range in diameter from 200 to 300 nanometers (Morcaski *et al.,* 2007).

The tegument compartment contains the majority of the virion proteins, with the most abundant tegument protein being the lower matrix phosphoprotein 65 (pp65), also termed unique long 83 (UL83) (Varnum *et al.,* 2004). Other major tegument proteins include the virion transactivator pp71 (upper matrix protein, UL82 gene product), the herpesvirus core virion maturation protein pp150 (large matrix phosphoprotein, UL32 gene product), the largest

tegument protein (UL48 gene product), and the UL99-encoded pp28 (Varnum *et al.*, 2004). In addition, the tegument also contains additional proteins that are present in small amounts and some cellular and viral RNA (Morcaski *et al.*, 2007).

The function of the tegument proteins can be separated into two classes:

(i) Proteins that play a structural role and are important for the assembly of virions and the disassembly of the particle during entry

(ii) Proteins which modulate the host cell response to infection (Morcaski *et al.*, 2007). The host cell endoplasmic reticulum-golgi intermediate compartment-derived lipid bilayer envelope surrounding the tegument contains at least 20 virus-encoded glycoproteins that are involved in cell attachment and penetration (Morcaski *et al.*, 2007). These include glycoprotein B (gB), gH, gL, gM, gN, and gO (Varnum *et al.*, 2004). Productive infection leads to the coordinated synthesis of proteins in three overlapping phases based on the time of synthesis after infection, namely, immediate early (IE) (0 to 2 h), delayed-early (24 h), and late (24 h) viral proteins.

Surface projections of envelope are distinct and spikes are dispersed evenly over the entire surface. The nucleocapsid is isometric, and surrounded by the tegument that consists of globular material which is frequently asymmetrically distributed and may be variable in amount (Tsaparas *et al.*, 2003). The nucleocapsid is sometimes penetrated by stain (although an intact envelope is impermeable to stain). Viral nucleocapsid is angular and has an icosahedral symmetry. There are 162 capsomeres per nucleocapsid, and capsomeres are hexagonal in cross section with a hole running half way down the long axis. The viral core consists of a fibrillar spool on which DNA is wrapped while the ends of the fibres are anchored to the underside of the

capsid shell. Incomplete virus particles often present, and they represent capsids lacking the envelope (Tsaparas *et al.,* 2003).

CMV is inactivated by a number of physical and chemical treatments including; heat (56□C for 30 minutes), low pH, ether, ultraviolet light and cycles of freezing and thawing (Richardson *et al*,2004). CMV virions contain one molecule of linear double stranded DNA. Total genome length is 120,000-220,000bp .Molecular virology techniques have been used to study variation among CMV strains. DNA strains have been shown to have similar but distinctive fragment migration patterns. Antigenic heterogeneity among CMV strains has been detected in cross neutralization and other serologic assays; however evidence for distinct serotypes is limited (Cicognac, 1993; Patel *et al.,* 2009).

The HCMV envelope is exceedingly complex and currently incompletely defined. Its genome encodes ORFs to at least 57 putative glycoproteins; far more than other Herpesviruses. However, the extent of transcription, translation and function of the majority of these glycoproteins remains unknown. Biochemical studies of the virions have revealed 14 structural glycoproteins; eight of which have been experimentally shown to reside in the envelope (Britt *et al*., 2004).

2.6.2 Immunity against cytomegalovirus infection

The cell-mediated immune response plays an important role in the control of HCMV infection. In particular, clinical observations indicate that the severity of HCMV disease parallels the degree of impairment of T-cell responses (Pass, 2001). Both major histocompatibility complex class I (MHC-I)- and MHC-II-restricted HCMV-specific cytotoxic T lymphocyte responses are generated and are important for virus clearance (Lindsley *et al.*, 1986). MHC-II-

restricted CD4 cells act as critical antiviral effectors as well as providing helper functions for maintaining HCMV specific CD8 T-cell responses (Komanduri *et al.*, 2001), and impairment of the HCMV-specific CD4 T-cell response has been associated with prolonged secretion of virus in young children (Tu *et al.*, 2004).

In a study of CD4 T-cell responses following primary HCMV infection in renal transplant recipients, the results provided evidence for the emergence of HCMV-specific CD4 T cells that were represented during the latent phase of infection and that had the capacity to lyse HCMV antigen-expressing cells in an MHC-II-dependent manner (Vanleeuwen *et al.*, 2006). Thus, MHC-II- restricted CD4 T cells likely play an important role in the control of productive infection and latency/reactivation. These findings have led to the hypothesis that HCMV-encoded modulation of MHC-II would enhance the capacity of the virus to limit the host immune response during both productive and latent phases of infection. During latency, interference with MHC-II expression leading to impairment of CD4 T-cell surveillance of latently infected cells may enhance the chances for long-term maintenance of the virus. In this respect, experimental latent infection of cultured granulocyte macrophage progenitor (GM-P) cells has been reported to result in a decrease in the expression of cell surface MHC-II molecules (Slobedman *et al.*, 2004).

Human cytomegalovirus clinical strains display genetic polymorphisms, possibly related to strain-specific tissue-tropism and HCMV-induced immunopathogenesis. A substantial portion of the genome encodes proteins with the potential to affect virulence through cell tropism, immune evasion, molecular mimicry, or interference with host chemokines (Hahn *et al.*, 2004).

2.7 Antibody and complement against human cytomegalovirus infections

Following primary infection, antibodies specific to numerous HCMV encoded proteins are readily detectable in serum (Britt, 1991; Landini and Michelson, 1988). These include structural tegument proteins (such as pp65 and pp150), envelope glycoproteins (predominantly gB and gH) as well as non-structural proteins such as the Immediate Early 1 protein (IE1, UL123) and a DNA binding protein (UL44). Viral neutralizing activity in vitro
is predominantly mediated by antibodies specific for gb and gH (Britt *et al*., 1988; Urban *et al*., 1996). Both mouse and guinea pig animal models suggest that antibody is important in protection from a lethal infective dose and in reducing fetal infection (Rapp *et al*., 1992; Harrison *et al*., 1995). In humans, pre-existing humoral immunity to cytomegalovirus also plays an important role in preventing congenital infection of the fetus during pregnancy (Fowler *et al*., 1992) and in preventing transfusion-associated infection in premature infants (Yeager *et al*., 1981). The role of humoral immunity in the pathogenesis of HCMV disease
in immunosuppressed patients is uncertain. Prior infection and thus the existence of preformed antibody to the may be an important factor in limiting reactivation and protecting from re-exposure: however there is very limited evidence for the benefits of administration of HCMV-specific antibody to immunosuppressed transplant patients who
are undergoing a primary infection or reactivation, with some studies reporting beneficial results and others suggesting no benefit (Guglielmo *et al*., 1994; Munoz *et al*., 2001).

2.7.1 HCMV, innate immunity and natural killer cells (nk) cells

NK cells were originally described for their ability to mediate cytotoxicity against certain tumours without prior activation; they are characterized by the lack of both T- and B-cell

markers, and are a component of the innate immune system. An important role for NK cells in the early control of viral infections is emerging (Tay *et al*., 1998; Biron *et al*., 1999). There is only limited evidence for the role of NK cells in the control of HCMV infection (Biron *et al*., 1989, 1999). However the virus encodes a variety of NK cell evasion mechanisms which provides indirect evidence for the importance of these cells in the innate response to HCMV.

In the MCMV murine model system the evidence for their protective role is much stronger. Newborn mice are highly susceptible to lethal MCMV infection until NK responses become apparent at 3 weeks. Adoptive transfer of NK cells into these mice or adult Mice with Severe combined immunodeficiency can confer protection. (Tay *et al*., 1998). Inbred mouse strains have differing resistance to Muri cytomegalovirus (Scalzo *et al*., 1992), and the dominant CMV1 resistance locus has been mapped to the NK cell gene complex on chromosome 6. The CMV11 resistance gene has now been shown to encode an activating NK cell receptor Ly49H (Brown *et al*., 2001; Lee *et al*., 2001; Daniels *et al*., 2001).

2.8 Pathogenesis of CMV

An understanding of the process of CMV viral replication will provide insights into its molecular mechanisms of infection and pathogenesis (Bale *et al.*, 1999). Following infection, or contact with the virus, the DNA enters the nucleus of the host cells and begins the process of replication and shedding, leading to the release of new virions (viral particles) into the blood and other body fluids. This replication cycle takes approximately 24hs and consists of 3 phases ; an immediate early phase (4hs) during which regulator proteins are made followed by an early phase (8hs) during which viral DNA polymers are made and a late phase (12hs) during which structural proteins and new progeny viral particles are assembled .

In an immunocompetent host, most of the virus is destroyed by CMV –specific cytotoxic T-cells, and the infectious process becomes asymptomatic. The presence of asymptomatic CMV infection is based on the detection of CMV in body fluids or on seroconversion from a negative to a positive status. The latter is defined as the appearance of IgM or a fourfold rise in the level of CMV specific IgG antibodies. In immunocompromised patients who are not receiving prophylactic treatment against CMV, symptomatic CMV infection; CMV disease or organ involvement may result (Wingard *et al.*, 1999).

There are various mechanisms by which CMV disease may occur and these include; direct cellular loss through viral infection, both of individual cells, and of larger foci because of propensity to spread, leading to coalescing cells. This close cell-cell interaction protects virus from antibody inactivation. Systemic hypoxic- Ischemic insult due to systemic hypotension and immunologic injury, involving antigen antibody complex deposition may also be involved in organs such as the kidney. Eventually, CMV attains a latent state and persistent infection within T- cells, endothelial cells and monocyte-derived macrophages ensues (Wingard *et al.*, 1999).

Cell mediated immunity which is required for resolution of symptoms, also contributes to symptoms while the role of antibodies are limited. Suppression of cell mediated immunity as seen in HIV infection allows recurrence of symptoms and can result in exacerbation of disease. The virus also has the ability to induce immunosuppression in the body (Cicognac, 1993). The degree of morbidity induced by CMV is influenced by the degree of immunosuppression. In transplant recipients, there are two factors that influence the degree of morbidity due to CMV infection; the type and extent of immunosuppression, and the type of transplant. Morbidity is highest among bone marrow transplant (BMT) patients, and lowest among kidney transplant patients. Multiple mechanisms of immune evasion by CMV could relate to the pathogenic role of

the virus. The expression of immune evasion genes US3, US6, and US11 of CMV in the blood of solid organ transplant recipients has been investigated (Patel *et al.,* 2009).

2.9 Pathology of CMV

CMV produces characteristic cytopathic changes on infected cells which appear large, rounded and contains ground glass appearing inclusion bodies in the cytoplasm. These infected cells are the hallmark of CMV infection and indicates the presence of CMV in a sample. The nuclear inclusion has the appearance of an owl's eyes because it is typically surrounded by a clear halo that extends to the nuclear membrane (Euright *et al.*, 2001).

2.10 Clinical manifestations of CMV

Following a first exposure to CMV, the virus lies dormant in the body for life and can be reactivated. Usually a reactivated infection causes few or no symptoms. CMV disease may manifest in any of the under listed ways.

2.10.1 Congenital infection

Congenital CMV infection is defined as the isolation of CMV from the saliva or urine of the neonate within 3 weeks of birth (Meyers and Flournoy, 1996). The virus may also be transmitted from the mother to the infant in breast milk. CMV is now the commonest cause of congenital infection and affects around 0.3%-1% of all live births. A total of 5-10% of congenitally infected infants have symptoms at birth while fatal disease occurs in 20% of these infants (Boeckh *et al.*, 2001).

