CYTOMEGALOVIRUS, RUBELLA VIRUS AND HERPES SIMPLEX -2 VIRUS INFECTIONS IN PREGNANT WOMEN ATTENDING THE KOMFO ANOKYE TEACHING HOSPITAL FOR ANTENATAL CARE (ANC) SERVICES.

By

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DECLARATION

I hereby declare that this submission is my own work toward the MSc (Clinical Microbiology) degree and that to the best of my knowledge, it contains no material previously published by another person or material which has been accepted for the award of any other degree of any University except where due acknowledgement has been made.

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LIST OF ABBREVIATIONS & ACRONYMS

AF	Amniotic fluid
AIDS	Acquired immunodeficiency syndrome
ANC	Antenatal clinic
CDC	Centre for Disease Control
CMV	Cytomegalovirus
CPE	Cytopathic effect
CRS	Congenital Rubella Syndrome
DNA	Deoxyribonucleic acid
EBV	Epstein-Barr virus
HIV	Human immunodeficiency virus
HLA CLASS I	Human Leukocytes Antigen class I
HSV-1	Herpes Simplex virus type 1
HSV-2	Herpes Simplex virus type 2
IGG	Immunoglobulin G antibodies
IGM	Immunoglobulin M antibodies
IU	International unit
KATH	Komfo Anokye Teaching Hospital
MMR	Measles, Mumps, Rubella vaccine
MRC-5	Human Fetal Lung Fibroblast cell lines
PCR	Polymerase chain reaction
PP	Phosphoprotein
RBV	Rubella virus
RNA	Ribonucleic acid
RT-PCR	Real time polymerase chain reaction

STD'S	Sexually Transmitted Diseases
TBM	3, 3', 5, 5' tetramethylbenzidin
TORCH	Toxoplasmosis, Rubella, Cytomegalovirus and Herpes simplex virus
VZV	Varicella zoster virus
WI-38	Human Lung Fibroblast cell lines

ABSTRACT

Cytomegalovirus (CMV), Herpes simplex-2 virus (HSV-2) and Rubella virus infections in pregnant women can result in undesirable neonatal outcomes. Determination of the susceptibility levels among pregnant women as well as factors influencing their susceptibility is an important first line approach to assessing at-risk individuals who then become the main target of any health interventions. A cross sectional study was carried out at the Komfo Anokye Teaching hospital (KATH), Kumasi to investigate the seroprevalence of CMV, HSV-2 and Rubella virus infections and associated probable factors influencing susceptibility levels among pregnant women attending the antenatal clinic. Structured questionnaire were administered to obtain socio-demographic data and Enzyme Linked Immunosorbent Assay, ELISA (Human diagnostic worldwide-Germany) was used to investigate the presence of IgM and IgG antibodies to CMV and Rubella. Only IgG antibody to HSV-2 was investigated. The seroprevalence of IgG antibodies to CMV, Rubella and HSV-2 was 95.60%, 92.31% and 68.13% respectively while the IgM antibodies to CMV and Rubella was 38.46% and 6.59% respectively. Age, parity, educational background and the use of protection during sex were factors that did not seemed to influence the acquisition of these infections even though it was observed that infectivity increases with age and number of delivery with individuals of basic or no education backgrounds becoming more susceptible. However, a small number of the subjects were susceptible to the rubella virus as majority of them already had antibodies and very few of them had either recent infection or reinfection and therefore were positive to both IgM and IgG antibodies. Nevertheless, none of the other associated factors such as history of miscarriage and or stillbirth, exposure to children (under 3), history of blood transfusion, sex with protection and oral sex practice had any association with CMV, Rubella and HSV-2 infection in this study. The results from this study revealed a very high seroprevalence of CMV, HSV-2 and Rubella among pregnant women which implies only few remain susceptible to primary CMV, Rubella and HSV-2 infections. This is good news since very few pregnant individuals are less likely to transmit these viruses to their foetuses. Further studies in newborns to determine abnormalities that result from primary infection in susceptible pregnant women would be worth carrying out.

CHAPTER ONE

INTRODUCTION

1.1 Background to the study

Children born with some deformities or disabilities or who develop disabilities later in life has been documented in the world over and this have become a major public health concern. These disabilities may be the result of exposure to congenital infections from certain microorganisms including viral infections during pregnancy (Jamison *et al.*, 2006). Three of such viral infections include the Cytomegalovirus, Rubella virus and Herpes simplex virus type 2. When these viruses infect immunocompetent individuals, their outcomes are usually mild and in most cases are asymptomatic or without any signs or symptoms. This has contributed to majority of infected individuals being unaware of their infection status (Brooks *et al.*, 2010). However, when such infections occur in pregnant women especially at certain stage of gestation, these viruses are able to cross the placenta and infect the foetus causing foetal damage thereby resulting in spontaneous abortion (miscarriage), stillbirth and wide range of malformations in newborns such as hearing loss, mental retardation, developmental delay, cerebral palsy, epilepsy, ocular abnormality, microcephaly, hydrocephaly, hydranencephaly (absence of the cerebral hemispheres), porencephaly (cavities in the brain), heart disease, cataract, intracranial calcification, microphthalmia, chorioretinitis, skin aplasia (failure of skin to develop), skin lesions, and psychomotor retardation (Ross & Boppana, 2005; Atreya *et al.*, 2004; Jones, 2003).

The ability of these viruses to cross the placenta, infect the foetus and cause damage depends, among other things, on the mother's immune status against the specific virus (Mendelson *et al.*, 2006). Although primary infections during pregnancy are known to be

significantly more damaging than secondary infections or reactivations, the trimester in which the infection occurred is also a determining factor. In the case of CMV and rubella, primary infection during the first trimester of pregnancy is very critical while that of HSV-2 is more serious during late pregnancy (Kimberlin, 2007; Edlich *et al.*, 2005; Eskild *et al.*, 1999)

Several studies carried out to determine the prevalence of these viral infections among the pregnant population has revealed high numbers of these infections among pregnant women. It has been reported that in Africa, about 80% of pregnant women are already immune to rubella virus while close to 100% of them would have antibodies to CMV with between 30-80% also having antibodies to HSV-2 (Weiss, 2004; Ho, 1990; Gomwalk & Ahmad, 1989). However, the intention of these studies has always been to determine susceptible pregnant women and to direct an appropriate health intervention towards them so as to curb any detrimental outcome.

Among the three viruses, vaccines are only available for rubella. Nevertheless, once infected with CMV, Rubella virus and HSV-2, antibodies produced offers some protection to the mother and also prevent or reduce the viral transmission to the foetus thereby preventing disabilities in newborns. With this in mind, several developed countries continue to see the pressing need to carry out research on these viruses in pregnant women to identify susceptible ones. Some of these countries even routinely screen pregnant women during their visit to antenatal clinic (ANC) for TORCH; an acronym for Toxoplasmosis, Rubella, Cytomegalovirus and HSV-2 as well as other pathogens such as *Treponema pallidum* which can cause harm to the fetus (Kaur *et al.*, 1999).

The focus of this study therefore was to determine the susceptibility levels among pregnant women as far as these viruses are concerned by looking for individuals with no antibodies to these viral infections among the participating subjects. The study also assessed some associated risk factors that increase the infection rate among pregnant women. This would not only provide current information on the numbers of pregnant women at risk of these viral infections but also provide some useful information for health promotion activities.

1.2 Problem Statement

In recent years, there has been an increase in genital herpes (HSV-2) as well as in the prevalence of CMV and rubella in the general African population (Weiss, 2004; Mbizvo, 2002; Cusini & Ghislanzoni, 2001). Though this on the positive note suggests a reduced number of susceptible individuals including pregnant women, there are still some reports of congenital infections with their consequent abnormalities in newborns which usually occur in primary infection among the few susceptible pregnant subjects (Lawn *et al.,* 2000). For example, in the United States, the HSV-2 seroprevalence has increased since 1970 by 30%, where currently, one out of five adults is infected (Cusini and Ghislanzoni, 2001; Fleming *et al.,* 1997). Comparing to the developing countries, significantly higher rates of HSV-2 have been observed in sub-Saharan Africa, where prevalence among adults spans from 30% to 80% in women and 10% to 50% in men (Weiss, 2004). Unlike HSV-2, the prevalence of CMV has been reported to be very high among some African population with Nigeria recording about 97.2% of CMV seropositivity among pregnant women (Akinbami *et al.,* 2011) while that of Western Sudan is about 72.2% CMV infected pregnant women (Hamdan *et al.,* 2011). In Ghana, studies carried out on the general population excluding pregnant women reported of 77.6% seropositivity (Adjei *et*

al., 2008) and 93.3% among voluntary blood donors (Adjei *et al.,* 2006). Almost the same observation was made with Rubella virus investigations. However, unlike CMV investigations, a study on Rubella infection in pregnant women was once carried out more than a decade ago where the prevalence was reported to be 92.6% (Lawn *et al.,* 2000). Globally, the incidence of rubella infection has been reduced as a result of the introduction of the Rubella vaccine years ago. However some African countries including Ghana have not yet included rubella vaccine in their routine childhood immunization program and it is therefore not surprising to see high prevalence of the infection in these countries. In Mozambique, the incidence is about 95% (Barreto *et al.,* 2006) while in Western Sudan a prevalence of 65.3% has been reported among pregnant women (Hamdan *et al.,* 2011). Most of these African countries like Ghana, vaccinate children against Measles and Mumps and not Rubella. Unlike most African countries, most advanced countries combine these two vaccines (measles and mumps) with the Rubella vaccine commonly known as Measles Mumps Rubella (MMR) vaccine in their immunization programs. This has resulted in massive reduction and elimination of rubella and CRS in most of these countries (Peltola *et al.,* 2008; Best, 2007; Lee & Bowden, 2000; Cutts & Vynnycky, 1999). Investigating therefore into the susceptibility levels of these viruses in pregnant women as well as some considered factors that seem to increase their susceptibility to these infections is worth carrying out since it would provide a current epidemiological data on these infections which would be helpful in health promotion activities.