Ninety percent (90) of the symptomatic survivors have long term sequelae while 15% of the asymptomatic survivors also have long term sequelae. CMV is now the second commonest cause of mental retardation after Downs's syndrome and causes more cases of congenital damage than Rubella (Meyers and Dandliker, 1998) Cytomegalic inclusion disease refers to any group of diseases caused by cytomegalovirus infection, and marked by characteristic inclusion bodies in enlarged infected cells. The classic disease is congenital, being acquired *in utero* from the mother and typically presents with intrauterine growth retardation, jaundice, hepatosplenomegaly, thrombocytopenia and encephalitis, with or without microcephaly. It is often difficult to differentiate, on clinical grounds between the several agents that may cause intrauterine infection. The severe thrombocytopenia, hepatitis, peumonitis and myocarditis attributed to the virus may be life threatening. Central nervous system involvement may lead to seizures, focal neurological signs, and mental retardation. Unlike rubella, there is no evidence that CMV is teratogenic.(Meyers and Dandliker, 1998).

Most of the damage is caused by destruction of target cells once they have been formed, and unlike rubella, the fetus can be damaged by infection during any stage of pregnancy. In 20% of cases (1% of those who are congenitally infected), the infection is so severe that mortality occurs during infancy, while the rest are likely to sustain serious abnormalities for the rest of their lives. Brain damage is by far the commonest abnormality on follow up, and thus may manifest as microcephaly, mental retardation and seizures. The spectrum of brain damage varies from mild to severe. Optic atrophy, deafness, and blindness may also be present (Atkinson and Downs, 1995).

The effects of congenital CMV infection are as follows; CNS abnormalities microcephaly, mental retardation, spasticity, epilepsy, periventricular calcification, eye-

choroidoretinitis, and optic atrophy, ear – sensorineural deafness, liver – hepatosplenomegaly and jaundice which is due to hepatitis., lung – pneumonia, heart – myocarditis, haematological thrombocytopenic purpura, haemolytic anaemia, neutropenia and lymphocytosis. Late sequelae include damage to the enamel forming organ of the teeth, resulting in yellow discoloration of the teeth and brittleness. This occurs in 40% of infants (Atkinson and Downs, 1995).

2.10.2 Perinatal infection

Despite the continued excretion of high titers of virus in the urine for many months, the vast majority of perinatally infected infants do not develop acute symptoms, although few cases of infantile pneumonitis have been reported (Meyers and Dandliker, 1998; You and Dimopoulous, 2001). This appears to be an exceedingly rare event.

2.10.3 Postnatal infection

The incubation period for CMV is thought to be 4-8 weeks. Primary CMV infection in the postnatal period is usually mild or asymptomatic. Occasionally, primary infection may be accompanied by the syndrome of infectious mononucleosis, with atypical lymphocytosis. This is similar to the syndrome produced by Epstein Barr virus (EBV) except that lymphadenopathy is uncommon, and Paul-Bunnell test is negative. CMV- induced mononucleosis can be symptomatically indistinguishable from EBV induced mononucleosis (Goldrich and Mori, 2004). Malaise, fever of up to 39.4^{o}C, chills, sore throat, headache, and fatigue can be the predominant features of both viruses. Many of the same clinical manifestations that are typical of EBV-induced mononucleosis (e.g. lymphadenopathy, splenomegaly, pharyngeal erythema) also can occur with CMV, although less frequently. Presentation of acute cytomegalovirus infection

in an immunocompetent host may be in form of mononucleosis. Patients with mononucleosis may present with nonspecific skin rashes such as; generalised maculopapular, urticarial, and scarlatiniform rashes. These rashes are not a direct cause of CMV proliferation within the skin, but are the result of an immunologic response to the virus.

The classic hypersensitivity drug rash which is associated with ampicillin therapy given to patients with EBVinduced mononucleosis also can occur with CMVinduced mononucleosis. The post perfusion syndrome is essentially CMV mononucleosis acquired by blood transfusion (Canpolat and Culbert, 1997). Sometimes the hepatitis picture predominates so that a diagnosis of non-non-B hepatitis is made.

2.10.4 CMV and organ transplant rejection

The risk of HCMV disease is 3–5 times greater in a seronegative than a seropositive recipient receiving an organ allograft from a seropositive donor, and disease is much more severe. Many centers "match" seronegative donors to seronegative recipients, although this is often thwarted by organ shortage. Disease often presents with specific organ involvement not seen in the normal subject. Interstitial pneumonitis due to HCMV carries a poor prognosis; disease in the gastrointestinal tract includes oesophagitis, gastritis and gastric ulceration, and colitis. HCMV retinitis may occur in severely immunosuppressed patients (Rubin, 2001; van der Bij and Speich, 2001).

There has been much circumstantial suggestion in the literature that CMV, even more so than other virus infections, may be somehow involved in the pathogenesis of rejection of solid organ allografts. There is suggestive epidemiological evidence that clinically significant HCMV infection is commoner in subjects who develop graft rejection, although it can be difficult to

dissect out whether HCMV infection is a consequence of the immunosuppressive therapy in use, or preceded the treatment of rejection episodes – the latter would make a causal association more plausible (Borchers *et al*., 1999). Possible mechanisms which have been invoked to explain how CMV might be causally associated with rejection have focussed mainly on changes which the virus might induce in endothelial cells and in the expression of Class II MHC molecules. It is postulated that CMV-induced upregulation of adhesion molecules on endothelial cells might promote the infiltration of allospecific T-cells (Borchers *et al*., 1999; Craigen and Grundy, 1996). There is some evidence for increased expression of Class II MHC molecules on cells in solid organs in association with CMV infection – given the evidence that, if anything, CMV may downregulate class II MHC molecules in isolated cell types in vitro, such upregulation seems more likely to be mediated by cytokine release related to virus infection, such as IFN-_ or others. There is some evidence from the model of rat CMV infection that rat CMVcan enhance chronic kidney allograft rejection in a transplant model (Lautenschlager *et al*., 1997) and that this is associated with increased vascular endothelial and tubular epithelial expression of ICAM-1 and increased interstitial inflammation (Yilmaz *et al*., 1996). One group has reported that CD13 (Aminopeptidase N, a cell surface zinc metalloproteinase) is incorporated into virions, and that this may be associated with the development of autoantibodies to CD13. In the allogeneic bone marrow transplant setting, the presence of antibodies to CD13 correlated with the development of GVHD, and it was suggested CMV induced CD13 specific autoimmunity was contributing to the mediation of the GVHD (Moller *et al*.,1999).

The other aspect of solid organ allograft rejection in which CMV has been implicated is the chronic vasculopathy which may be a feature of chronic rejection. In cardiac transplantation, the most common cause of death following transplantation is cardiac allograft vasculopathy, an

obliterative progressive vascular disease of the coronary arteries which is believed to be a formof chronic rejection (Orbaek, 1999).

A number of studies have indicated an association between CMV and cardiac allograft vasculopathy (Weill, 2001), and it has been postulated that CMV may promote vasculopathy by the sort of mechanisms discussed above. Again, these suggestions are largely based on circumstantial evidence, although the virus may enhance the development of allograft vasculopathy in the rat CMV model of heterotopic heart or aortic transplantation, (Hosenpud, 1999; Koskinen *et al*., 1999).

2.10.5 CMV infection in the immunocompromised patients

Primary CMV infection in immunocompromised individuals is far less likely to be asymptomatic. These patients develop spiking pyrexia, which resolves within a few days (Canpolat and Culbert, 1997). Some may develop a viraemia with septicemia - like syndrome in the presence or absence of hepatitis. Pneumonitis may develop, which is associated with grave prognosis .The virus may disseminate to involve the retina, causing CMV retinitis which is the commonest occular opportunistic infection, and the most common cause of visual loss in people with AIDS (You and Dimopoulous, 2001). CMV may disseminate to the gut, where it may cause an asymptomatic infection or ulceration or haemorrhage by the erosion of nearby blood vessels. In patients with CMV – induced immuno suppressive syndrome the patient becomes unable to deal with opportunistic infections such as Pseudomonas. AIDS patients may develop low grade encephalopathy and CMV adenitis.

In addition, kaporsi sarcoma has been associated with past CMV infection which also is the most common infection in bone marrow transplant recipients. Infection by this virus occurs

in over one half of the patients. The virus is an immunomodulator and CMV disease exacerbates an ongoing immunosuppression in transplant recipient, thereby increasing the risk for bacterial and fungal infection such as *Norcadia asteriodes, Mycoplasma,* and *Pneumocystis carini*.(sissons *et a*l, 2002).

2.10.6 Relationship between CMV and cancer

Cancer is the result of multiple genetic changes that can be mediated through gross chromosomal changes, which have the potential to be cytogenetically detectable (Solomon *et al.*, 1991). The CBMN-Cyt assay is a widely used cytogenetic method to measure DNA damage by (Cobbs *et al.*, 2002) scoring chromosome fragments or whole chromosomes that form micronuclei (MN) as a result of not engaging with the mitotic spindle and (Schwartzbaum *et al.*, 2006) nucleoplasmic bridges (NPBs) which originate from dicentric chromosomes whose centromeres are pulled to opposite poles of the cell at anaphase (Fenech *et al.*, 2003; Fenech, 2007) This technique is a comprehensive means of scoring cellular events in the form of not only chromosome damage, but also cell death. Thus, the CBMN-Cyt provides a better understanding of the underlying mechanisms involved in the sensitivity of cells to genotoxic exposures. MN have been used as a biomarker of cancer risk in lung and breast cancers (Cheng *et al.*, 1996; Mozdarani *et al*., 2005; El-zein *et al.*, 2006; Wu *et al.*, 2007; El-zein *et al.*, 2008). This assay is an ideal means for evaluating the effects of chromosome damage modulated by HCMV and radiation; since it can identify and score an optimal amount of damage resulting from different chromosomal changes (*i.e.*, MN, NPBs and apoptosis).

Human cytomegalovirus has been reported to increase the risk of a number of cancers including prostate and cervical carcinomas, Kaposi's sarcoma and gliomas (Cobbs *et al.*, 2002;

Shirashi *et al.,* 2008). It is a herpesvirus that affects up to 80% of adults (depending on the source population), however, most infections remain subclinical (Ho *et al.,* 1990; Albrecht *et al.,* 2004). After infection, the virus may enter into a latency period that can persist for years and be reactivated in response to immunosuppression or genotoxic exposures, which allows the virus to actively initiate mutagenesis through the disruption of cellular pathways, such as apoptosis and cell proliferation (Boldogh *et al.,* 1983; Boldogh *et al.,* 1991; Scheurer *et al.,* 2008). The virus has also been reported to induce genetic alterations that can potentially enhance the genotoxicity of environmental factors (radiation, chemicals) (Albrecht *et al.,* 1989). The oncogenic properties may also stem from the ability of HCMV to transactivate proto-oncogenes, which result in mutations in these normal cells that can then lead to cancer development (Boldogh *et al.,* 1990; Boldogh *et al.,* 1991). Furthermore, other researchers have detected HCMV DNA and antigens present in glioblastoma, anaplastic and low-grade glioma tissues (Scheurer *et al.,* 2008) When combined with DNA damaging agents, such as ionizing radiation, HCMV's oncogenic potential is enhanced (Boldogh *et al.,* 1990; Boldogh *et al.,* 1991). (Ohagen *et al*., 2004) demonstrated the ability for radiation to induce latent HCMV activation and expression. Similar studies focusing on pancreatic cancer have also found a significant association with radiation and the activation of HCMV promoters and virus replication (Egami *et al.,* 2008).

Activated HCMV can induce chromosomal damage with subsequent genetic instability that facilitates the development of cancer. The mechanism for virus replication has been difficult to ascertain (Albrecht *et al.,* 1989) but new *in vitro* testing techniques have provided insight into modes of replication and inhibition of HCMV (Bresnahan *et al.,* 2000) and furthermore, it has been shown to replicate in the presence of radiation (Shanley, 1986). In addition, Abubakar *et al*., 1990 has provided evidence illustrating HCMV's ability to induce chromosomal aberrations

when activated in the body. These aberrations, when coupled with exposure to genotoxic agents such as ionizing radiation, are hypothesized to increase the risk of malignancies at the site of infection (Deng *et al.,* 1992).

Epidemiological studies have explored the association between genetic alterations induced by ionizing radiation and glioma development (Gurney and Kadan, 2001) Ionizing radiation has been shown to cause chromosomal aberrations, mainly double strand breaks, which facilitate an ongoing cycle of DNA damage, dysregulation of cellular pathways and overall genomic instability (Deng *et al.*, 1992; Bonassi *et al.,* 2001). The DNA alterations caused by radiation are thought to be the promoters of malignant glial cell transformation and development. (Bondy *et al.*, 1996; Bondy *et al.,* 2001; Bondy *et al.,* 2008), Lui *et al.,(* 2009), reported that exposure to ionizing radiation was consistently observed as an independent risk factor for brain tumours with higher levels of chromosome damage, with an increased sensitivity to radiation in glioma patients compared to controls. Exposure to therapeutic high-dose radiation has also been shown to increase risk of gliomas (Hodges *et al.*, 1992). Other studies have concluded that exposure to therapeutic cranial radiation is a likely risk factor for the development of secondary brain tumours of glial origin in cancer survivors. (Edick *et al.*, 2005; Relling *et al.*, 1999).

HCMV spreads mainly by the sexual and transfusion routes and an association with cervical cancer has been suggested (Odida and Schmauz, 1996). Based on previous studies, it may be suggested that HCMV is a cofactor in the oncogenesis of cervical cancer, however, it is not determinative and might not be directly associated with the oncogenic processes of oral cancer (Yang *et al.,* 2004). Moreover, HCMV was the only virus associated with thyroid tumour and the presence of HCMV in mammary tissues may be common and might occur less frequently in thyroid tissues than in breast tissues (Tsai *et al.,* 2005). Further, late exposure (in

adulthood) to a common virus, such as human cytomegalovirus, has been hypothesized as a risk factor for breast carcinomas (Richardson, 1997).

Other researchers have further provided evidence of an association between HCMV IgG levels and breast cancer in young women (Breathnach *et al.,* 1999). By measuring HCMV IgG levels in stored plasma from 208 women with breast cancer and 169 controls, higher mean HCMV IgG levels were found in women with breast cancer. This could be the result of a more recent infection with HCMV, and potentially confirms that late exposure to HCMV is a risk factor for breast cancer (Richardson *et al.,* 2004). Interestingly, it is consistent with other previous study to suggest HCMV as well as human herpesvirus-8 are the only two studied DNA tumour viruses closely related to relapse-free and overall survivals of breast cancer(Tsai *et al.,* 2007). It has been suggested that HCMV could be associated with breast cancer because it is a ubiquitous virus that is shed in breast milk, as well as in saliva, urine, cervical secretions, and semen, HCMV persistently infects epithelial cells (Richardson *et al.*, 2004). However, whether the association between HCMV and breast cancer is causal requires further investigation.

2.11 Immunosuppression by CMV

It is frequently stated that HCMV is "immunosuppressive." At a clinical level, the association is somewhat anecdotal: obviously CMV disease frequently arises in the context of immunosuppression, iatrogenic or otherwise, and it is difficult conclusively to implicate HCMV in the causation of immunosuppression. The disease associated with primary HCMV infection in the normal immunocompetent host is characterized by marked T cell proliferation, but not obviously associated with immunosuppression. The possession of a large number of gene functions which modulate the expression of MHC molecules, cytokines and NK cell interactions,

is really the most specific evidence that HCMV exerts “immunosuppressive” effects, although it is obvious these effects do not prevent the normal host from mounting a sustained and effective immune response against the virus. In the whole organism the effect of HCMV- induced downregulation of MHC molecules may be counteracted by other influences – for instance, it has been shown that IFN-_ and TNF_ can

upregulate MHC Class I molecules and counteract the downregulating effect of the HCMVgenes (Hengel *et al*., 1995).

Much of the in vitro evidence for “immunosuppression” comes from experiments in which investigators have added HCMV to cultures of peripheral blood mononuclear cells and then used a readout such as T cell proliferation in response to exogenous mitogens or a similar assay. Given that HCMV, particularly recent clinical isolates, can infect differentiated macrophages, it can be envisaged that infection in this sort of in vitro system at high multiplicities may well cause impairment of such assays. More recently, it has been shown that HCMV can exogenously infect dendritic cells (DC) in vitro (Raftery *et al*., 2001), with enhanced expression of their costimulatory molecules and partial downregulation of MHC molecules, with upregulation of apoptosis-inducing ligands CD95L(FasL) and tumour necrosis factor related apoptosis-inducing ligand (TRAIL). This would result in HCMV-infected DC potentially being able to delete activated T-cells.

There is emerging evidence that dendritic cells may well be a site of HCMV latency and, although it can be envisaged this sort of mechanism might operate in vivo, it is difficult to know how valid it is to extrapolate from in vitro experiments in which large numbers of DC are infected to the situation likely to obtain in vivo, where HCMV infection of DC would seem likely to be a low frequency event. Murine DC have also been shown to be permissive for murine

CMV infection in vitro: again, MCMV infected DC were unable to deliver the signals necessary for T-cell activation and it has consequently been suggested they may be involved in CMV-induced immunosuppression in the mouse (Andrews *et al*., 2001).

In summary, the issue of whether HCMV can exert generalized immunosuppressive effects in vivo is unresolved. The clinical settings in which disseminated HCMV disease occurs are usually characterized by multiple variables operating simultaneously – such as pre-existing immunosuppression due to other disease or the administration of immunosuppressive drugs, the simultaneous administration of anti-rejection therapy, and other opportunistic infections. Given the in vitro evidence, it is plausible to suggest that CMV disease may be causally associated with immunosuppression but the case has to be regarded as not proven.

2.12 Epidemiology of cytomegalovirus

Human cytomegalovirus is common in the general population. Thus, between 30 and 100% of adults show serological evidence of prior CMV infection (Weller, 1971;Gold and Nankervis, 1982). Although most the infections are mild or symptomless, there are two major groups of patients who may manifest serious CMV infections. One group includes congenitally infected newborn infants, and the other group is patients receiving immunosuppressive therapy. Between 0.3 and 2% of newborn infants excrete CMV in their urine (Stagno *et al.*, 1981; Peckham *et al.,* 1983;) and up to 100% of allograft recipients show evidence of the viral infection (Glenn, 1981). Because of recent developments in chemotherapy, treatment of CMV disease is becoming possible (Oberg, 1983). The need for a rapid CMV diagnosis is increasing accordingly.

This virus is ubiquitous, being found universally throughout all geographic locations and socioeconomic groups, and infects between 50% and 85% of adults in the United States by 40 years of age (Leinikki *et al.*, 1996). CMV infection is more widespread in developing countries and in areas of low socio economic condition (Cabau *et al.*, 2000). In the developing countries, very young children are often virtually universally CMV antibody positive (Bello, 1984). The early acquisition and high prevalence of CMV antibody has been ascribed to low socio economic conditions, poor hygiene, and overcrowding (Bello, 1984). However reports from studies carried out on the people of the New Guinea highland villages with primitive standards of hygiene and who often lived apart from childhood until marriage as well as studies carried out on people of the New Hebrides and Solomon island who lived in crowded population but are scrupulously clean, the prevalence of CMV infection was higher in the latter than the former group. It was therefore concluded that close personal contact, rather than poor hygiene was more important for the acquisition and spread of CMV.(Jemal, 2011).

Human cytomegalovirus infections have been recognized in every human population that has been studied (Krech *et al.*, 1971; Gold and Nankervis, 1976). It is endemic without seasonal variation (Gold and Nankervis, 1976) and is acquired early in life in most populations with the exception of people in economically well developed countries of Northern Europe and North America. The patterns of HCMV acquisition vary greatly based on geographic and socioeconomic backgrounds of the population and the seroprevalence increases generally with age (Alford *et al.*, 1981). In the developing world, acquisition of HCMV is nearly universal in early childhood. Studies have shown that most preschool children (>90%) in South America, Sub-Saharan Africa, East Asia, and India are HCMV antibody positive (Gold and Nankervis, 1982; Stagno, 2001). In contrast, seroepidemiologic surveys in Great Britain and in certain

populations in the United States have found that less than 20% of children of similar age are seropositive (Huang *et al.*, 1980; Gold and Nankervis,1982).

In Chengdu, China a population survey observed 60% of children 4 to 7 years of age were HCMV seropositive (Liu *et al.*, 1990). Similiarly, 58% of children 4 to 12 years of age in Taipei, Taiwan, 61% of hospitalized pediatric patients from a low income population in Rio de Janeiro, Brazil and 56% of children aged 1 to 4 years in Jamaica were HCMV antibody positive (Prabhakar *et al.*, 1992; Shen *et al.*, 1992; Suassuna *et al.*, 1995). In Finland the HCMVseroprevalence rate increased from 27% in children 7 months of age to 41% in children 8 years of age in a cohort of children followed for 8 years (Aarnisalo *et al.*, 2003). In a population survey in Parma, Italy, age-specific HCMV seroprevalence increased from 28% in two year olds to 96% in 45–54-year old residents (Natali *et al.*, 1997).

Similiarly, in Spain, the CMV seroprevalence rate in children 2 to 5 years of age was 42% increasing to 79% in adults 31 to 40 years of age (de Ory *et al.*, 2004). Recent studies in blood donors have demonstrated that populations in Asia and Africa continue to have CMV seropositivity rates of 95–100% (Urwijitaroon *et al.*, 1993; Lu *et al.*, 1999; Pultoo *et al.*, 2001; Kothari *et al.*, 2002) whereas in Germany the HCMV seropositivity rates in blood donors are lower ranging from 30% in 18 to 20 year olds to >70% in adults >65 years of age (Hecker *et al.*, 2004).

2.13 Modes of transmission of cytomegalovirus

Although the exact mode of HCMV acquisition is unknown, it is assumed to be through direct contact with body fluids from an infected person. The differences in age-related prevalence probably reflect differences in child rearing practices, sexual *behaviour*s, and

possibly, living conditions. Breastfeeding, group care of children, crowded living conditions, and sexual activity have all been associated with high rates of HCMV infections. Sources of virus include oropharyngeal secretions, urine, cervical and vaginal secretions, semen, breast milk, blood products, and allografts (Hayes *et al.*, 1972; Reynolds *et al.*, 1973; Lang, 1975; Alford *et al.*, 1980). Presumably, exposure to saliva and other body fluids containing infectious virus is a primary mode of spread and because infected infants typically excrete significant amounts of HCMV for months to years following infection. Even older children and adults shed virus for prolonged periods (>6 months) following a primary HCMV infection. In addition, a significant proportion of seropositive individuals continue to shed virus intermittently. An important determinant of the frequency of congenital and perinatal HCMV infections is the seroprevalence rate in women of child-bearing age. The incidence of congenital HCMV infection is directly related to the seroprevalence rates. Studies from United States and Europe have shown that the seropositivity rates in young women range from less than 50 to 85% (Krech *et al.*, 1971; Gold and Nankervis, 1982).

In contrast, most women of child bearing age in less well developed regions are HCMV antibody positive (Schopfer *et al.*, 1978; Stagno *et al.*, 1982; Vial *et al.*, 1985). Prospective studies of pregnant women in the United States have shown that the rate of HCMV acquisition in young women of lower income is about 6% per year compared with about 2% in women of middle to upper income background (Stagno *et al.*, 1986).

Perinatal HCMV acquisition, including congenital infection contributes significantly to the spread of HCMV in the population because infected infants excrete large amounts of virus for prolonged periods of time.

An additional and less well appreciated mode of virus spread is through breast milk. It is estimated that over 80% of breast-fed infants of persistently infected mothers will be exposed to HCMV as a result of breast feeding (Hayes *et al.*, 1972; Stagno *et al.*, 1980). Similar to congenital infections, infants infected through breast feeding will excrete virus for prolonged periods of time, making them ideal vectors for the spread of virus. Children continue to acquire HCMV infection throughout childhood and the rate of infection continues to increase during adolescence and early adulthood secondary to sexual exposure. Significant titers of infectious HCMV can be found in semen and cervical secretions, suggesting that exposure to the body fluids could result in the transmission of HCMV (Jordan *et al.*, 1973; Willmott,1975; Drew *et al.*, 1981; Chandler *et al.*, 1985a,b).