1.3 Justification and Relevance of study

Cytomegalovirus, Rubella virus and Herpes Simplex-2 virus are typical viral pathogens that cause congenital infections in pregnant women and thereby cause foetal or neonatal

abnormalities with high foetal morbidity and mortality. The basic epidemiological information concerning these infections including their prevalence and some associated factors that increases the susceptibility of pregnant women to these infections is helpful to health planners, care providers and also for health promotion activities (Hamdan *et al.*, 2011). In Ghana, screening pregnant women for these infections are only carried out upon a Clinician‘s request. This has also contributed to lack of data on the prevalence of these viruses in pregnant women in Ghana. Evidence from several studies indicates that susceptible pregnant women are more prone to giving birth to infants with abnormalities caused by these viruses in first time exposure than their counterparts who already have antibodies to these infections. Therefore, a study to determine the susceptibility levels among pregnant women and some associated factors increasing their susceptibility rate is inevitable. This study is therefore to determine prevalence of these viruses among pregnant women and it is to extend the few studies that have been carried out on the general population.

1.4 Objective of the Study

The study was aimed at determining the current susceptibility levels of Cytomegalovirus (CMV), Rubella and Herpes Simplex-2 Virus (HSV-2) infections and some considered associated factors that influence susceptibility in pregnant women attending the Komfo Anokye Teaching Hospital (KATH), Ghana, for antenatal care (ANC) services.

1.4.1 Specific Study Objectives

The specific objectives of the study were;

1. To determine the seroprevalence of Cytomegalovirus, Rubella and Herpes simplex-2 virus in pregnant women who visit the Komfo Anokye Teaching Hospital for antenatal care.

2. To determine pre-disposing risk factors to these infections.

CHAPTER TWO

LITERATURE REVIEW

2.1 CYTOMEGALOVIRUS

2.1.1 Historical Background

The Cytomegalovirus (CMV) is a β-herpes virus, belonging to the family herpesviridae, a large group of double-stranded enveloped DNA viruses (Brooks *et al.,* 2010; Mendelson *et al.,* 2006). Cytomegalovirus is the largest member of this herpes virus family. More than 150 members of the herpes family have been identified to date and in humans, eight different herpes viruses are known. Five of these viruses are usually known by their common names: Herpes Simplex Virus type 1 and type 2 (HSV 1 & 2), Varicella Zoster Virus (VZV), Human Cytomegalovirus (CMV) and Epstein Bar Virus (EBV) instead of their official names: Human Herpes Virus 1 to 5. The other three which include Human herpes viruses 6, 7 and 8 are still known by their official names because they have no other names (Brooks *et al.,* 2010)

The characteristic cytomegalic cells of CMV disease were first noted by Ribbert in 1881 in the kidney and parotic glands of a syphilitic neonate, and confirmed by Jesionek and Kiolemenoglu in 1904 but for a long time the disease was thought to be of protozoan nature. Other researchers later detected similar cells in other infants and saw remarkable similarities of these cells to those seen in herpes zoster and herpes simplex-2. The term "salivary gland virus" was coined as a result of the prominence of these cells in salivary glands and in 1926 a guinea pig model of salivary gland virus disease confirmed the viral agent of this disease as transmissible through saliva. As knowledge and experience advanced, a neonatal illness with petechiae, hepatosplenomegaly, and brain calcifications

was characterised and correlated with the presence of cytomegalic cells. Wyatt *et al.* (1950) coined the term “generalised cytomegalic inclusion disease”. Fetterman (1952) having heard of the presence of the viral inclusions in kidney tubules cells begun using urine as a sample for diagnosis especially in infants. Weller *et al.* (1957), Smith (1956) and Rowe *et al.*, (1956) isolated the virus giving credence to reports of earlier researchers. Weller *et al.* (1970) proposed the term "cytomegalovirus" for this viral agent, from two Greek words cyto meaning cell and megalo, meaning large as a results of the cytopathic effect in cell culture and subsequently isolated CMV from the urine of infants with generalised disease (Riley, 1997).

Numerous diseases were detected to be associated with CMV in the mid-1950s and 1980s, and connection between congenital CMV infection, deafness and cognitive difficulties later in life were also established. Correlation between CMV and CMV-mononucleosis, transmission of CMV via transfused blood during cardiac surgery and CMV disease in transplant and AIDS patients (Ho 2008; Riley 1997) also followed later.

2.1.2 Biology of Cytomegalovirus

The CMV is species-specific and has been isolated from many animal species. However the human CMV is believed to exist in only human, that is humans are the only reservoir. The CMV (also known as herpes virus 5) has characteristic herpes viral appearance with a central double stranded DNA genome of about 235 thousand base pairs which is surrounded by an icosahedral capsid composed of 162 capsomeres (Brooks *et al.,* 2010). The capsid is surrounded by a region called the tegument which is also surrounded by a loose envelope containing embedded viral glycoprotein complexes (Brooks *et al.,* 2010; Davison & Bhella, 2007). The tegument is composed of about 20 proteins which includes

Phosphoprotein 65 (pp65), pp150 etc., with pp65 being the major representative. These proteins are highly immunogenic and capable of deregulating the cellular cycle of the host cell. The pp65 is of special interest because it is used as a diagnostic tool in the pp65 antigenemia assay. Pp65 can be found in the nuclei of a small fraction of polymorphonuclear granulocytes in active infection. These granulocytes have acquired the pp65 from infected endothelial cells (Kas-Deelen *et al.,* 2001; Grefte *et al.,* 1994). Upon in-vitro infection, the pp65 protein is transported to the nucleus of infected fibroblasts immediately after fusion of the virion with the cell membrane.

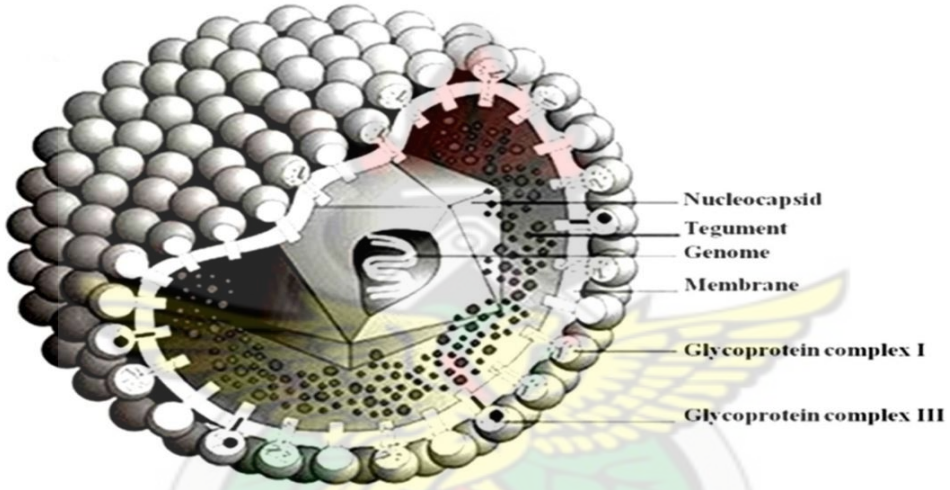


Figure 1: Basic structure of a mature CMV virion, dsDNA–double stranded DNA

Source**: http://www.intechopen.com/source/html/42576/media/image1.jpeg**

2.1.3 The immune system and Cytomegalovirus

After an individual is exposed to the CMV for the first time (primary infection), CMV IgM antibodies are produced and persist for 3 or 4 months, however it can also be produced during recurrent infections (reactivation and reinfection) (Lazzarotto *et al.,* 2008). CMV IgG antibodies on the other hand are produced after occurrence of the infection and remains throughout life (Giessen *et al.,* 1990). The IgG antibody usually indicates past CMV infection. After the infection, host defence in immune-competent

individuals combines both cellular and humoral immune responses against the virus thereby preventing severe CMV disease in the vast majority of infections (Mendelson *et al.,* 2006). As a result of the combined defence especially due to cellular immune response, infected cells are destroyed and therefore further replication and dissemination of the virus are halted. The virus enters a stage of latency and may be reactivated during a state of decreased immunity which can be by the use of immunosuppressive drugs (Sissons *et al.*, 2002). Reinfection with a second strain or reactivation of latent CMV when occurs result in active CMV infection (Sissons *et al.*, 2002; Baldanti *et al.*, 1998a). While CMV persistently infects most cell types during active infection, it appears to remain latent in myeloid lineage cells (Sissons *et al.*, 2002; Slobedman & Mocarski, 1999). These myeloid precursor cells differentiate to peripheral blood monocytes and factors associated with differentiation can lead to the reactivation of CMV (Streblow & Nelson, 2003; Hummel & Abecassis, 2002).

In the case of maternal past infection, some protection has been reported of foetus and therefore not all maternal infection results in foetal transmission and damage. Only 35–50% of maternal primary infections and 0.2–2% of secondary infections lead to foetal infection, out of which only 5–15% in primary infection and about 1% in secondary infections are clinically affected (Raynor, 1993; Boppana *et al.,* 1992; Stagno *et al.,* 1982). Nevertheless, protection due to maternal antibodies can also be overcome by reinfection of CMV-seropositive women with a second CMV strain during pregnancy, resulting in transmission and possible symptomatic infection (Boppana *et al.,* 2001). The importance of the humoral immune response is also demonstrated by CMV specific antibodies reducing the generation of pp65 positive granulocytes by inhibiting uptake of

pp65 from infected endothelial cells in vitro (Kas-Deelen *et al.,* 2001) but the role of the humoral immune response to CMV infection is yet to be fully understood.

2.1.4 Epidemiology of Maternal and Congenital CMV infection

The virus is known to be transmitted by several ways including direct contact with saliva (kissing), breast milk, urine, blood, cervical secretions and semen (Kenneson & Cannon, 2007; Yamanishi *et al.,* 2007; Jones, 2003; Demmler, 1991). The ubiquitous nature of the organism and the variety of media by which it can be carried and transmitted probably has made it one of the most prevalent viral infections in the world. The occurrence of CMV has been observed to increase with age, but the patterns of its acquisition vary significantly among different populations according to geographic location, sexual behaviour, breast feeding and socioeconomic factors (Kenneson & Cannon, 2007; Ross & Boppana, 2005; Jones, 2003; Demmler, 1991).