The natural history of HCMV infection in adolescents and adults has been shown to parallel sexually transmitted diseases(STDs) (Knox *et al.*, 1979; Sohn *et al.*, 1991). Homosexual men and women attending STD clinics have an increased incidence of HCMV infection (Drew *et al.*, 1981). Thus, it should be considered an STD in adults that can effectively spread through a sexually active population.

2.14 Treatment of CMV infections

In the management of CMV disease, four different strategies can be utilized; antiviral therapy,prophylaxis, pre-emptive and suppressive treatment. Ganciclovir, a nucleoside analogue of guanine is a potent inhibitor of CMV replication *in vitro*. Valganciclovir, a prodrug of ganciclovir has also been found to be useful in treatment of CMV infection. The use of both drugs has been associated with myelosuppression which is often dose dependent. Foscarnet, a pyrophosphate analogue with in vitro activity against all human Herpes viruses as well as HIV

has also been useful in treatment of CMV retinitis in AIDS patients. Nephrotoxicity and electrolyte imbalance are the most common toxicity associated with foscarnet (Hilt and Buchhotz, 2001).

Foscarnet may be used as a second line therapy in CMV patients with ganciclovir resistance. Oral ganciclovir has been licensed for CMV prophylaxis in patients with advanced AIDS. Routine prophylaxis however has not become standard in most HIV care settings, mainly due to the high cost of prophylaxis, potential toxicity and the inconvenience of taking 12 capsules per day.(Hilt and Buchhotz, 2001).

2.15 Prevention and control of CMV infections

Optimal prevention of CMV disease would be vaccination. However, until today there is no effective vaccine available. The Towne strain reduced the severity of disease without affecting the infection rate. Studies are currently going on to determine an alternative approach which is the use of subunit recombinant or DNA vaccines (Einsele *et al.*, 2005).

Currently, there is no approved vaccine for CMV, but two vaccines are in phase II studies: one is a recombinant vaccine containing the major envelope glycoprotein B of the virus with the adjuvant MF59 (gB/MF59) that induces high levels of neutralising antibodies, is safe and immunogenic in adults and infants, preventing also maternal CMV infection (Pass, 2009; Pass *et al*., 2009). The other vaccine is the live attenuated CMV Towne strain that stimulates neutralising antibodies comparable to those induced by wild type virus and protects renal transplant patients from severe CMV after transplantation (Adler, 2008; Griffiths, 2009).

The main interventions for the prevention of CMV infection should be aimed at women who wish to become pregnant, women who care for children and immunocompromised

individuals. These individuals in whom exposure to CMV can be most detrimental will be the target groups for possible administration of a future vaccine.

Possible approaches to preventing congenital CMV infections include improved hygiene behaviour of seronegative pregnant women, administration of CMV hyperimmune globulin (HIG) to pregnant women with primary infection, and vaccines, once available, administered to girls or women before pregnancy(Adler, 2007).

2.14.1 Prophylactic vaccination

The development of an effective prophylactic vaccine for HCMV-associated diseases remains a significant challenge. As our knowledge of the immune response to HCMV infection has progressed, various strategies have been explored, and considerable advances have also been made in the field of HCMV vaccine development. Initial HCMV vaccine development was based on the Jennerian concept of using an attenuated form of the virus as a vaccine (Adler *et al*., 1998).

A renewed interest in this approach has emerged with the codevelopment of a recombinant technology that has allowed the design of a chimeric virus that may be more immunogenic than the parent viral strain (Heineman *et al*., 2006). More recently, a number of attempts have been made to design prophylactic HCMVvaccines that are based predominantly on subunit vaccine technologies. These vaccine formulations have been delivered as recombinant proteins (Frey *et al*., 1999) and/or viral vectors (poxvirus/ adenovirus) (Adler *et al*., 1999). Most of these concepts have been extensively discussed in a number of reviews from our group and others (Plotkin, 2002).

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Study Area

Zaria is the second largest city in Kaduna state. The population of Zaria is about 19,434 people in 1953, by 1963, the population rose to about 166,170 people and in the demographic census of 1991, the region was put to a total population of about 489,858 people together (Hore, 1970). The region transverse about 70Km from the West to East and roughly covers 8,950 square kilometers. According to Hore (1970), Zaria is located on a plateau at a height of about 0.67 km above the mean sea level and more than 643.71 km away from the sea and possesses a tropical Savanna climate with distinct wet and dry seasons. The area belongs to the Precambrian basement complex of northern Nigeria.

In 1976, the Federal Government took over all the Teaching Hospitals in the country, the control of constituent Institutions of the Institute of Health passed from the Ahmadu Bello University to the Federal Ministry of Health. An autonomous board was set up in 1977 under the supervision of the Federal Ministry of Health to overlook the affairs of the institute. The staff were formally transferred from the University to the Management Board of the Institute of Health. With the promulgation of the Teaching Hospital decree no 1. 10 of 1st January 1985. The Ahmadu Bello University Teaching Hospital (ABUTH) became legally and operationally separated from the University.

The Ahmadu Bello University Teaching Hospital is spread out between Kadun and Zaria in Kaduna State and Malumfashi in Katsina State. A distance of about 120km radius around Zaria. The administrative headquarter is located on the main campus of the University in Samaru, Zaria.

Service departments include Family Medicine, Obstetrics and Gynaecology, Paediatrics, Radiotherapy and Oncology, Community Medicine among others. The out patients Departments include Antenatal Clinics, Ear, Nose, and Throat clinics,Opthalmology clinic, and General out patient departments. ABUTH has also been designated a center of excellence in Radiotherapy and Oncology. Clinic days for Oncology patients include Tuesdays, as general clinic day, Thursdays for new patients and Fridays for patients on the machine i.e patients receiving treatment.

3.2 Study Population

This study was conducted in the Oncology Unit of Ahmadu Bello University Teaching Hospital, Zaria. Patients with histopathologically confirmed cases of cancer were considered as the case study population whereas patients' relatives and any other non-oncology patient were considered as the control population.

3.3 Sample size

The sample size was determined through the formular below,

$$n=\frac{Z^2PQ}{L^2}$$

n=sample size

Z=Score of a given confidence interval usually 1.96 for 95% confidence.

P=Prevalence value of a previous research. 25% (Sanadgol, 2011)

Q= 1-p

L=Permissible error of estimate at 0.05(5%).

$$n=\frac{1.96^2\times 25\%\times(1-25\%)}{0.05^2}$$

$$n=\frac{0.7203}{0.0025}$$

n=288.

3.4 study design

Blood Samples were collected at ABUTH Oncology clinic with the aid of the hospital based supervisor.The sample collection was spread across 6-months such that i can attain the sample number required. After which samples were transferred to the postgraduate laboratory department of microbiology for further analysis.

3.5 Patients Counselling and Recruitment

Participants were counselled and recreiuted with a verbal explanation about the importance of the study especially to the cancer patients as well as the control population. All necessary questions were adressed and the patients that were willing to partake in the study were isued a copy of the questionnaire.

3.6 Ethical approval

Ethical approval was obtained from the Ethical Committee of Ahmadu Bello University Teaching Hospital.

3.7 Inclusion and Exclusion criteria

3.7.1 Inclusion criteria

All Histopathologically confirmed cases of cancer that are attending the ABUTH oncology clinic who agreed to participate in this study by filling the informed written consent as well as the questionnaire. And patient's relatives who wished to participate as a control population and also filled the consent form.

3.7.2 Exclusion criteria

Non Histopathological cases as well as Oncology patients who did not wish to participate by declining to fill the consent as well as patients whose health condition has deteriorated immensely.

3.8 Sample collection

A tourniquet was used to tie the upper hand of the patient to make the veins more visible. A clean cotton swab soaked in methylated spirit was used to clean the skin surface and a sterile syringe was used to obtain 2-3mls of blood sample. The blood sample was transfered into a well labelled sterile plain bottle. The syringe was discarded into a safety box while the sample botlle was labelled accordingly. Blood samples were allowed to clot for 30 minutes after which they were centrifuged at 1000rpm for 10 minutes to seperate the serum. Each resultant serum was carefully transfered into another sterile serum storage screw-capped container with the help of a pasteur pipette. All the sera obtained were stored at -20□C until ready to use.

3.9 Cytomegalovirus IgG titer quantitation

3.9.1 Test principle

Purified CMV antigen is coated on the surface of microwells. Diluted patient serum is added to wells, and CMV IgG specific antibody,if present, binds to the antigen. All unbound materials are washed away. After adding enzyme conjugate it binds to the antibody-antigen complex. Excess enzyme conjugate is washed off and Tetramethylbenzidine chromogenic substrate is added. The enzyme conjugate catalytic reaction is stopped at a specific time. The intensity of the colour generated is proportional to the amount of IgG specific antibody in the sample. The results are read by microabsorbance reader compared in a parallel manner with calibrator and controls.

3.9.2 Reagent preparation

Washing buffer was prepared by adding distilled water to the 10* wash concentrate provided in the kit to bring it to a total volume of 1 liter.

3.9.3 Specimen Labelling

All the serum samples obtained were brought out of the freezer (-21□C) and allowed equilibrate to room temperature. Using a razor blade, the masking tape was cut into small pieces, 1 small piece of the masking tape was attached on to each sample bottle, then the sample bottle was labelled accordingly.

The ELISA kits were manufactured by Diagnostic automation (immuno diagnostics). Microtiter plates were labelled A-H horizontally and 1-12 vertically, The first seven vertical wells of each test plate i.e A one 1- A one 7 were used for a blank, Negative Calibrator, Cut-off

Calibrator, Positive Calibrator, Pos, Negative Control, Positive Control consecutively Therefore, the first sample 90 were given a sample number from A one 8 , A one 9, A one 10 - A one 11, and A one 12. then B one 1, B one 2 – B one 12, C one 1 – C one 12, and so on uptill H one 1 to H one 12. Subesequently. The second 90 samples were labelled A two 8, A two 9, A two 10 to A two 11,and A two 12. then B two 1, B two 2, B two 3 to B two 12 and so on uptil H two 1- H two 12. Then, the last 90 samples were labelled from A three 8, A three 9, A three 10, A three 11, A three 12. Then B three 1 to B three 12 up to H three 1 to H three 12.

The indication of one, two, and three after each letter is to distinguish samples for the first, second and third kit respectfully,.

3.10 Cytomegalovirus IgG titer quantitation

All samples and reagents were brought to room temperature (i.e 20-25□C). The reagents were gently mixed by swirling before being used. Two hundred µl of the sample diluent was dispensed with a multichannel pipette into each well of the microtiter plates, then 5µl of each test sample, negative control, Positive control, and calibrators were dispensed with a single use micropipette into each corresponding well, (1:40 dilution). The well content was mixed thoroughly .One hundred µl of the diluted sera, calibrators and controls were dispensed into each corresponding microwell and for the blank well (i.e A1 well position), 100µl of the sample diluent was dispensed. The holder was tapped to remove any air bubbles and allowed to incubate for 30 minutes at room temperature. The liquid content of the microwells were emptied into a waste container and the microwells washed 3 times with the washing buffer. The wells were dried by carefully blotting with a tissue paper repeatedly to remove all the trapped washing solution.

Using a multi channel pipette, 100 µl of enzyme conjugate was added into each well and incubated for 30 minutes at room temperature. The content was emptied and wells washed with the washing buffer 3 times. The same blotting procedure was repeated to free the wells from excess water before adding the next reagent. One hundredµl of TMB chromogenic substrate was dispensed into each well with a multi channel pipette and allowed to incubate at room temperature for 30 minutes and finally 100µl of stop solution was added to each well to stop the reaction. The results were read at an optical density of 450nm using a microplate reader.

3.11 Total Human IgG Assessment

Nine sets of microtiter plates were arranged, three plates for the dilution process. The plates were labelled accordingly. One hundredµl of normal saline was dispensed with a pipette into each well on the microtiter plate. The first five vertical wells were left for the dilution of the standards, well 1 was left for the blank, well 2 was for 1:2 dilution, well 3, 1:4; well 4, 1:8;and well 5, 1:16; Tenµl of each seru sample was dispensed into appropriate wells that contained the normal saline and mixed thoroughly. The second set of plates containing 100µl of the normal saline were subdiluted by adding 100µl from the first set of plates. Lastly into each well of the 3rd set of plates, 100µl of the specimen diluent and 100 µl of the second dilution were mixed thoroughly.

One hundred µl from the 3rd dilution was dispensed into wells of the total human IgG test kit, mixed for 10 seconds and incubated at room temperature for 45 minutes covered with foil paper. After incubation the wells were washed with the washing buffer. The washing was repeated four times to achieve the best possible result. The wells were blotted with a tissue paper repeatedly until all the residual washing liquid was evacuated. Then 100µl of Horseradish

peroxidase conjugate goat anti-human IgG was dispensed into each well. Incubation was carried out at room temperature for 45 minutes. The content of the wells was removed and the wells washed with the washing buffer 4 times, followed by blotting to remove residual droplets. One hundredµl of tetramethylbenzidine(TMB) substrate was dispensed into each well, mixed for 5 seconds and allowed to incubate at room temperature for 15 minutes. Further reaction was stopped by adding 100µl of the stop solution (0.5N sulphuric acid) into each well. The result was read using a microtitre reader at an optical density of 450nm.

3.12 Human Immunodeficiency Virus Test principle

Incubated HIV antigen is coated on a strip, HIV antibodies if present in the test serum will react with the antigen and the results read accordingly.

3.13 HIV test methodology

All the patients coming to Oncology Department for the first time were required to run HIV test. These confirmed cases along side the control populations were subjected to HIV screening by the use of "Determine" test kit. The serum sample was dropped on the test strip and allowed to react for 5 minutes.

3.13 Data analysis

Data generated from this study was analysed using Statistical Package for Social Science (SPSS) Version 19 for Windows. Students t-test was used to compare the IgG levels recorded among the control populations as well as the cancer patients. Human IgG and CMV IgG was compared based on age-groups and educational level and other variables with more than two levels was compared using One-way analysis of variance(ANOVA). Inter relationship between

cancer and other underlying illnessess such as HIV infection, Diabetis, Hypertension and ulcer were also taken in to account. The level of significance was set at $p \leq 0.05$.

CHAPTER FOUR

4.0 RESULTS

A total of 267 samples were collected: 178 samples were collected from cancer patient (which served as the case study) while 89 samples were collected from non – cancer patients (which served as the control population). The total human and levels of both cases and controls were measure and the levels of recorded were analyzed using students t – test. All the samples analysed were tested positive for both CMV IgG and total human IgG i.e both cancer and non – cancer population was found to be 100%. The only difference was observed in the quantity (titre) analyzed, the prevalence of human IgG levels obtained were also 100% though the titres were found to vary under a certain conditions such as residential type, marital status and other factors. Values were considered statistically significant at 5% i.e ($p \leq 0.05$).

Out of the 179 females, 137 of them are cancer patients while 42 of the females were non cancer patients. In the same vein, the mean levels of males and females were compared, female cancer patients recorded a higher mean CMV level of (1.71 mg/ml) as opposed to the female control populations which recorded a mean CMV level of (0.53 mg/ml), this perceived difference was however statistically significant ($p = 0.03$). On the other hand among the 88 male population, 41 of the males were cancer patients and 47 of the males were non – cancer patients and male cancer population recorded a lower mean CMV IgG (1.05 mg/ml) than the male control population (1.20 mg/ml).

Male cancer cases had a mean total human IgG of (1.50mg/ml) whereas male control population had a mean of (1.73 mg/ml) and the difference was not statistically significant ($p=0.53$). the total human IgG levels recorded among female cancer population was (2.49 mg/ml) whereas the female control population recorded a lower mean hman IgG values of (o.76 mg/ml),

this difference was found to be statistically significant (p= 0.2). On the other hand male cancer patients had a mean human IgG of (1.50mg/ml) and male non – cancer recorded a higher mean human IgG value. (1.73mg/ml) this difference was not statistically significant.

Majority of the cancer patients were within the age of fourty years and above where as the control population had the highest age range between 21 – 25 years. The highest meanIgG levels recorded among the non control was between the ages 16 – 20 years (2.20 mg/ml) While the least CMV IgG levels recorded was between the ages 40years (0.44mg/ml). there is no statistically significant difference between the varying age groups analysed (p=0.367). Analyses of the IgG levels among the cancer patients. Revealed that the age group that are 40years had the highest IgG levels (1.93 um /ml) followed by age group 36 – 40 years (1.57 mg/ml) and the least values were recorded among the age group 21 – 25 years (0.09 mg/ml), the difference was also not statistically significant (p=0.231).

The highest total human IgG values were recorded among the control population that fell within the age range. 21 – 25years (2.94 mg/ml) followed by age group 26 – 30years (2.23mg/ml) and the least total humanIgG (control) recorded among the age of 40 (0.44mg/ml) for the cases how ever, age group 36 – 40 had the highest mean IgG of (2.30 mg/ml), followed by age group 40 (2.81 mg/ml). this difference was found not to be statistically significant (p = 0.43)

Table 4.3 shows relationship of IgG levels with places of residence among the control urban inhabitants were found to have the highest mean IgG levels (1.40mg/ml) followed by rural shelters (0.93 mg/ml) and the least IgG values were recorded among the urban dwellers (0.65 mg/ml) the difference between the means was found to be statistically significant (p=0.04).

The cases however, revealed mean IgG level of (1.57mg/ml) among the urban settlers followed by the rural settlers (1.25mg/ml) and the least was recorded amongst the semi urban settlers (0.84 mg/ml). the difference between the means were however found not to vary statistically.

Assessment of the total human IgG among the control revealed that the semi-urban dweller recorded the highest mean human IgG (2.03mg/ml) and the least was recorded among the urban dwellers though there is difference between the means, but statistical evidence reveals there is no significant difference.

The highest mean total human IgG among the cancer patients was recorded among the urban dwellers (2.27mg/ml) and least among the semi urban dwellers (1.22mg/ml). the difference as however statistically significant (p= 0.034).

Table 4.1: Distribution of mean CMV IgG and total human IgG levels according to gender.

	CANCER		NON CANCER		
Gender	**Number of samples**	**Mean IgG Values mg/ml**	**Number of samples**	**Mean IgG Values mg/ml**	**Total**
Females	137		42		179
CMV IgG		1.71		0.53	2.24
Total Human IgG		2.49		0.76	3.23
Males	41		47		88
Total Human		1.50		1.73	3.23
CMV IgG		1.05		1.20	2.25

t=162, p=0.688 ## t=0.63, p=0.802

Table 4.2: Differences of mean CMV IgG and Total human IgG According to Age.

Age	No of sample	NON – CANCER		CANCER		
		Mean Human IgG mg/ml	Mean CMV IgG mg/ml	No of sample	Mean Human IgG mg/ml	Mean CMV IgG (mg/ml)
0 – 5	0	0	0	0	0	0
6 – 10	0	0	0	1	1.30	1.08
11 – 15	0	0	0	5	1.25	0.08
16 – 20	12	1.64	2.20	5	0.69	0.92
21 – 25	22	2.94	2.11	1	0.13	0.09
26 – 30	15	2.23	1.55	7	1.04	0.72
31 – 35	11	1.48	1.03	13	1.74	1.22
36 – 40	10	0.92	0.63	25	2.30	1.57
≥ 40	19	0.44	0.30	121	2.81	1.93
	89			178		

f=0.320, p=0.900 ## f=1.025, p=0.405

Table 4.3: shows the effect of places residence on the mean CMV and total human IgG

Place of Residence	CANCER		NON-CANCER		Total
	No. of samples	Mean IgG values mg/ml	No. of samples	Mean IgG values mg/ml	
Rural	16		12		28
Human IgG		1.25		0.93	2.18
CMV IgG		1.86		1.39	3.25
Urban	156		67		223
Human IgG		1.57		0.68	2.25
CMV IgG		2.27		0.97	3.24
Semi urban	6		10		16
Human IgG		0.84		1.40	2.24
CMV IgG		1.22		2.03	3.25

f=0.360, P=0.699 ## f=1.171, p= 0.313

Educational levels of the subject were also assessed; 161 of the subjects were found not have any form of formal education and only 54 subjects out of the 267 have attained education up to the fertiary level. The highest meanIgGlevel recorded among the cases (cancer), was in the group that has no formal education; (1.94mg/ml) and the least IgGlevels recorded in the group was among the patients that had only secondary school qualifications (0.51mg/ml).The control population have the highest meanIgGlevels among these with secondary school certificate only (1.75mg/ml) and the leastIgGlevel was recorded among those without any formal education. Despite the virid difference between the means, but statistical analysis shows non significant difference.

Cancer patients that had no any form of formal education has a mean of total humanIgG of (2.81 mg/ml), and the least values were recorded among those that had secondary education. Among the non – cancer patients observed, those subject with secondary school education only had the highest meanIgGvalues (2.49mg/ml) then followed by these with tertiary qualificators (2.42 mg/ml). The difference between these means was not significant.

Marital status of the study population does not affect the mean of both total humanIgGand IgGthe highest population recorded among cancer patients was within the manied category, followed by singles and only of the 178 cancer population was a widow the same thing applies to the non – cancer population, manied subjects had the highest record (44) followed by singles and the least population was recorded among the separated.

Analysis of the meanIgGlevels among the non cancer patients revealed that the separate subjects had the highest mean IgG (2.34 mg/ml) followed by the singles (2.31mg/ml), the least record was amongst the widowed population. (2.14mg/ml).

Among the cancer patients however the patients that have been separated recorded the highest meanIgG (1.76mg/ml) followed by the married population (1.66mg/ml) and the least

was recorded among the widowed statistical analysis shows no difference between these categories of marriage considered and the meanIgGvalues.

The highest total human IgG was recorded among the non – cancer widowed patients (3.11mg/ml) followed by the cancer patients that have broken marriages (2.42mg/ml) and the least record was obtained among the widowed cancer patients (0.18mg/ml). depite these varations between the means, there is no statistical difference between the parameters assessed and the means total human)IgG obtained (table 4.5).

Table 4.4: Effect of educational status on the mean IgG level of cases and controls.

	CANCER		NON – CANCER		
Levels of Education	No of Samples	Mean IgG Values mg/ml	No of Samples	Mean IgG Values mg/ml	Total
No formal Education	140		21	0.42	161
Human IgG		2.81		0.29	3.23
CMV IgG		1.94			2.23
Primary	19		11	1.19	30
Human IgG		2.05		0.82	3.24
CMV IgG		1.41			2.23
Secondary	5		17	2.49	22
Human IgG		0.73		1.75	3.22
CMV IgG		0.51			2.26
Tertiary	14		40	2.42	54
Human IgG		0.85		1.66	3.27
CMV IgG		0.58			2.24

#f=0.488, p=0.691 ## f=0.081, P=0.970

Table 4.5: Analysis of the IgG levels obtained with relationship to marital status

	CANCER		NON-CANCER		
Parametres	No of samples	Mean IgG Values mg/ml	No of samples	Mean IgG Values mg/ml	Total
Married	128		44		172
Human IgG		2.40		0.83	3.23
CMV IgG		1.66		0.57	2.33
Separated	9		3		12
Human IgG		2.42		0.80	3.22
CMV IgG		1.76		0.59	2.34
Single	40		25		65
Human IgG		2.04		1.28	3.32
CMV IgG		1.42		0.89	2.31
Widowed	1		17		18
Human IgG		0.18		3.11	3.29
CMV IgG		0.12		2.02	2.14

f=1.457, p=0.228 ## f=2.503, p=0.061

Figure 4.1 illustrates a slight variation in the meanIgGlevels in the two different marriage types: monogamy and polygamy. Those subjects practicing monogamy had higher mean humanIgGlevel of 3.24mg/ml than those difference was not statistically significant. (t= 0.502, p= 0.480). the mean IgGvalues obtained among those that practice monogamy was (2.21mg/ml) as opposed to the values obtained from the ones practicing polygamy (2.25mg/ml). this variation was not significant (t= 0.853, p=0.357).