Generally, prevalence of CMV infection is higher in developing countries and among those with a low socioeconomic status (Suarez *et al.,* 1994). Seroprevalence from individuals in North Africa is 84.0% (Green *et al.,* 1993) while in West Africa, for that matter Ghana, there are limited epidemiological data on CMV especially among pregnant subjects; the very scanty data available only reveals the prevalence among the general Ghanaian population which is reported to be 77.6% (Adjei *et al.,* 2008) while among Ghanaian blood donors, the figure is estimated to about 93.3% (Adjei *et al.,* 2006). This suggests low levels of susceptibility among the above mentioned Ghanaian groups. However, the prevalence and susceptibility levels in pregnant women are not known. In Asia, the infection prevalence is 74% (Green *et al.,* 1993) with Thailand having one of the highest prevalence of almost 100% (Wang *et al.,* 2000). The infection rate however, is not

as high in the developed countries as compared to the under-developed and developing countries and even infections that occur in the developed countries are usually prevalent among those of low socio-economic class (Suarez *et al.,* 1994) probably because of crowding living condition as well as poor hygiene practices believed to be practiced by individuals of low socio-economic status and by individuals in under-developed and developing countries. In the United States for instance, 40% to 60% of children of low socio-economic status are infected before the age of 6, and by age 20 to 25 years, almost 80% to 100% have acquired the infection (Staras *et al.,* 2006; Betts, 1983). However among middle-upper class adults only 40% to 60% are CMV seropositive (Yow *et al.,* 1988). Conversely, developed countries such as Germany and England have even lower prevalence of 45% and 54% respectively (Hecker *et al.,* 2004; Tookey *et al.,* 1992). These unlike their African counterparts indicate high susceptibility levels in these countries. Nevertheless the rate of infection is reduced in these nations probably because of high socio-economic status of it inhabitant as well as good hygiene practices in these countries.

Again, most developed countries have seen the pressing need for devoted research into the prevalence of the virus among pregnant women. In this case, they are able know susceptible pregnant women and direct an appropriate intervention towards them so as to prevent primary maternal infection during pregnancy. Other developing African countries have followed suite, where in Nigeria the current prevalence among pregnant women is estimated to be 97.2% (Akinbami, *et al.,* 2011), that of Western Sudan is about 72.2% (Hamdan *et al.,* 2011).

CMV can be transmitted vertically in three different ways; transplacental, intrapartum or breastfeeding (Ross & Boppana, 2005). Congenital infection generally occurs when CMV is transferred across the placenta from a CMV-infected mother to the foetus. The trimester in which CMV infection and transmission occurs appears to influence the clinical outcome. Maternal seroconversion during the third trimester results in high transmission rates but has low incidence of neurological sequelae (Gindes *et al.,* 2008; Munro *et al.,* 2005).

However, congenital CMV infection in the first trimester is more likely to cause neurological sequelae, particularly sensorineural hearing loss (Pass *et al.,* 2006). Congenital CMV infection like that of maternal CMV infection is more prevalent in under-developed countries and among lower socio-economic groups in developed countries, where crowding and over population is more common. The infection occurs in approximately 0.2%-2.5% of all live births (Pass, 2002; Lagasse *et al.,* 2000; Stagno, 1999; Nelson & Demmler, 1997; Hicks *et al.,* 1993). Not all CMV-infected infants suffer adverse outcomes due to congenital CMV infection. Symptomatic CMV infection at birth with long term neurological sequelae, such as developmental delay and hearing loss, occurs in 10 to 25% of congenital CMV cases (Ross & Boppana, 2005; Boppana *et al.*, 1999). A further 10% of asymptomatic at birth, CMV-infected infants will develop symptoms during infancy (Ross & Boppana, 2005). Sensorineural impairment is common and often occurs in up to 65% of symptomatic congenital CMV infection and 5 – 10% of asymptomatic infections, which is significantly higher than impairment in the general population which is about 0.1 – 0.4% (Ross & Boppana, 2005; Fowler *et al.*, 1997; Whitley *et al.*, 1997; Williamson *et al.*, 1990).

Though, both primary and recurrent infections in mothers during pregnancy may result in congenital CMV infection, primary infections are more likely to result in congenital infection than recurrent infection. It is estimated that the transmission rate in the former is about 32-40% whereas that of the latter is about 1-3% (Kenneson and Cannon, 2007; Enders *et al.,* 2001; Peckham *et al.,* 2001; Adler, 1999).

2.1.5 Associated factors for Maternal CMV infection

Several factors have been associated with CMV susceptibility, both at infancy and at child bearing ages (maternal age). These include the geographic location, maternal socio-economic status, occupation, race, age and parity among other factors (Sheevani *et al.,* 2005). Research has shown that behaviours that expose an individual to body fluids known to be a medium of CMV transmission also contribute to the risk of infection. The virus can be found and therefore isolated from urine, saliva, cervical and vaginal secretions, semen, breast milk, tears, blood products and transplanted organs (Kenneson & Cannon, 2007; Yamanishi *et al.,* 2007; Jones, 2003; Bowden, 1991; Demmler, 1991;) which when exposed to increases ones susceptibility. Individuals living under crowded conditions with low socio-economic status and poor sanitation common in developing countries have high prevalence to CMV infection while among young adults; sexual activity (behaviour) has been ascertained to be one of the main factors of CMV infection (Zanghellini *et al.,* 1999; Fowler & Pass, 1991; Sohn *et al.,* 1991; Collier *et al.,* 1990).

However, young females who have had exposure to infants particularly between the ages of 3 and below have also been showed to have high seroprevalence than their counterparts who have not had exposure to infants (Stadler *et al.,* 2012). This is also true for both parent and pregnant women who have had exposure to children who are either their own

or not, both at home and at work (American Academy of Paediatrics, 2003; Chin, 2000; Boppana *et al.,* 2001). Once infected, children less than 3 years of age excrete virus in both saliva and urine for an average of 24 months and therefore seronegative women not excluding pregnant women who have contact with young children are more likely to become infected than are women who do not. This makes infants and children important sources for the spread of CMV. The infection has also been demonstrated to be transmitted sexually. Thus, studies have proved that the number of sexual partners also influences the chance of contracting the virus, increased sexual partners increases the risk (Robain *et al.,* 1998; Collier *et al.,* 1990). Pregnant women may also have a higher risk of acquiring infections because of pregnancy-induced immune depression (Yip *et al.,* 2006). The daily activities that put the pregnant women, mothers and workers of day care centres exposed to children to risk includes touching, changing of diapers, washing and feeding of young children (Stadler *et al.,* 2012). CMV has also been known to be the significant cause of transfusion-acquired infections in patient populations even though transmission by blood is rare; about 3 - 5%, studies still reveals that transmission of CMV by blood still occurs (Roback, 2002). Several studies have revealed very high seroprevalence among subjects with history of blood transfusion as well as high seroconversion rate and prevalence among blood donors (Ojide *et al.,* 2012; Adjei *et al.,* 2006; Hecker *et al.,* 2004).

2.1.6 Clinical presentation, treatment and management of Cytomegalovirus infections

The virus is usually known to cause a mild and self-limiting disease and treatment became essential when the virus was observed to cause severe disease in immunocompromised individual such as those with the human immunodeficiency virus

(HIV), patients of organ transplants and neonates of infected mothers as a result of congenital infection (Ahmed, 2011). The CMV is an opportunistic pathogen and causes severe illness in the above mentioned group and has a very high tendency of resulting in a significant morbidity and mortality (Sun *et al.,* 2008; Arthurs *et al.,* 2008; Li *et al.,* 2007). After the incubation period, that is 4-8 weeks, most infected persons within the general population would not show any signs or symptoms of the infection; would be asymptomatic and only few individuals probably show signs of infection including flu-like symptom, CMV hepatitis, encephalitis, intestinal pneumonitis, retinitis and mononucleosis-like syndrome (Emery, 2001; De Jong *et al.,* 1998; Studhal *et al.,* 1992), but in the group most at risk, the severity may result in neurological defects, mental retardation, deafness and other defects in newborns whereas immuno-compromised patients like HIV patients and organ transplants patients may suffer severe diseases and even death.

Currently the well-known antiviral drugs for the treatment of CMV infections includes Ganciclovir, Valganciclovir, Foscarnet and Cidoforvir which are also being explored for the treatment of congenital CMV infections (Harter & Michel 2012; Ahmed, 2011). The availability of these antiviral drugs has provided major advances in the treatment and prevention of CMV infection as well as significantly improving the outcomes for immunocompromised host.

Prevention they say is better than cure and though prevention of the CMV infection may seem almost impossible due to the ubiquitous nature of the organism, efforts made in preventing primary maternal infections as well as congenital CMV infections is directed towards avoiding all possible factors that put mothers and pregnant women at risk of acquiring the infection. This may include adapting proper hygienic practices for

susceptible pregnant women, avoiding exposure to children, administration of CMV hyper-immune globulin to pregnant women with a primary infection (Buxmann *et al.,* 2012; Onorato *et al.,* 1985) and vaccines administered to girls or women before pregnancy. However vaccines studies for CMV are still ongoing.

2.1.7 Laboratory diagnosis of CMV

2.1.7.1 Serological method

Prenatal screening for antibodies to CMV together with some other agent such as *T. gondii* is a routine practice in some parts of the world (Kaur *et al.,* 1999*),* commonly referred to as TORCH. Despite the significant cause of morbidity and mortality worldwide, by these microbial agents, widespread implementation of screening programmes has been queried due to several factors, including lack of consistent and reliable serologic methods, cost, and misinterpretation of results (Abdel-Fattah *et al.,* 2005; Khan *et al.,* 2000; Garland & Gilbert 1993).

Despite these considerations, serologic testing for CMV has been shown to be a valuable diagnostic tool when ordered judiciously. The detection of IgM antibodies in maternal sera though helpful However this is not without complications because though the presence of IgM antibodies to CMV occurs in all primary infections except in some immuno-compromised individuals, They may also be present during reactivation from latency or reinfection (secondary infection) with different strains and can also remain for months (Lazzarotto *et al.,* 2008) and therefore detection of IgM to CMV in a particular serum sample is not conclusive for a primary CMV infection (Stuart *et al.,* 2007; Deyi *et al.,* 2000). Also, the mere presence of IgG class of antibodies to CMV in pregnant women

for instance is not enough to differentiate between past exposure (i.e., low risk of congenital infection) and recent, acute infection (i.e., increased risk of congenital infection).