Meanwhile the association of consumption offmits and vegetables was also compared to theIgGvalues (table 4.6) about 140 of the case studies consume fruits and vegetable randomly (i.e without any specific pattern) and only 14 out of the 178 consume fruit and vegetables on a daily basis as opposed to the control which had 39 participants consuming fruit and vegetables on a daily basis.

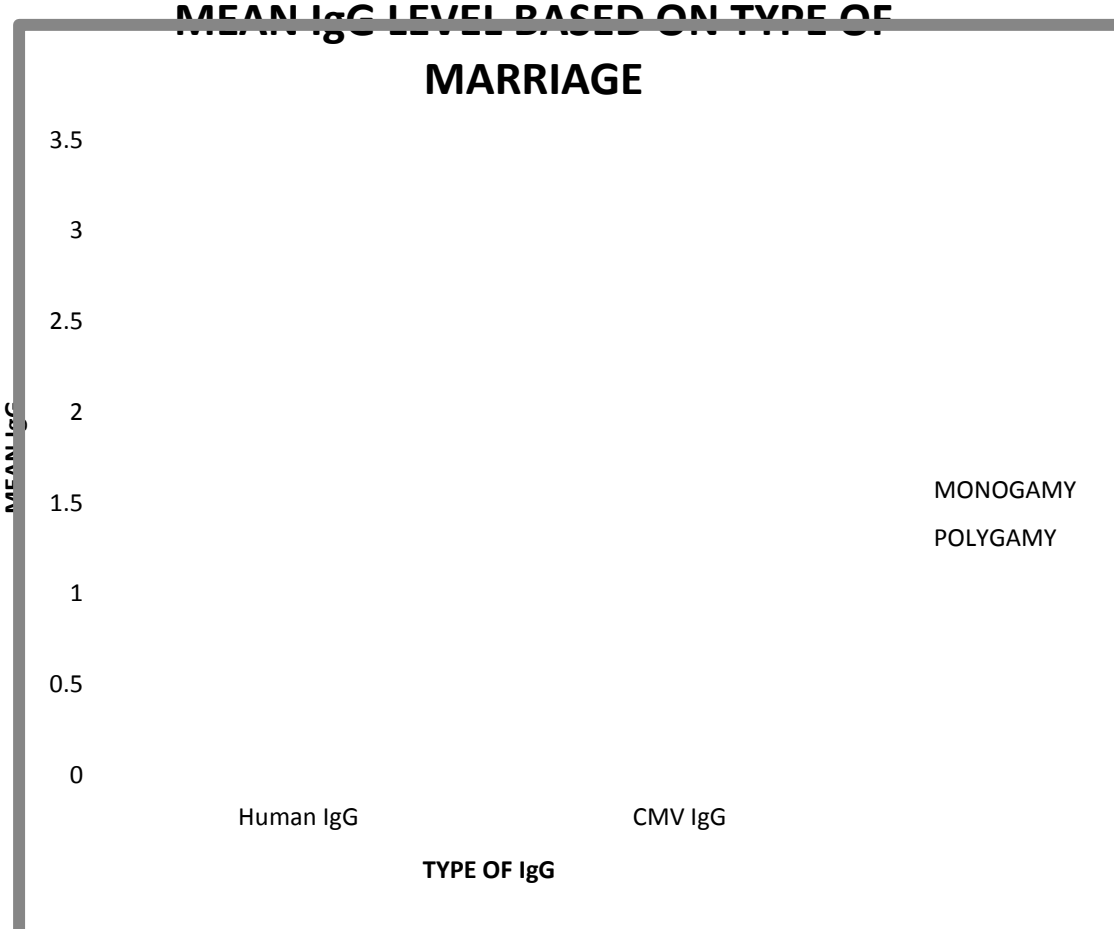
The highest mean IgGlevel was recorded among the cancer patients that had no specific pattern of fruits and vegetables consumption (1.92mg/ml), followed by those that consume on weekly basis (1.07mg/ml) and the least was recorded among those that consume on a daily basis (0.59mg/ml). On the other hand, the control population recorded the highest meanIgGvalues on those that have a daily consumption (1.66mg/ml), followed by those with a weekly pattern (1.20mg/ml) and the least with those that had no specific pattern of consumption of fruits and vegetables (0.31mg/ml). the difference between these mean values was found not to have any significant statistical difference.

The mean total humanIgGvalues recorded among the cancer patients without any regular pattern of fruits and vegetables consumption was the highest (2.77mg/ml) and the least was recorded among those that have a daily habit of consumption of fruits and vegetables (Table 4.6) (0.86mg/ml) among the non cancer cases examined the subjects that have the habit of consuming fruits and vegetables daily had the highest mean total humanIgG (2.41mg/ml) whereas those that consume without any specific pattern recorded the least (0.46mg/ml). The

difference between the means was statistically significant (p= 0.04) Table 4.7 revealed that the mean IgG values do not vary significantly with the feeding habits of both cases and controls. (p= 0.240, and p= 0.6331).

However, the total humanIgGvalues recorded among the cancer cases that consume fresh products (2.45mg/ml) was the lower than those that consume processed products (2.54mg/ml) and those that consume food from a variety of sources (i.e fresh, processed canned) had the least mean total humanIgGvalues (1.65mg/ml).

The control population with the highest mean total humanIgGvalue was found amongst the subjects that do not have any specific form of feeding habits (1.59mg/ml) and the least was recorded amongst those that had habit of consuming fresh products. Despite these differences observed between the means of cancer cases and the control, statistical analysis shows that there is significant difference between the cancer cases and the control with regards to feeding on fresh products. (p= 0.014)



t=0.502, p=0.480 and ## t= 0.853, p=0.357

Figure 4.1:Mean IgGlevel based on type of marriage

Table 4.6: Effect of fruits and vegetable consumption on the mean IgG levels.

	CANCER		NON CANCER		
Frequency of consumption of fruits & vegetables	No of Samples	Mean IgG Values mg/ml	No of samples	Mean IgG values mg/ml	Total
Daily	14		39		53
CMV IgG		0.59		1.66	2.25
Human IgG		0.86		2.41	3.27
Weekly	24		27		51
CMV IgG		1.07		1.20	2.27
Human IgG		1.52		1.71	3.23
No specific pattern	140		23	0.31	163
CMV IgG		1.92		0.46	2.23
Human IgG		2.77			3.23

f=0.477, p= 0.622 ## f= 0.210, p=0.811

Table 4.7: Analysis of the food sources of the study participants.

		CANCER		NON CANCER	
Food sources	No of samples	Mean IgG values mg/ml	No of samples	Mean IgG values mg/ml	Total
Fresh products	106		33		139
CMV IgG		1.71		0.53	2.24
Human IgG		2.45		0.76	3.22
Processed products	9		3		12
CMV IgG		1.73		0.58	2.31
Human IgG		2.54		0.85	3.38
Canned products	13		5		18
CMV IgG		1.58		0.61	2.19
Human IgG		2.37		0.91	3.28
No specific pattern	50		48		98
CMV IgG		1.14		1.09	2.24
Human IgG		1.65		1.59	3.24

f=1.417, p=0.246 ## f= 0.576, p=0.631.

Cervical cancer was found to have the highest prevalence 40% followed by breast cancer with 25% and nasopharyngeal cancer occupying the 3rd position with 15%. Analysis of mean IgG levels in relation to type of cancer shows that breast cancer had the highest mean human IgG (3.30 mg/ml). While Cervical and colorectal cancer had the least mean human IgG (3.22mg/ml and 3.22 mg/ml respectively). However Oropharyngeal cancer recorded mean CMV IgG levels of (2.31 mg/ml) at the highest position,followed by prostate cancer which recorded mean CMV IgG levels of (2.29 mg/ml), then cervical cancer patients had a record of (2.26 mg/ml) and least mean CMV IgG levels was recorded in patients with bladder tumour (2.05 mg/ml). There was no significant difference between the types of cancers and the total human IgG levels recorded. (human IgG: f=0.833, P= 0.528; CMV IgG: f=0.791, p=0.557) (Table 4.8).

Mean human IgG level was highest among those diagnosed with HIV (3.28 mg/ml) and least among those with high blood pressure (3.23 mg/ml). The difference in mean human IgG levels among the various diseases diagnosed was not significant(f=0.318, P=0.864). Ulcer patients on the other hand had the highest(2.40 mg/ml) mean CMV IgG level followed by patients with high blood pressure (2.26 mg/ml) and HIV patients had the least mean CMV IgG level (2.17 mg/ml). although this perceived difference was not statistically significant (f= 0.491, p= 0.742) (figure 4.2).

The mean CMV IgG levels of control subjects (2.17 mg/ml) were compared with that of cancer patients (2.23 mg/ml). The difference between the two mean CMV IgG values were found to be statistically significant (t=2.198, p=0.029).(Table 4.9).

Table 4.8 Mean IgG levels in relation to the type of cancer

Cancer type	Number of samples	Percentage of occurrence (%)	Mean human IgG mg/ml	Mean CMV IgG mg/ml
Cervical	67	40	3.22	2.26
Breast	42	25	3.30	2.24
Nasopharyngeal	25	13	3.23	2.20
Oropharyngeal	5	3	3.24	2.31
Kaposi sarcoma	6	4	3.25	2.14
Colorectal	12	11	3.22	2.20
Prostate	10	6	3.24	2.29
Salivary gland	3	2	3.30	2.11
Laryngeal	4	2	3.08	2.24
Bladder tumour	4	2	3.10	2.05

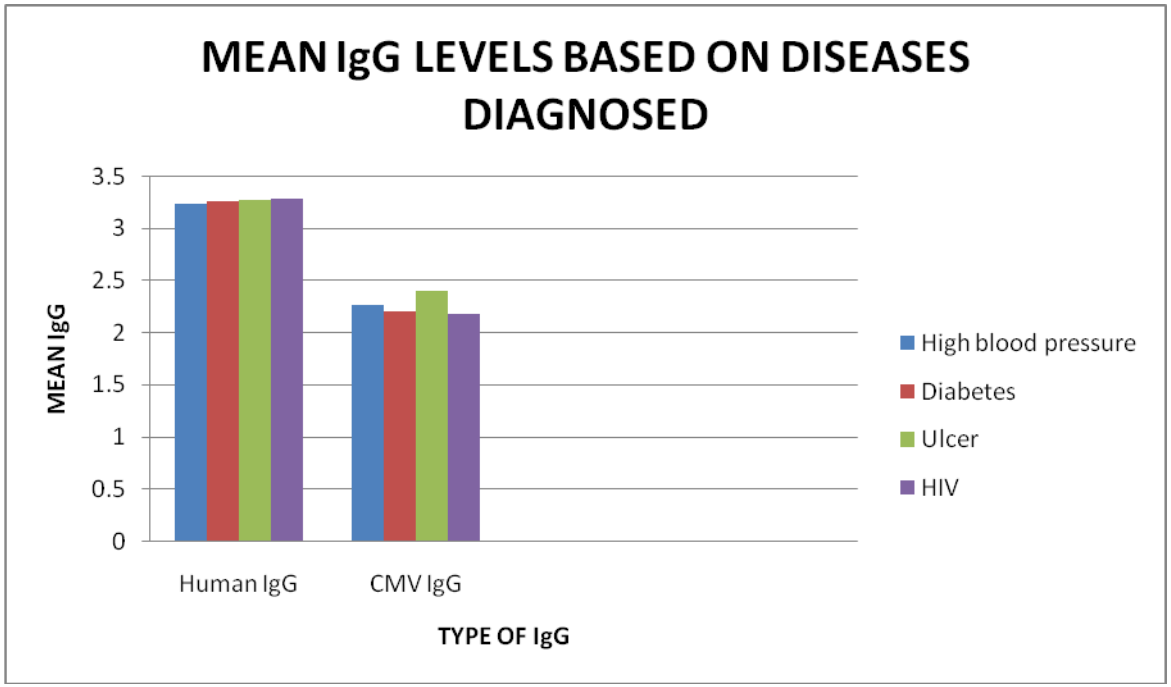


Figure 4.2: Mean IgG level based on other diseases diagnosed

Table 4.9: Comparison CMV IgG and total human IgG between Cancer patients and control.