The recommended method for serological diagnosis of asymptomatic maternal primary infection is seroconversion, this is however rare because universally, serial serological screening of pregnant women is not done in any part of the world. Antibody avidity is so far the best practical method of diagnosing maternal primary infection apart from seroconversion (Lazzarotto *et al.,* 2008) and therefore combining antibodies to CMV IgM and low avidity ant-CMV IgG makes it the most accurate means of diagnosing maternal primary infection. Foetal CMV infection can be effectively be diagnosed detection of IgM in fetal blood after a positive PCR test on Amniotic fluid especially after 21 weeks gestation (Fabbri *et al.,* 2011). However, diagnosis of foetal CMV IgM antibodies alone cannot predict whether the newborn will be symptomatic or not, but the detection of other non-viral factors such as Beta-2 microglobulin and platelet counts as well as ultrasound examination is able to reveal foetal abnormalities which in most cases is evident or indication of potential symptomatic fetus (Fabbri *et al.,* 2011; Guerra *et al.,* 2008; Nigro *et al.,* 2005).

2.1.7.2 Virus isolation in tissue culture

Tissue culture has been considered as the gold standard in detecting foetal infection with 100% specificity (Mendelson *et al.,* 2006). Culture is usually done on the amniotic fluid using either primary cell lines such as human embryonic cells and human fore-skin cells or continuous culture such as MRC-5 and WI-38 cells (Hodinka, 1999). CMV produces a typical CPE which is recognisable within an average time of 10 – 30 days (Leland & Ginocchio, 2007).

2.1.7.3 Detection of CMV by PCR

PCR has become the preferred method for rapid viral diagnosis in recent years but the major limitation is the possible contamination leading to false positive results (Mendelson *et al.,* 2006). The PCR makes use of several components or instruments for DNA extraction, amplification and gel electrophoresis and though it is generally agreed that PCR for CMV is more sensitive than virus isolation in tissue culture, finding CMV in amniotic fluids itself is not enough to confirm fetal infection and therefore other clinical examination such as ultrasonographic examination is important in confirming fetal infection that might result in fetal abnormalities although sometimes ultrasonography could also fail (Guerra *et al.,* 2008). One other benefit with the use of PCR is that results can be repeated in the case of controversial results. The introduction of quantitative PCR based assays such as the real time PCR, sensitivity and specificity has been increased while contamination has been minimised especially during amplification (Boeckh *et al.,* 2004; Gouarin *et al.,* 2004; Piiparinen *et al.*, 2004).

2.2: HERPES SIMPLEX-2 VIRUS

2.2.1: History and Biology of Herpes Simplex -2 virus

Herpes has been known for almost 2,000 years but the term *Herpes Simplex* only appeared for the first time in Richard Boulton's A System of Rational and Practical Chirurgery in 1713 and in the 1940s it was finally recognised as a virus. Since then several work has been done on the virus and a lot of information is known concerning the virus. The HSV-2 which is also known as human herpes virus-2 like the HSV-1, CMV and other members of the herpes family is a double stranded enveloped virus with a linear

DNA genome. This virus belongs to *Alphaherpesvirinae*, a subfamily of the *Herpesviridae* family (Brooks *et al.,* 2010; Ryan & Ray, 2004). The structure is no different from that of CMV and other herpes viruses. The major structural components that are evident as seen in other herpes viruses includes a central core containing the viral DNA, an inner core called the tegument that serves to join the envelope to the capsid with the envelope consisting of viral glycoproteins and host cell membranes (Mettenleiter *et al.,* 2006).

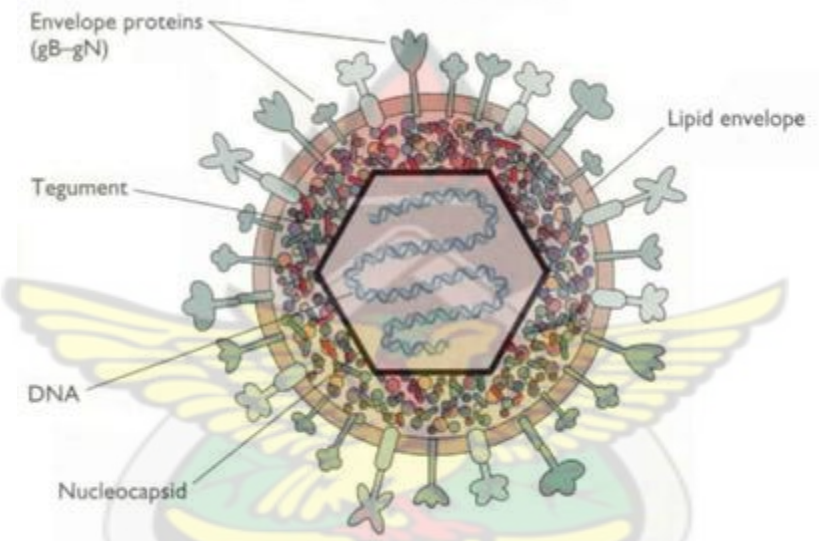


Figure 2: Structure of Herpes Simplex Virus

Source:
http://www.bio.davidson.edu/people/sosarafova/Assets/Bio307/jehodge/page01.html

2.2.2: Epidemiology of Maternal and Neonatal HSV-2 Infection

Herpes Simplex Virus type 2 (HSV-2) is known to be the most common cause of genital ulcer disease worldwide (Cheng *et al.,* 2000; Beyrer *et al.,* 1999). The virus is primarily contracted during sexual intercourse, meanwhile many infected individual are ignorant of their infectious status in spite of symptoms (Andria *et al.,* 1999; Brugha *et al.,* 1997). Herpes simplex virus type 1 (HSV-1) has always been recognised as a infantile infection

which is usually passed on through non-sexual contacts or through oral route but in recent years, has been seen a major contributor of genital herpes cases and is therefore recommended that diagnosing genital herpes should involve testing for both HSV-1 and HSV-2 (Mendelson *et al.*, 2006; Nilson & Myrmel, 2000). The highest occurrence of the HSV infections occurs in women in their childbearing age and therefore poses a risk of maternal transmission of the virus to the foetus or neonate; this has raise a major health concern (Cusini & Ghislanzoni, 2001).

The occurrence of the infection also varies with different geographic settings. In advanced nations like the United States, a recent report from the CDC suggests an overall seroprevalence of 16.2% (CDC, 2010). This however shows a massive decline of the infection in the US as reported by early researchers that the infection is on the rise at least by 30%, making every one out of five individual positive to the infection (Cusini & Ghislanzoni, 2001; Fleming *et al.*, 1997). Comparing the developing countries, higher seroprevalence of HSV-2 have been reported in sub-Saharan Africa, with prevalence in adult females ranging from 30% to 80% and while that of adult males range from 10% to 50% (Weiss, 2004; Smith & Robinson, 2002). A study among volunteers coming for STD testing revealed that 87% of them were infected (Agabi *et al.*, 2010). Earlier study in Zimbabwe reported an HSV-2 prevalence of 42.2% amongst women of childbearing age (Mbizvo, 2002) but a recent study by Munjora *et al.*, 2010 in Zimbabwe indicates that the figure has increased to 49.1% and this confirms the increasing rate of the infection in the world especially in Africa (Munjora *et al.*, 2010). However, in patients with decreased immunity like the HIV Patients and those with other STD's, higher prevalence has been recorded compared to the individuals within the general population. A latest study in Zimbabwe again showed a prevalence of 89.3% HSV-2 amongst HIV-1 infected participants as compared to 35.8% amongst the HIV-1 uninfected (Munjoma *et al.*, 2010).

Prevalence of the infection among pregnant women in the developed world though high, is lower than that in the developing countries and for that matter sub-Saharan Africa; it is reported that 22% of pregnant women in Senegal have the infection (Diawara *et al.*, 2008). In the Italy about 3% of women become infected with HSV-2 during pregnancy (Ciavattini *et al.*, 2007) while in Norway, the figure is much lower; about 2.6% of susceptible women acquired HSV-2 infection during Pregnancy (Suligoi *et al.*, 2000).

Pregnant women who acquire the infection close to delivery are at high risk of transmitting the virus from cervix or lower genital tract to their newborns during vaginal delivery and this can result in serious consequences in the neonates; The risk of neonatal herpes infection varies from 30% to 50% when it occurred during late pregnancy (last trimester), whereas early pregnancy infection carries a lower risk of about 1% (Kimberlin, 2007; Eskild *et al.*, 1999; Brown *et al.*, 1997). High transmission rate in late pregnancy is partly due to the adequate production of antibodies needed to combat viral replication before delivery. The most likely outcomes in neonatal herpes Infection include eye or skin lesions, meningoencephalitis, psychomotor retardation, or foetal malformations (Straface *et al.*, 2012). Unlike as in the case of CMV and Rubella maternal infection Stillbirth is usually not associated with maternal herpes infection. The reason is that transplacental passage of the virus rarely occurs (Eskild *et al.*, 2002) and in rare cases where transplacental passage occurs, it has been associated with spontaneous abortion, intrauterine growth retardation and preterm labour (Arvaja *et al.*, 1999; Ciavattini *et al.*, 2007; Brown *et al.*, 1997).

2.2.3: Associated factors for Maternal HSV-2 Infection

Several factors have been associated with the acquisition of genital HSV-2 infections. These factors increase the susceptibility of an individual to the infection. However, some

studies have considered some of these factors to be a risk factor while others have been merely considered as associated factors since their association with the acquisition of the infection were not statistically significant. Prominent among these factors are ones sexual behaviour or sexual orientation (Gupta *et al.,* 2007; Paz-Bailey *et al.,* 2007). Since the infection is sexually transmitted, it is not unusual to see higher prevalence among those with multiple sexual partners and also among individuals who engage or practice oral genital sex nevertheless the use of condom has been proved to reduce the risk of contracting the virus (Dipankar *et al.,* 2011; Bucher *et al.,* 2004; Jonsson & Wahren, 2004) and is therefore recommended during pregnancy to avoid the infection.