Type of sample	Number of sample	Human IgG# mg/ml	CMV IgG## mg/ml
Control	89	3.21	2.17
Cancer	178	3.25	2.23

t=0.754, p= 0.452 ## t=2.198, P=0.029*

*Statistically significant difference exists

Moreover mean human IgG (f=1.572,p=0.198) and CMV IgG (f=1.323, p=0.269) did not vary significantly with the type of treatment received. Patients placed on combined therapy of chemotherapy and radiotherapy had the highest mean CMV IgG (3.3 mg/ml 7) followed by those receiving radiotherapy only (3.28 mg/ml). Those receiving chemotherapy only had the least mean human IgG (3.21 mg/ml). Patients receiving Chemotherapy and radiotherapy only had the highest mean CMV IgG (2.40 mg/ml) While those patients that underwent surgery had the least CMV IgG (2.13 mg/ml) (table 4.10).

Exercise as a factor was also compared among the study participants, the mean IgG was fond to vary significantly between those that exercise their bodies (2.34mg/ml) as opposed to those that do not exercise their bodies (2.20mg/ml). (p= < 0.001) table 4.11). even though the number of cancer cases that do not exercise their bodies were 142 and opposed to the number that do not exercise their bodies 36, upon assessing their total human IgG values those that do not exercise their bodies recorded higher value of (2.66mg/ml) and those that undergo exercise have a value of (1.25mg/ml). The control population however, recorded (2.01mg/ml) among those that under go exercise and a mean of (0.58mg/ml) to those that do not exercise their bodies, the difference between the means of exercise and those without exercise among the control was statistically significant (p= < 0.003).

There is statistically significant difference between the IgG values assessed based on excerise, those that exercise on a daily basis had the highest mean IgGas against those that had no specific form of exercise (p= 0.017).The IgGvalues observed among the non control cases revealed a higher mean IgG among those that under go exercise on a monthly basis (1.58mg/ml) followed by those that exercise on a daily basis (1.55mg/ml) and the least value obtained was among those that do not have specific form of exercise (1.28mg/ml) the difference in the mean was not statistically significant (p= 0.789).

The cancer cases however had the highest mean IgGamong those that exercise on monthly basis (1.26mg/ml) followed by those that do not exercise on no specific pattern (0.91mg/ml) and the leastIgGvalue recorded was among those that exercise on a daily basis (0.66mg/ml) the difference was not statistically significant. Similarly, the frequency of exercise of exercise was also assessed, 20 out of the 44 cancer cases that do exercise their body do not have specific pattern of exercise their body not have specific pattern of exercise and had a total humanIgGof (1.35mg/ml and the leastIgGwas recorded was among those cancer patients that do exercise on a daily basis (0.9mg/ml)

The non – cancer cases that exercise on a daily basis has the highest mean humanIgG of (2.28mg/ml) and the least mean total humanIgGlevels was recorded among those that do not have specific pattern of exercise (1.900mg/ml).

Table 4.10: Differences of mean IgG levels in relation to the treatment patterns received by the cancer patients.

Treatment type	Number of sample	Mean human IgG mg/ml	Mean CMV IgG mg/ml
Chemotherapy	82	3.21±0.03	2.22±0.02
Radiotherapy	84	3.28±0.02	2.26±0.03
Combined chemotherapy and radiotherapy	4	3.37±0.01	2.40±0.19
Surgery	8	3.27±0.03	2.13±0.03

f=1.572, p=0.198 ## f=1.323, p=0.269

Table 4.11 Effect of exercise on the IgG levels of both CMV and total human

	CANCER		NON CANCER		
Do you exercise your body?	No of samples	Mean IgG values mg/ml	No of samples	Mean Ig G values mg/ml	Total
YES	36		58		94
Human IgG		1.25		2.01	3.26
CMV IgG		0.90		1.44	2.34
NO	142		31		173
Human, IgG		2.66		0.58	3.24
CMV IgG		1.81		0.39	2.20

t=0.334, p=0.564 ## t= 13.522, p= <0.001*

* Significant difference exists at $p \leq 0.05$.

Table 4.12: differences in the mean IgG levels based on frequency of excersise

Frequency of exercise	Cancer		Non Cancer		Total
	No. of samples	Mean IgG values mg/ml	No. of samples	Mean IgG values mg/ml	
Daily	3		7		10
Human IgG		0.98		2.28	3.26
CMV IgG		0.66		1.55	2.21
Weekly	8		13		21
Human IgG		1.23		1.99	3.22
CMV IgG		0.89		1.44	2.33
Monthly	5		10		15
Human IgG		1.71		2.13	3.20
CMV IgG		1.26		1.58	2.37
No specific pattern	20		28		48
Human IgG		1.35		1.90	3.23
CMV IgG		0.91		1.28	2.19

f=0.358, P=0.699 ## f=4.150,p=0.017*

CHAPTER FIVE

5.0 DISCUSSION

Out of the 267 samples examined,as expected all the samples were positive for the total human IgG. All humans must have an IgG even a one day old baby due to its ability to cross the placenta. In healthy adults, IgG constitutes approximately 75% of the total serum immunoglobulins. IgG is approximately equally distributed between intra- and extravascular serum pools.The positivity however depends upon many factors, since the entire IgG profile was examined. Individual variation based on their varying immune system, their consistency of contact with foreign agents (antigens), good personal hygiene, marital status, age, and history of any previous disease will also contribute greatly to the IgG levels. Due to these factors mentioned above. It is difficult to provide an established reference standard for total human IgG although some scientist are of the opinion that individuals presenting with a total human IgG of <2.5g/l with non detectable IgA or IgM can be considered to have an underlying immunological problem.

All the samples proved positive to both CMV IgG and total human IgG. The only observed difference was in the quantity of the antibodies analysed. out of 267 blood samples were collected and analysed, 179 of the study population were females out of which 125 participants were cancer patients and only 40 individuals were male cancer patient. This wide variation in gender is due to the fact that, the most predominant type of cancer all over the world is cervical cancer, followed by breast cancer although one sample out of the 42 breast cancer patients was a male.

Comparism of the mean IgG levels based on gender revealled that female cancer patients had higher mean CMV IgG levels than female control population. This observed difference was statistically significant. This goes to suggest possible CMV infection among the cancer patients. On the other hand, there is no significant difference between male cancer

patients and male non cancer patients. In general, The total mean CMV IgG of all the females was also found to be higher than that of the males (Though the difference is not statistically significant) (p=0.04) . This observed difference may likely be due to the fact that females are more prone to infectious agents than males, factors such as, child bearing, sexual activities may also contribute to the higher CMV IgG levels recorded in females than in males.

The total human IgG observed between female cancer patients and female non cancer patients was observed to be statistically significant(p=0.02), as oppossed to their male counter parts (P=3.45). However an assessment of the cancer cases versus the control population proved statistically significant difference between the cancer cases and the control (p=0.041). The non cancer cases were found to have higher mean human IgG than the cancer cases.

Most of the study participants were adults of ages 40years and above this agrees with other previous surveys that revealed high prevalence of cancer cases more commonly among adults than in children. Moreover, the control population were also mostly adults, because they were the patients' relatives accompanying them to the hospital for treatment.The highest age range recorded among the cancer patients was 40years and above whereas the control population had their highest range between 21-25years.

The highest mean IgG profile was recorded among the subjects that were between ages 16-20years. This finding is in consistent with the findings of other researchers which revealed that total IgG would gradually decrease in children but the level starts to increase after a certain period tending towards adulthood. Mean CMV IgG levels of the control population that were between ages 16-20 years was the highest, this finding is in consistent with the findings of which states that CMV is acquired early in life and by adulthood most people must have come in contact with the virus but due to its asymptomatic nature the

infection goes unnoticed. The least mean CMV IgG levels was recorded among the contol population that were between ages 40years and above. There is no statistical difference between the means observed with the age groups considered.

On the other hand the mean CMV IgG levels observed among the cancer patients revealled that the age group with the highest mean was 40years and above, this is due to the fact that most of the cancer patients were between ages 40years and above and CMV is a virus associated with progresssion of tumour cells. The least CMV IgG levels was recorded among the age group of between 21-25years. This finding is statistically significant (p=0.002).

There was statistically significant difference between the control population and cancer patients with regards to Total human IgG. The control population recorded higher mean values than the cancer cases.(p=0.001), this difference in the Total human IgG values possibly suggests the effect of the immunosuppressive treatment procedures undertaken by these cancer patients. However,Comparism of Total human IgG values with age brackets reveils that the control population with the highest mean IgG levels were between ages 21-25years followed by those of age 26-30years and the least IgG values was recorded among the age group 40years and above, This finding is in consistence with the finding of that states that at certain age in life the immunity of an individual begins to rise tending towards adulthood and the immunity weans out as one begins to grow older.

Assessment of the total human IgG values of the cancer patients shows that the patients that were between the age group of 36-40years had the highest values and the least values were recorded among (Bale 1999). This observed difference in mean values was not staitstically significant.

Moreover, Place of residence, marital status, type of marriage and the level of education of the study participants, were analysed as part of the risk factors associated with

CMV infection as well as factors that may influence the level of total human IgG values. Even though, Urban dwellers are expected to have better personal and environmental hygiene than rural dwellers because of the general assumption that a clean environment is considered averagely healthy.

In this study the mean CMV IgG recorded among the control yielded that semi urban inhabitants was found to be the highest, followed by that of the rural inhabitants and the least record was found among the Urban dwellers. Whereas the cancer cases that had the highest record of mean CMV IgG were the urban dwellers, followed by the rural settlers and the least record was among the semi urban inhabitants this observed difference in mean values was not statistically significant.This difference was found not to have any significant effect. one simple reason for the lack of difference may be due to the ubiquity of microorganisms, because micro organisms are found in every environment, although sometimes an environment may predispose the individuals to, or prevent them from, becoming infected with some microbial agents. This finding is consistent with the study carried out by Bello in 1984 which states that early acquisition and high prevalence of CMV antibody has also been ascribed to low socioeconomic conditions, poor hygiene, and overcrowding (Bello, 1984).

However reports from studies carried out on the people of the New Guinea highland villages with primitive standards of hygiene and who often lived apart from childhood until marriage as well as studies carried out on people of the New Hebrides and Solomon island who lived in crowded population but are scrupulously clean, the prevalence of CMV infection was higher in the latter than the former group. It was therefore concluded that close personal contact, rather than poor hygiene was more important for the acquisition and spread of CMV.

Comparing the mean values of the total human IgG among the control revealled that the semi urban dwellers had the highest values, followed by the rural dwellers and the least

values were recorded among the urban setllers. On the other hand, the cancer cases with the highest mean total human IgG were those living in the urban areas and the least was recorded among the semi urban dwellers.

Most of the case studies had no any formal education and just a few obtained primary and secondary education, there was no significant difference observed between those with tertiary, secondary, primary or no formal education. 161 of the study participants did not have any form of formal education and only 54 participants out of the 267 had tertiary education. Among the control the highest CMV IgG record was found among those with secondary education and the least values were recorded amond those without any form of education. The cancer cases with the highest CMV IgG values were those without any form of formal education and the least values were found among those that attained secondary education. The difference was not statistically significant (p=0.345).