Age and sex is an important risk factors as far as genital herpes infections are concern; women are known to be prone to the infection than men and the prevalence of HSV-2 infection have been observed to very low in childhood and early adolescent and increases with age, reaching the uttermost around 40 years of age (Dipankar *et al.,* 2011; Cusini & Ghislanzoni, 2001). While early onset of sexually activities put people at higher risk of the infection, other studies have associated ethnicity, poverty, cocaine abuse, and bacterial vaginosis as factors that facilitate a woman's risk of infection before pregnancy (Cherpes *et al.,* 2003; Gottlieb *et al.,* 2002). HIV-1 among other sexual transmitted diseases is considered to be highly associated with high HSV-2 seropositivity. Genital ulcers caused by the HSV-2 increases the risk of HIV infection. A current study in Zimbabwe attests to this fact where 89.3% of HIV patient had antibodies to the (Munjoma *et al.,* 2010). The income status as well as the level of education in a particular study was significantly associated with the acquisition of the infection (Dipankar *et al.,* 2011)

2.2.4 Immunity in Herpes simplex -2 virus Infection

The virus transmitted by sexual means, first infects epithelial cells of the genital tract and migrates to nerve tissues; neurons of the sensory ganglia where they remain in a latent state (Straface *et al.,* 2012). The virus is normally found in the lumbosacral ganglia and reactivation can occur during suppressed immunity (Gupta *et al.,* 2001). Like CMV and rubella, IgM are produced during primary infection and also in recurrent infection, this usually last for few to several weeks while IgG are produced few days from the onset of primary infection last for lifetime. The presence of pre-existing maternal antibodies however only reduces fetal viral transmission to certain degree but does not prevent intrauterine or perinatal transmission of HSV-2 as in rubella (Peckham *et al.,* 2001). The risk of transmission of the virus to the infant during child birth decreases from about 30-50% in the case of maternal primary infection to about 1-3% in non-primary maternal infection (Anzivino *et al.,* 2009; Brown *et al.,* 2007)

2.2.5 Clinical presentations in pregnant mothers and neonate, treatment and management of HSV-2 infection

The infection may present no symptoms (asymptomatic) as it happens in many cases resulting in many infected individuals unaware of their infection status (Brugha *et al.,* 1997; Brown *et al.,* 1997). In symptomatic cases clinical presentation of genital herpes usually occurs after an incubation of a period of 2–20 days and lasts up to 21 days (Cusini & Ghislanzoni, 2001; Desselberger *et al.,* 1998). Presentations in women include blistering and ulceration of the external genitalia and cervix causing vulval pain, dysuria, vaginal discharge, and local lymphadenopathy (Desselberger *et al.,* 1998). Primary infection can however result in complication when the infection becomes systemic; this

therefore results in symptoms such as fever, headache, and myalgia. Meningitis is one other complication that may occur but this rarely happens (Gottlieb *et al.,* 2002; Suligoi *et al.,* 2000).

In neonate, the identification of the infection in the absence of vesicular rash has proved to be difficult however several studies have associated maternal primary HSV infection, maternal fever, vaginal delivery, postnatal HSV contact, vesicular rash (skin lesion) in mothers before or during pregnancy with possible neonatal infection which can result in any of the following presentations; chorioretinitis, cerebral palsy, skin aplasia (failure of skin to develop), hypothermia, lethargy, seizures, severe respiratory distress, proteinosis, hepatosplenomegaly, thrombocytopenia, cerebrospinal fluid pleocyosis, hydranencephaly (absence of the cerebral hemispheres), porencephaly (cavities in the brain), intracranial calcification, microphthalmia chorioretinitis and psychomotor retardation (Caviness *et al.,* 2008; Kimberlin *et al.,* 2001; Eskild *et al.,* 1999; Brown 1997)

Since the 1970's, there has been an effective antiviral agents treatment for infected pregnant mothers and neonatal HSV disease (Kimberlin *et al.,* 2001). Studies have shown that the administration of acyclovir and valacyclovir at the recommended doses is effective in the treatment of neonatal herpes simplex infections as well as pregnant women with first clinical episode or recurrent infection. The main purpose of the therapy is to prevent the widespread dissemination of the virus and also reduce it replication within the central nervous system especially in neonatal herpes simplex infection (Kimberlin *et al.,* 2001). In pregnant women, the drugs have also been proved to reduce the frequency of clinical presentation and virus shedding during delivery and therefore decreasing the need for caesarean delivery (Andrews *et al.,* 2006; Sheffield *et al.,* 2006; Major *et al.,* 2003; Watts *et al.,* 2003). However when primary infection occurs during

the third trimester, it is suggested that caesarean section be carried out especially in pregnant women who have developed primary clinical infection within the last few days to delivery (Patel *et al.,* 2001; Brown *et al.,* 1997). In cases where vaginal delivery has been started and is irreversible, both the mother and the neonate should be administered with intravenous acyclovir (Ciavattini *et al.,* 2007).

2.2.6 Laboratory diagnosis of HSV-2

Diagnosis of genital herpes (HSV-2) must not be based solely on clinical presentation since it has a sensitivity of 40% and specificity of 99% with about 20% rate of false-positive (Sauerbrei & Wutzler, 2007) and there should be confirmed with either serological test or viral detection (PCR and viral culture).

2.2.6.1 Serology and detection of HSV-2 by PCR

Diagnosis of HSV infection relies on both serological and virological methods, however the diagnostic process may be problematical due to the nature of the viral infection. The presence of HSV-2 antibodies especially IgG in pregnant women is an indication of recurrent infection if vesicles are found in the genital tract (Arvin *et al.,* 2006). Detection of viral DNA from any specimen including cerebrospinal fluid using polymerase chain reaction, has improved the probability of obtaining a speedy diagnosis, nonetheless, negative results does not rule out the presence of an infection (Kimberlin, 2007; Malm & Forsgren 1999; Aurelius *et al.,* 1991). The detection IgM in a neonate is highly significant in establishing neonatal herpes infection (Leventon-Kriss *et al.,* 1983).

2.2.6.2 HSV-2 detection by virus isolation in cell culture

The standard laboratory method to confirm current HSV infection is virus isolation and typing in cell culture since HSV grows readily in tissue culture. The virus may be isolated within 2–4 days from swabs taken from herpetic skin and laryngeal or genital lesions however, the probability of obtaining a positive HSV culture in infected infants is greater with culture from the skin lesions or conjunctivae (Kimberlin *et al.,* 2001). This method however has its own limitation where the sensitivity of the test can be compromised by poor sampling and transportation of the specimen and in cases where lesions are healed, they may result in negative culture (Berardi *et al.,* 2011).

2.3 RUBELLA VIRUS

2.3.1 History and Biology of Rubella virus

Rubella virus is a single stranded RNA virus of paramyxovirus group. It is a togavirus and the only member of the genus rubivirus and the cause of Rubella, a childhood disease commonly known as German measles. The disease was first described in the mid-eighteen century with Friedrich Hoffmann being the first to clinically describe the disease in 1740. This was later confirmed by de Bergen in 1752 and later again in1758 by Orlow of whom were all Germans (Wesselthoeft, 1949). This disease was difficult to distinguish from measles and scarlet fever since they nearly produces similar clinical presentations until in 1814 where George Maton gave a more vivid description of the disease and therefore suggested that the disease should be considered different from that of measles and scarlet fever. The disease had it common name as "German measles" from the fact

that all the early scientists of the disease were Germans (Best *et al.*, 2005). Henry Veale, an English Royal Artillery surgeon was the first to describe an outbreak of the disease in India and later in 1866 became the author of the now well-known name of the disease, "Rubella" (Lee & Bowden, 2000; Ackerknecht & Hernz, 1982). Alfred Fabian Hess, in 1914 base on some work on monkeys theorized that the Rubella was cause by a viral agent (virus). This was later confirmed in 1935 by Hiro and Tosaka by passing the disease to children using a filter nasal washing from an acute case (Hess & Fabian, 1914).

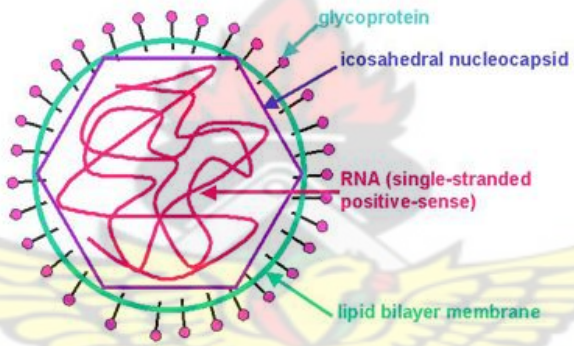


Figure 3: Structure of the rubella virus.

Source: http://pathmicro.med.sc.edu/mhunt/rubella.htm

2.3.2 Pathogenesis and Immunity

The infection is acquired by inhalation of aerosols or nasopharyngeal secretion containing the virus. The virus then infects the cells of the upper respiratory tract and enters the cell by receptor-mediated endocytosis. It is believed that replication probably begins in the respiratory tract. From there the virus spread and replicates in the lymphoid tissue of the upper respiratory tract, viraemia proceeds causing systemic infection after about 7 – 9 days and last until the appearance antibody on about day 13 – 15 (Brooks *et al.*, 2010).

When viraemia has occurred, the virus spread to many organs including placenta where it can infect the fetus in pregnancy leading to congenital infection and it subsequent congenital rubella syndrome (CRS) (Deorari *et al*., 2000; Coulter *et al.*, 1999).

Maternal immunity, either after vaccination or naturally derived, is generally protective against intrauterine rubella infection (Bullens *et al.,* 2000). Immune mothers usually transfer their antibodies to their offspring which protect them for about 4 – 6 months after birth (Brooks *et al.,* 2010). A measure of antibody level of 10-15 international unit (IU) is universally considered to be a positive immunity status, i.e one is immune (Mendelson *et al.,* 2006). Like most infections, majority of the initial antibodies elicited are IgM which normally lasts for some week and has been used detecting recent rubella infection as well as congenital rubella (Best, 2007) even though it is not confirmative in itself. IgG is initially present in lower titre and rises with time while persisting throughout life. Majority of naturally infected victims develop life-long immunity while in vaccinated subjects immunity has been shown to be preventive against viraemia with protection usually lasting for more than 16 years, nevertheless few failure in vaccination have been reported where there is development of partial immunity and therefore protection offered wanes and last for about 5 to 8 years instead (Mendelson *et al.,* 2006; Banatvala & Best *et al.,* 1990). Immunity or past infection does not guarantee protection from reinfection. A study done in Italy followed immunized subjects for 5 years to demonstrate an evidence of reinfection after vaccination and it was found that 9.8% of the subjects showed an indication of reinfection (Cusi *et al*., 1993). In maternal reinfections, some cases of CRS have resulted especially in maternal reinfection before the 12th week of pregnancy (Robinson *et al.,* 1994) even though the risk of CRS at this stage of the gestational period is very low (Best, 2007).

2.3.3 Epidemiology of maternal and congenital rubella

Rubella is a well-known childhood disease (Gomwalk & Ahmad, 1989) which usually produces a mild febrile symptoms and a rash which is usually self-limiting. The infection commonly spread mainly through the family either by direct contact or inhalation of nasopharyngeal secretions from infected persons (WHO, 2000) such that by the age of 10 years, most African children would be immune to the virus (Gomwalk & Ahmad, 1989). The mildness and or asymptomatic nature of the disease in some cases; about 20 - 50% (Brooks *et al.,* 2010) may probably be a contributing factor for it easy spread among family. In recent times, though the infection has declined with the implementation of rubella vaccination more than 40 years ago, they are still considered as an important public health problem around the world especially in the third world countries. Most advanced countries have effectively reduced the occurrence of infection and many are at the verge of eliminating CRS (Peltola *et al.,* 2008; Lee & Bowden, 2000) while few others have eliminated rubella and congenital rubella (Best, 2007). The situation however is different for most developing countries with particular reference to Africa. The infection rate is so high that the prevalence in Mozambique is almost 100% and that of Ghana about 13 years ago was 92.6% (Barreto *et al.,* 2006; Lawn *et al.,* 2000). Nigeria and Sudan are equally high with 76% and 65.3% seroprevalence in pregnant women respectively (Hamdan *et al.,* 2011; Onyenekwe *et al.,* 2000). The only good news about the higher seropositivity is the fact that less people are susceptible to the infection (Lawn *et al.,* 2000) this however does not exclude the occurrence of CRS since the few susceptible are vulnerable to the infection and have higher probability of resulting in CRS in pregnancy. The high levels of rubella infection among some African population may probably be due to some of the same reasons why CMV is so high in Africa; crowding living condition, increase direct contact especially in families, poor knowledge of the

infection and absence of the rubella vaccination (Cutts & Vynnycky, 1999). The Rubella virus readily invades the placenta and fetus during gestation (Coulter *et al.*, 1999). This usually results in serious congenital defects such as deafness and blindness (Deorari *et al*., 2000). It estimated that approximately 30%–50% of foetuses of women who become infected with Rubella virus during the first 3 months of pregnancy will be adversely affected by the virus. However current finding indicates that maternal infection or exposure in the first trimester result in nearly 80% rate of fetal infection (Best, 2007) and this drop to 25% in the late second trimester and increasing again in the third trimester from 35% at 27–30 weeks' gestation to nearly 100% beyond 36 weeks' gestation (Gabbe *et al.,* 2002). Estimation made in 1996 by WHO reported that 22 000 babies were born with CRS in Africa, and in South-East Asia the estimation was about 46 000 and close to 13 000 in the Western Pacific and very few countries in these regions had introduced rubella-containing vaccine by the year 2008, and therefore the current burden of CRS in these settings is thought to be similar to that estimated for 1996 (WHO, 2012).

2.3.4. Rubella and associated factors

Rubella has been associated with some factors which other studies have considered as risk factors. These factors contribute to ones susceptibility to the acquisition of the infections. Factors such as history of miscarriage or spontaneous abortion, blood transfusion and exposure to young children are the main factors that have been associated with higher rubella infectivity rate (Brooks *et al.,* 2010). In Nigeria, a study reported IgG antibodies to rubella in 86% of women who had experienced miscarriage before (Onyenekwe *et al.*, 2000).

2.3.5 Pathology, prevention and control

The rubella in the absence of pregnancy usually presents a mild and self-limiting disease which usually resolves after some few weeks thereby resulting in lifelong immunity. There is usually the appearance of maculopapular rash about two to three weeks after first time exposure. The rash appears on the face and then spreads to the trunk and then to the extremities. There may also be other symptoms such as low-grade fever, sore throat, lymphadenopathy and general malaise (Lee & Bowden, 2000). However some complications such as arthritis and arthralgia may be seen in adults, surprisingly, these symptoms are more severe in adult females than in men (Wolinsky *et al.,* 1996). Thrombocytopenic purpura and encephalopathy may be more severe complications in rubella infections (Brooks *et al.*, 2010; Wolinsky *et al.*, 1996; Frey, 1994).

There is no specific treatment of rubella nevertheless management is directed towards symptoms so as to reduce discomfort. In the case of CRS in newborns, management focuses on dealing with the complications. The control of rubella has always the golden tool of prevention. Live attenuated vaccine have been in existence since 1969, this vaccine is available either as a single antigen or combined with measles and mumps vaccines. The primary purpose of the rubella vaccine however, is to prevent congenital rubella infections (Brooks *et al*., 2010).

2.3.6 Laboratory diagnosis of rubella

Diagnosis of rubella in pregnant women is very important especially in suspected cases to rule a primary infection that has a high probability of resulting in CRS. The assessment of maternal primary infection are usually relies on the detecting of specific IgM antibodies to the rubella virus, seroconversion and or greater four folds rise in IgG antibodies. As in many other viral infections, IgM alone cannot provide an evidence of recent infection and

therefore some employs the use of IgG avidity testing to establish primary or recent infections. The test for IgM and IgG can be demonstrated serological by the use ELISA.

The laboratory methods used for virus detection are virus isolation in tissue culture or amplification of viral nucleic acids by RT/PCR. However, using those methods for detection of rubella virus in Amniotic fluid (AF) might be unreliable, particularly in AF samples due to low viral load. According to Mendelson and others in their review, stated that several studies have showed that rubella virus may be present in the placenta but not in the fetus, or it can be present in the fetus but not in the placenta, leading to false negative results (Mendelson *et al.,* 2006). Thus, according to one opinion, detection of rubella virus in AF does not justify the risk of fetal loss following these invasive procedures (D'Aalton & DeCherney *et al.,* 1993), while according to another opinion, laboratory diagnosis of fetal infection should combine a serological assay (detection of rubella specific IgM) with a molecular method (viral RNA detection) in order to enhance the reliability of the diagnosis (Tang *et al.,* 2003). A recent study showed 83–95% sensitivity and 100% specificity for detection of Rubella virus in AF by RT/PCR (Mace *et al.,* 2004).

CHAPTER THREE

MATERIALS AND METHODS

3.1 STUDY SITES AND SAMPLING STRATEGY

3.1.1 Study Site

The study was conducted at the Antenatal Clinic (ANC) and the Virology Laboratory of the Komfo Anokye Teaching Hospital and the Department of Clinical Microbiology, all in Kumasi. The samples were collected at the clinic and analyzed at the Virology Laboratory.

Figure 4: A section of Kumasi Metropolis, showing location of KATH

Source: http://maps.google.com.gh/maps?q=MAP+OF+KUMASI+SHOWING KATH

3.1.2 Sampling Period

The study took place from January 2013 to March 2013. Ethical clearance was obtained from the Committee on Human Research, Publications and Ethics (CHRPE) at the Komfo Anokye Teaching Hospital, and the School of Medical Sciences, KNUST.

3.1.3 Study Population

The study populations were pregnant women attending the antenatal clinic (ANC) at the Komfo Anokye Teaching Hospital (KATH), Kumasi.

3.1.4 Eligibility Criteria

3.1.4.1 Inclusion Criteria

All pregnant women attending antenatal care and consenting to participate in the study were sampled.

3.1.4.2 Exclusion Criteria

1. Pregnant women in need of emergency care or having risk during pregnancy.

2. Antenatal respondents reporting for repeat visits during the study period.

3.1.5 Sampling Strategy

Every respondent attending antenatal care who met the eligibility criteria and consented to the study was sampled until the required sample size.

3.1.6 Sample size

One hundred and fifteen (115) blood samples were collected within the sampling period, however, only 91 were used. This was due to resource constraints. Only 89 samples were also tested for IgG antibodies to Rubella virus because of the nature (design) of Rubella ELISA test kits used.

3.1.7 Contact Process

Pregnant women attending KATH ANC were approached and the rational of the study explained to them. Signed informed consent was then obtained from those willing to participate. Using structured questionnaire, their socio-demographic data, and associated factors to Cytomegalovirus, Rubella virus and Herpes simplex-2 virus were obtained (Appendix 1).

3.1.8 Sample collection, Labelling and Confidentiality

Five millilitres (5mls) of participants blood were collected into a BD Vacutainer with SST II Advance Semi-separator gel (BD, Belliver Industrial Estate, Plymouth, PL6 7BP, United Kingdom), mixed by turning it 5 times upside-down.

3.1.8.1 Sampling procedure

The blood samples were taken by well-qualified and experienced Phlebotomists from the Komfo Anokye Teaching Hospital (KATH) who offered to help with the sampling process during the study period.

A good-sized vein was identified, usually in the antecubital fossae or on the dorsum (back) of the hand. A tourniquet was then applied proximal to the site of venepuncture to ensure engorgement of vein with blood. A 5ml syringe was used to take the blood sample. The site of venepuncture was cleaned with an alcohol swab. The needle was then inserted into the vein looking for blood flashback in the bevel of the syringe. The vacutainer with SST II Advance Semi-separator gel allowed self-filling of blood (approximately 5mls of blood). Once enough blood was withdrawn, the tourniquet was removed, with the needle still in place, a cotton swab was taken and placed over site of needle insertion

(venepuncture) and the needle was gently removed. Direct pressure was applied with the cotton swab over the puncture site to stop any bleeding (about 2 minutes), after which the swab was removed to ensure bleeding has stopped. If not, the swab was affixed with gauze tape. The tubes were centrifuged at 3500 rpm for 5 minutes at the virology department of the Komfo Anokye Teaching Hospital (KATH) after which, the serum was aliquoted into cryo tubes. The cryo tubes were packed in a cryo box for transport and storage.

3.1.8.2 Labelling

The individual subject samples were labelled with a specific study number that also corresponded with their identification number on questionnaire and the informed consent form.

3.1.9 Transfer, separation and storage of serum

The sera were then transported the Virology laboratory of the department of Clinical Microbiology, School of Medical Sciences, KNUST, where the sera were screened for IgM and IgG antibodies to CMV, Rubella virus and only IgG antibodies to HSV-2 using commercially obtained ELISA kits from the Human Diagnostic Worldwide, German.