The total human IgG values recorded among the cancer patients that had no any form of formal education was found to be the highest, and the least values were recorded among those with secondary school qualifications. In contrast the control population with the highest mean human IgG were those with secondary school qualifications,

The exact mode of CMV acquisition remains unknown (Alford *et al.,* 1980) Thus CMV is a virus that can be transmitted through a number of ways one of which is sexual means, the results obtained showed that Marital status and category of marriage practised had no significant effect on the levels of mean CMV IgG obtained.The highest mean CMV IgG recorded among the controls that were widowed was found to be the highest, followed by the values obtained from those with broken homes (separated), then the singles and the least values were recorded among the married ones. On the other hand the married cancer patients had the highest CMV IgG values, followed by the singles, then the widowed and the least

values were found among the separated couples.there was significant difference between the married cancer cases and non married control population. (p=0.23).

As illustrated in barchart format in chapter four the category of marriage practised by the study participants was also assessed and the most practised form of marriage was monogamy,followed by polygamy and not a single participant was found to have been practising polyandary. Mean CMV IgG levels of the participants practising monogamy was found to be lower than the means of those practising polygamy, since CMV can be transferred through sexual means, this may be the reason for the higher mean values recorded among the monogamous participants as oppossed to the polygamous ones.

However, the total human IgG values obtained from the subjects that were in a monogamous family settings was found to be higher than the values of those subjects that are ina polygamous family settings. The difference observed was not statistically significant. , the polygamous family members were found to have higher mean CMV IgG than the monogamous ones. This can be due to greater sexual activity in the former than the latter hence enhancing the spread of the virus from one partner to another. The difference however is not statistically significant.

Patients with different types of cancers were considered in this study, however Cervical cancer patients were found to have the highest prevalence (40%), followed by breast cancer patients (25%) then, nasopharyngeal cancer (13%,), and colorectal cancer(11%). Only the 10 most predominant cancer types were considered for this analysis because the other types lack representative sampling. Among the cancer patients examined, individuals with breast cancer had the highest mean CMV IgG levels then the cervical patients and colorectal cancer patients recorded the least mean IgG profile of respectively, this higher values of mean CMV IgG levels observed in breast cancer patients can be correlated to the work of (Breathnach et al,1999) which reported a high levels of CMV

among women with stage 4 breast cancer. Breast cancer is one of the most frequently diagnosed malignancies of women in many populations. Several studies have provided evidence that viruses exist in patients with breast cancer and suggest viruses can be one of the risk factors for breast cancer.

Comparison of the means of CMV IgG levels of both case study (cancer patients) and the control (non- cancer patients) revealled a statistically significant difference (P=0.029) between the mean CMV IgG of case study and that of the control. Cancer patients had a higher mean CMV IgG as against the control and this agrees with the findings of (Ali et al, 2006) which reported 100% CMV prevalence rate among HIV/AIDS patients in Iraq.

The treatment pattern (Chemotherapy, Radiotherapy, Combined Chemotherapy and Radiotherapy) had no statistically significant difference.this finding is in contrst with the findings of (Kuo *et al*., 2008) In a pilot study conducted to ascertain the rate of CMV reactivation during treatment, it revealed that 99% of the CMV-seropositive patients experience CMV reactivation during the course of chemotherapy. In this study also, the cancer patients that were receiving both chemotherapy and radiotherapy were found to have the highest mean CMV IgG levels while those that underwent surgery had the least CMV IgG.

In addition the high CMV IgG levels observed (99%) in both the case study and the control is due to the ubiquity of the CMV and its asymptomatic nature in healthy individuals. It was also observed that most of the subjects that were above 30 years had high levels of CMV IgG. This agrees with the work of Weller(1971) and Gold and Nankervis (1982). Mean CMV IgG was highest among the subjects that had Oropharyngeal cancer, CMV is a virus associated with the salivary gland (Melnick, 2011), Cervical cancer patients also recorded a high mean CMV IgG, but the least mean was obtained with kaposi sarcoma patients.

Association of cancer cases with other underlying diseases such as HIV, diabetis, high blood pressure, and stomach ulcer was also considered in this study and the findings revealled that The cancer patients that had stomach ulcer were found to have the highest mean CMV IgG levels, followed by the cancer patients that are hypertensive,the least mean CMV IgG was obtained from those cancer patients that had HIV as well. this perceived difference is not statistically significant.

The highest total human IgG levels was obtained from the cancer patients that were diagnosed with HIV as well while the least mean total IgG level was found among the subjects that had high blood pressure, though the difference was not statistically significant.

Fruits and vegetables are known to boost the immune system due to their high content of vitamins and minerals, In this study, it was found that the subjects that consumed fruits and vegetables on a daily basis had the highest mean total IgG as against those that consumed fruits and vegetables on a weekly, monthly, or with no specific pattern. This perceived difference is due to the fact that the body system of those individuals who consumed fruits and vegetables daily utilised the vitamin content of the fruits and vegetables to enhance the function of the cells of the immune system and enables the immune system to function effectively against any invading pathogen. There was statistically significant($p=0.04$) difference between those that consume fruits and vegetables as against those that had no specific pattern of consumption. The control population that have a daily habit of fruits and vegetables consumption had a slightly higher mean total human than the cancer patients although the difference is not statitically significant.($p=0.987$).

There is a general assumption that healthy life style reduces the cances of cancer in an individual, so the feeding habits of the study participants was also analysed, the cancer patients that feed mostly on fresh products were 106, those who feed on processed products were only 9 and the ones that feed on canned products were 13 the remaining 50 patients do

not have any specific form of feeding. On the other hand the control population without any specific form of feeding were the highest in number (48) , followed by the ones that consume fresh products (33) and those that feed on canned and processed products were 3 and 5 respectively. The feeding habits of the study participants was found not to have affected the mean CMV IgG levels significantly.

Alternatively, the mean total human IgG levels obtained from the cancer patients that consume fresh products was the lowest followed by the means of those who consume processed products and the least mean values was recorded among those without any specific feeding habits. Meanwhile, The control population with the highest mean total human IgG were those without any specific feeding habits, followed by the subjects that were feeding on canned products, then those that feed on processed products and the least values were recorded among those that feed on fresh products. This observed difference was not statistically significant.

Exercise on a regular basis improves the function of the cells of the body and makes their functions more easier and effective.The mean CMV IgG levels was found to vary significantly between those that exercise their bodies and those that do not, the number of cancer patients that do not excersise their bodies were 142 out of 178. And o nly 36 of the pateints excersise their bodies.($p=0.017$).

In addition, the frequency of excersise matters alot the highest mean CMV IgG was recorded among those subjects that exercise on a monthly basis, followed by those that excersise on a daily basis and the least mean values were observed among those that do not have regular form of excersise, the means were however not statistically significant.

Similarly, 20 patients out of the 44 cancer cases that answered yes to the excerise were found not not have any regular form of excersie but their mean total human IgG was

the higher than those that excersise on adaily basis. The difference between the means was however not statistically significant.

The cancer patients that excersise without any regular pattern had the highest total huaman IgG, followed by those that excersise on a monthly basis, then those that excerise on a weekly basis and the least mean values was obtained by those that excerise on a daily basis. The difference between the means was not statistically significant

Mean total human IgG levels observed in the control population that excersise on a daily basis were the highest, followed by those that excersise on a monthly basis, then those that excersise on a weekly basis and the least values were obtained from those that excercise without any regular pattern. . There was no statistically significant difference among the controls with regards to frequency of exercise.

CHAPTER SIX

6.0 CONCLUSION AND RECOMMENDATIONS

Dermographic data generated from this study reveals that female cancer cases have higher rate of occurence than males, and cancer is more prominent in the age group 40years and above.The most predominat type of cancer is cervical cancer, followed by breast cancer.

Based on the information obtained in this research, CMV is endemic in our population as already established in the literature, CMV is present in every population sampled, this is why this research recorded 100% CMV IgG in all the samples. The only difference was found when the levels of IgG was determined.The mean HCMV IgG levels of the case study was found to be higher(2.23mg/ml) than that of the control(2.17mg/ml) this statistically significant difference between the IgG levels goes a long way in supporting the argument that CMV can be classified as an onco virus.

Moreover, The high prevalence of CMV IgG observed in this study confirms the ubiquity of CMV in our population, as suggested by other researchers that CMV is endemic in almost every population sampled, but due to its asymptomatic nature of infection, it mostly goes unnoticed. During Immune suppression, CMV has been shown to reactivate from latency and cause serious illness to the immunocompromised host. Although most the researches carried out previously focused on CMV in pregnancy, a renewed interest about finding CMV among cancer patients is gaining grounds. More recent studies about this virus have suggested possible viral involvement in the progression of tumours. The high mean CMV IgG observed among the cancer patients as against the control is a possible indication of the argument that CMV can be classified as an oncogenic virus, although further research has to be conducted to affirm the argument.

Moreover, the high mean CMV IgG observed among the patients that were receiving chemotherapy and radiotherapy is also an indication of CMV reactivation during treatment to contribute to the progression of these tumours.

Marital status or more importantly the category of marriage practised can also be reported to have affected the spreading capacity of CMV, those that practised polygamy were found to have greater values than those practising monogamy. The highest mean CMV IgG recorded among oropharyngeal cancer patients also confirms possible involvement of CMV in the progression of these tumours because CMV is known to evade detection by the immune system through a number of ways.

On the other hand, total human IgG profile was, as expected 100% although the titers were found to vary based on several factors. It can however be concluded that consumption of fruits and vegetables as well exercise have great roles to play in enhancing the functions of a person's immune system this is so because the highest mean IgG profiles were found among the subject that consume fruits and vegetables on a daily basis as well as those that exercise their bodies regularly. The mean total IgG for the case study was found to be 3.21mg/ml, and 3.25mg/ml for the control population. Further more patients with HIV infection as well as cancer were found to have high total IgG (3.28mg/ml).

Out of the 267 samples examined,as expected all the samples were positive for the total human IgG. All humans must have an IgG even a one day old baby due to its ability to cross the placenta. In healthy adults, IgG constitutes approximately 75% of the total serum immunoglobulins. IgG is approximately equally distributed between intra- and extravascular serum pools.The positivity however depends upon many factors, since the entire IgG profile was examined. Individual variation based on their varying immune system, their consistency of contact with foreign agents (antigens), good personal hygiene, marital status, age, and history of any previous disease will also contribute greatly to the IgG levels. Due to these

factors mentioned above. It is difficult to provide an established reference standard for total human IgG although some scientist are of the opinion that individuals presenting with a total human IgG of <2.5g/l with non detectable IgA or IgM can be considered to have an underlying immunological problem.

On the other hand, total human IgG profile was, as expected 100% although the titers were found to vary based on several factors. It can however be concluded that consumption of fruits and vegetables as well exercise have great roles to play in enhancing the functions of a person's immune system this is so because the highest mean IgG profiles were found among the subject that consume fruits and vegetables on a daily basis as well as those that exercise their bodies regularly. The mean total IgG for the case study was found to be 3.21mg/ml, and 3.25mg/ml for the control population. Further more patients with HIV infection as well as cancer were found to have high total IgG (3.28mg/ml). On the other hand Educational status, marital status, Feeding habits were found to have no significant difference statistically. However, educational status, marital status, feeding habits were found to have no significant difference statistically.

6.1 Recommendations

1 .Combined chemotherapy alongside CMV treatment will help in better management of cancer patients. Testing for CMV IgG among patients with immunosuppressive diseases should be done routinely in the hospitals

2. Vaccines should be developed and administered to the population at risk.

3. Improvement in the standard of personal and enviromental hygiene is strongly recommended to prevent interpersonal transfer.

4. Blood banks should include screening for CMV to the list of viruses normally screened prior to transfusion.

6.2 Limitations

1. Financial constrains.
2. Long standing quest for ethical approval.
3. Sampling challenges.
4. Industrial action by the University Union (ASUU).

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