3.2.0 Reagent preparation and Laboratory testing of patients' serum samples

3.2.1 Preparation of Reagent and Working Washing Solution

All reagents were brought to room temperature (25ºC) before used according the manufacture's instruction. The working washing solution was prepared by diluting 1 part (eg. 50ml) of the Washing solution with 20 parts (1000ml) of fresh deionised water (distilled water).

3.2.2 Washing Procedure

The washing procedure was very critical because insufficient washing would result in a poor precision or falsely high absorbance. An automatic washer was used for the washing procedure. Washing was repeated for 4-5 times and it was ensured that the washers filled all the wells completely and were aspirated off efficiently after 30 seconds. The remaining liquid was removed by tapping the plate upside down on a tissue paper.

3.3.3 Sample Preparation and Dilution

The subjects' sera were diluted with the dilution buffer specially prepared for testing for IgM and IgG antibodies to the CMV, Rubella and HSV-2 as provided by the manufacturer. 1 part (10 µl) of serum was diluted with 100 parts (1ml) of the dilution buffer. Diluted samples were incubated for 5 minutes prior to further processing. The sample dilution buffer contains anti-human IgG to prevent rheumatoid factor interference and competition from specific IgG present in the specimen.

3.3.4 Laboratory testing using Enzyme Linked Immunosorbent Assay (ELISA)

3.3.4.1 Immunoglobulin M (IgM) to CMV and Rubella

The microtitre strip wells were arranged according to the number of samples. 100µl of the negative control were aliquoted into wells B1 and C1 while well A1 was left blank according to the manufacturer's instruction. 100µl of the positive control were aliquoted into wells D1 and E1. 100µl of patients' serum were then aliquoted into each of the remaining microplate wells (91 of them). The test is incubated at 17 ºC – 25 ºC (room temperature) for an hour and then washed for a minimum of 3 cycles with at least 350µl filling washing solution. The wells were blotted on an adsorbent paper until completely dried. 100µl of anti- IgM conjugate (anti- human IgM antibodies, peroxidase conjugate)

were added to each well and incubated for 30 minutes of 17 ºC – 25 ºC. The washing procedure was repeated after which 100µl of substration solution (3,3´,5,5´-tetramethylbenzidin (TBM) hydrogen peroxide) were added to each well and incubated in the dark at 17º C - 25º C for 15 minutes. 100µl of stopping solution was added to each well. The ELISA microtitre plate reader (Human Diagnostic Worldwide, Germany) was set to zero using the substrate blank in well A1. The absorbance was measured at 450nm within 30 minutes after terminating the reaction using a reference wavelength of 630nm–690nm. The principle underlining the ELISA test can be found in the Appendix 5

CALCULATION OF CUT-OFF POINT

The automated microtitre plate reader used in this study automatically generates the result of the test by comparing the cut off values with the individual absorbance of the microtitre well. The cut off value according the manufacturer is calculated using the below formula;

- The mean absorbance value of negative control in well B1 and C1 (MNC) were calculated as MNC = (B1 + C1) / 2
- The mean absorbance value of the positive control value (MPC) in wells D1 and E1 were calculated as MPC = (D1 + E1) / 2 and
- The Cut off value were computed as Cut Off Value = MNC + (0.2 X MPC)

3.3.4.2 Immunoglobulin G (IgG) to CMV, Rubella and HSV-2

The microtitre strip well was arranged according to the number of samples. 100µl of the negative control were aliquoted into wells B1 and C1 while well A1 was left blank according to the manufacturer's instruction. 100µl of the positive control were aliquoted into wells D1 and E1 (in the case of CMV and HSV-2) and into wells F1 and G1 (in the

case of Rubella). Wells D1 and E1 in rubella testing were aliquoted with 100µl rubella cut-off control. 100µl of patients' serum were then aliquoted into each of the remaining microplate wells (91 wells for CMV and HSV-2 and 89 wells for Rubella). The test is incubated at 17 ºC – 25 ºC (room temperature) for an hour and then washed for a minimum of 3 cycles with at least 350µl filling washing solution. The wells were blotted on an adsorbent paper until they are completely dried. 100µl of anti-IgG conjugate (anti-human IgG antibodies, peroxidase conjugate) were added to each well and incubated for 30 minutes of 17 ºC – 25 ºC. The washing procedure was repeated and 100µl of substration solution (3, 3´, 5, 5´- tetramethylbenzidin (TBM) hydrogen peroxide) were added to each well.

The test was incubated in the dark at 17º C - 25º C for 15 minutes after which 100µl of stopping solution was added to each well. The ELISA microtitre plate reader (Human Diagnostic Worldwide, Germany) was set to zero using the substrate blank in well A1. The absorbance was measured at 450nm within 30 minutes after terminating the reaction using a reference wavelength of 630nm – 690nm.

CALCULATION OF CUT-OFF POINT (CMV AND HSV-2)

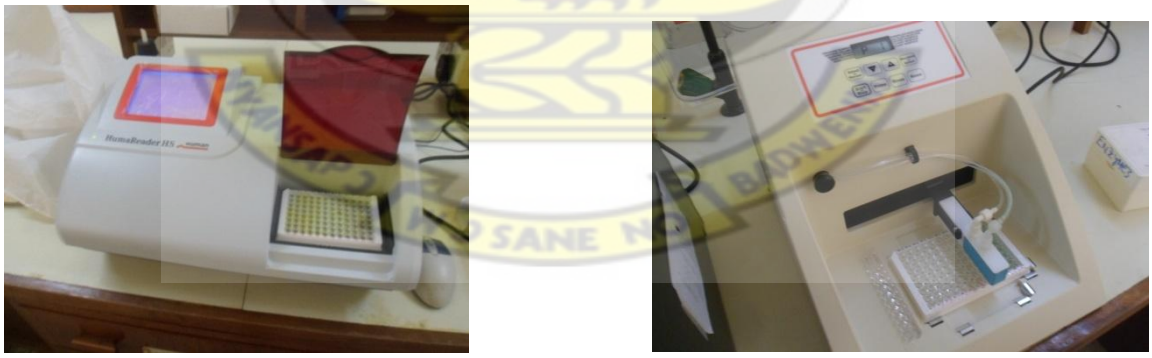
- The mean absorbance value of negative control in well B1 and C1 were calculated as MNC = (B1 + C1) / 2
- The mean absorbance value of the positive control value (MPC) in wells D1 and E1 calculated as MPC = (D1 + E1) / 2 and
- The Cut off value were calculated as Cut Off Value = MNC + (0.1 X MPC)

CALCULATION OF CUT-OFF POINT FOR RUBELLA

- The mean absorbance value of negative control in well B1 and C1 for Rubella were calculated as MNC = (B1 + C1) / 2
- The mean absorbance value of Cut-off Control in well D1 and E1 were computed as MCC = (D1 + E1) / 2
- The mean absorbance value of the positive control value in wells F1 and G1 for Rubella were calculated as MPC = (F1 + G1) / 2
- The Cut off value were calculated as COV = MNC + (0.1 X MPC)

3.3.4.3 INTERPRETATION OF RESULTS

Results of patient sample greater than the absorbance of the cut-off value (COV) were conventionally considered positive for the various antibodies tested. All patient results less than that of the cut-off value were considered negative for the different antibodies tested.



a **b**

Figure 5: Automated Microtitre plate reader (a) and microtitre plate washer (b) used for the work.

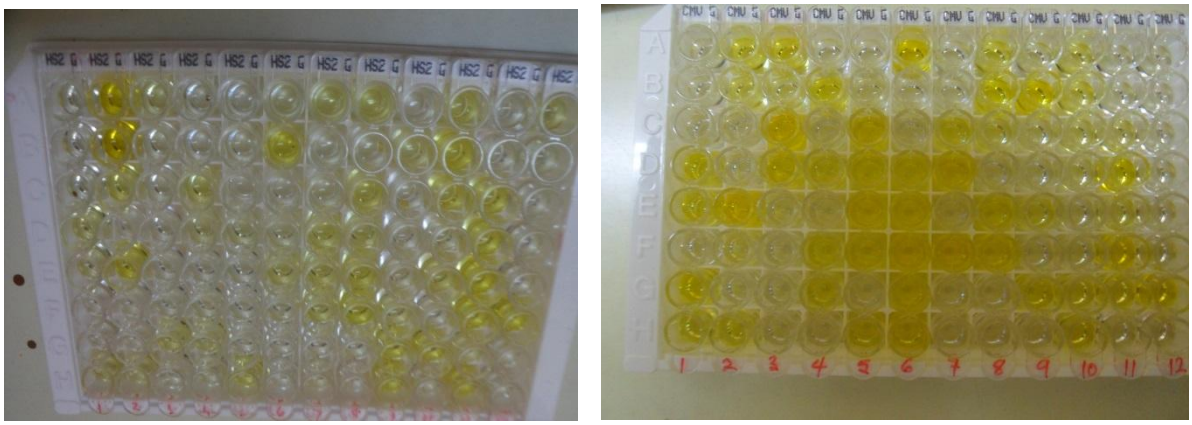


Figure 6: Microtitre plate showing antibody reactivity

3.3.4.4 ANALYSIS OF RESULTS

The results were statistically analysed using the Stata Statistical package. Significant differences between the proportions and the groups or variables were determined using both Chi-square and one-Way Anova. P-values of 0.05 were considered significant.

CHAPTER FOUR

RESULTS

4.1 Socio-demographic characteristics of pregnant women

A total of 91 pregnant women were enrolled in the study. They were between the ages of 16-46 years with only 1 person under 18 years. Most of them were married (89.01%), with very few (10.99%) being unmarried (Table 1).

Table 1: The Distribution of the Socio-demographic Characteristics of Pregnant Women

Variable	Number (n)	Percentage (%)
Age		
16 – 25	12	13.19
26 – 30	32	35.16
31 – 35	29	31.87
36 – 40	15	16.48
41 – 46	3	3.3
Marital Status		
Married	81	89.01
Unmarried	10	10.99
Educational Background		
No education	6	6.59
Basic	48	52.75
Secondary	17	18.68
Tertiary	20	21.98
Parity		
Nulliparity	30	32.97
Primiparity	17	18.68
Multiparity	41	45.05
Grandparity	3	3.3

4.2 Overall prevalence of IgM and IgG antibodies to CMV, HSV-2 and Rubella

Out of the total respondents, 87 (95.60%) and 35 (38.46%) were seropositive to CMV IgG and IgM respectively. A total of 84 (92.31%) and 6 (6.59%) women were seropositive to Rubella IgG and IgM respectively. For both CMV and Rubella, all the women who had IgM antibodies also were positive to IgG antibody. Seropositivity to HSV-2 IgG was 62 (68.13%) as shown figure 7 below

About 65.93% respondents had IgG antibodies to both HSV-2 and CMV and 62.92% of them also had IgG antibodies to both HSV-2 and Rubella while a total of 89.89% of the pregnant women had IgG antibodies to Rubella and CMV. Among the married, 30 (37.04%) and 77 (95.06%) were positive to IgM and IgG antibodies to CMV respectively. It was also observed that, 74 (95.06%) and 5 (6.17%) of them had IgM and IgG antibodies to Rubella were married while 56 (69.14%) of the married subjects had IgG antibodies to HSV-2.

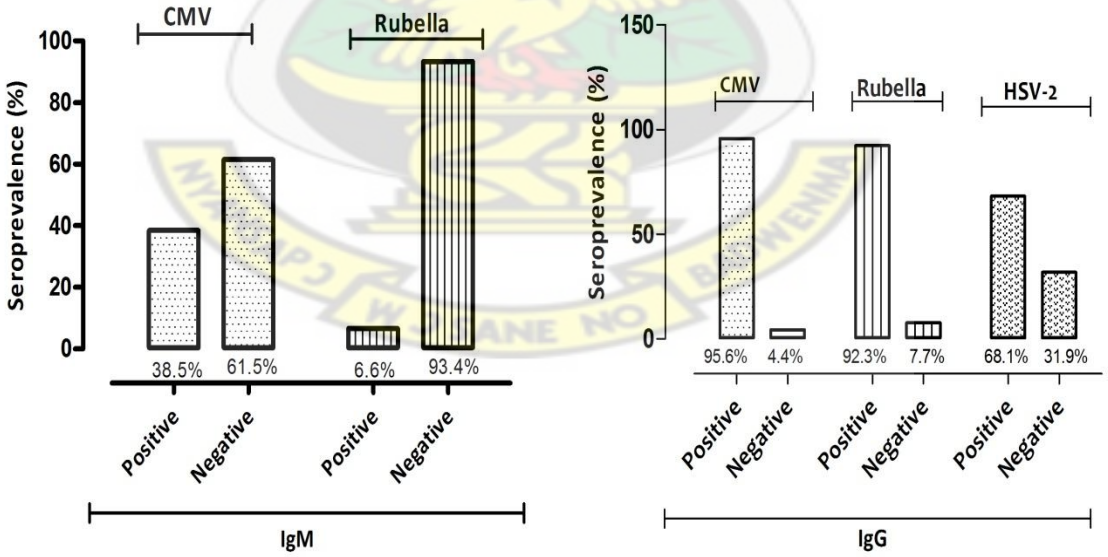


Figure 7: The prevalence of IgM and IgG antibodies to CMV, Rubella and HSV-2

4.3 Age distribution of respondents stratified by CMV, HSV-2 and Rubella antibodies

Among the age groups, majority of the respondents who showed evidence of past infection to CMV (34.48%) and Rubella (36.90%) were within 26 – 30 age groups while most with high seropositivity to HSV-2 (38.71%) were between the ages of 31 and 40 years. There was a significant difference between the proportions of seropositivity of CMV (IgG) among the age groups (P = 0.012). However, no significant difference was observed between the other tested parameters and the age distributions (Table 2).

Table 2: The prevalence of CMV, Rubella and HSV-2 and the age of respondents

Age groups	*CMV (n = 91)*				*Rubella (n =89)*				*HSV-2 (n = 91)*	
	IgM (35)	*P –Value*	*IgG (87)*	*P –value*	*IgM (6)*	*P- value*	*IgG (84)*	*P – value*	*IgG (62)*	*P - value*
16 -25	5 (14.29)		11 (12.6)		0 (.00)		10 (11.90)		4 (6.45)	
26 -30	12(34.29)		30 (34.48)		5 (83.33)		31 (36.90)		22 (35.48)	
31 -35	12 (34.29)	**0.205**	28 (32.18)	**0.012**	1 (16.67)	**0.893**	26 (30.95)	**0.073**	24 (38.71)	**0.070**
36 – 40	4 (11.43)		15 (17.24)		0 (0.00)		14 (16.67)		11 (17.74)	
41 – 46	2 (5.71)		3 (2.30)		0 (0.00)		3 (3.57)		1 (1.61)	

One –way Anova was used to estimate the difference between proportions and the groups. Percentage value in parenthesis respondents.

P is significant at 0.05

4.4 Educational background against CMV, Rubella and HSV-2 prevalence

Educational background showed a significant difference between IgG CMV outcomes (P = 0.002). All basic educational level participants were seropositive to the IgG CMV antibodies and so were individuals with no education and secondary education. The only individuals who were seronegative to CMV IgG all belong to the category of tertiary educational level. Also, most of the respondents who were seropositive to IgG of Rubella (53.57%) and HSV-2 (54.84%) had only basic education (Table 3). There were no significant difference between the Rubella and HSV-2 seropositivity among educational background.

Table 3: Educational background against CMV, HSV-2 and Rubella antibodies

Education	*Total No. respondents*	*CMV (n = 91)*				*Rubella (n =89)*				*HSV-2 (n = 91)*	
		IgM (35)	*P –Value*	*IgG (87)*	*P -value*	*IgM (6)*	*P- value*	*IgG (84)*	*P –value*	*IgG (62)*	*P - value*
No education	6 (6.59)	3 (8.57)		6 (6.90)		0(0.00)		6 (7.14)		5 (8.06)	
Basic	48 (52.75)	20 (57.14)		48 (55.17)		4(66.67)		45 (53.57)		34 (54.84)	
Secondary	17 (18.68)	8 (22.86)	**0.115**	17 (19.54)	**0.002**	0(0.00)	**0.730**	14 (16.67)	**0.722**	12 (19.35)	**0.487**
Tertiary	20 (21.98)	4 (11.43)		16 (18.39)		2(33.33)		19 (22.62)		11 (17.74)	

One –way Anova was used to estimate the difference between proportions and the groups. Percentage value in parenthesis respondents.

P is significant at 0.05

4.5 Parity of the respondents against CMV, Rubella and HSV-2 prevalence

All 44 participants with 2 or more children (multi and grand parity category) had IgG antibodies to CMV, while 88.6% and 70.5% of them showed evidence of past infection to Rubella and HSV-2 respectively. Subjects of nulliparity (no children) and primiparity (only one child) equally showed high seropositivity to CMV, Rubella and HSV-2. Though, there was a significant difference between CMV seropositivity (P = 0.041) among the parity groups, it appears that difference proportions of seropositivity of CMV, Rubella and HSV-2 could be due to chance and not parity as an associated factor (Table 4).

Table 4: Parity of respondents (subjects) and the prevalence of CMV, Rubella and HSV-2 antibodies

Parity	*Total No. respondents*	*CMV (n = 91)*				*Rubella (n =89)*				*HSV-2 (n = 91)*	
		IgM (35)	*P –Value*	*IgG (87)*	*P-value*	*IgM (6)*	*P-value*	*IgG (84)*	*P - value*	*IgG (62)*	*P - value*
Nulliparity	30 (32.97)	10 (28.57)		27 (31.03)		2(33.33)		10 (33.33)		16 (25.81)	
Primiparity	17 (18.68)	7 (20.00)		16 (18.39)		1(16.67)		17 (20.24)		15 (24.19)	
Multiparity	41 (45.05)	16 (45.71)	**0.706**	41 (47.13)	**0.041**	3(50.00)	**0.957**	38 (45.24)	**0.545**	29 (46.77)	**0.193**
Grandparity	3 (3.30)	2 (5.71)		3 (3.45)		0 (0.00)		1 (1.19)		2 (3.23)	

One –way Anova was used to estimate the difference between proportions and the groups. Percentage value in parenthesis respondents.

P is significant at 0.05

4.6 Associated factors of respondents against CMV, Rubella and HSV-2

The trend that was observed among the associated factors and the relationship with the seropositivity levels of CMV, Rubella and HSV-2 among the respondents was almost the same for all, though none of these factors seemed to be significantly associated with the seropositivity of the respondents to any of the antibodies tested. Among the respondents that had history of miscarriage and or stillbirth, 15 (about 42.86%) out of 35 and 43 (49.43%) were positive to IgM and IgG antibodies of CMV respectively (Table 5), while 3 (50%) and 39 (46.43%) had IgM and IgG antibodies to rubella (Table 6) with 33 (53.23%) of them having IgG antibodies to HSV-2 (Table 7). However, there was no significant difference between seropositivity to these viruses among individuals with history of miscarriage and or stillbirth and those who do not.

Among those who have had interaction with children (exposure to children) by touching, feeding, changing of diapers and or bathing them, 25 (71.43%) and 55 (63.22%) had IgM and IgG antibodies of CMV respectively (Table 5) while 54 (65.06%) of them had IgG to rubella (Table 6). For respondents with history of blood transfusion, 11 (12.64%) and 8 (12.90%) respectively had antibodies to CMV and HSV-2 with respect to IgG (Table 5, 6 & 7).While 64 (73.56%) of the respondents who had been sexually involved with Multiple partner were seropositive to CMV (IgG), 47 (75.80%) were seropositive to HSV-2. 25 (28.78%) of respondents who use protection (condom) during sex had IgG antibodies to CMV while 17 (27.42%) had antibodies to HSV-2 (Table 6 & 7). However, only 19 (30.65%) of those who practise oral sex with their partners had antibodies to HSV-2(Table7).

Table 5: Associated factors (characteristics) of respondents stratified by CMV IgM and IgG reactivity profile

Parameter	Total	CMV IgM			CMV IgG		
		Negative (56)	Positive (35)	p-value	Negative (4)	Positive (87)	p-value
History of Miscarriage/Stillbirth							
No	47(51.65)	27(48.21)	20(57.14)	0.5183	3(75)	44(50.57)	0.6174
Yes	44(48.35)	29(51.79)	15(42.86)		1(25)	43(49.43)	
Exposure to Children							
No	33(36.26)	23(41.07)	10(28.57)	0.2635	2(50)	31(35)	0.6221
Yes	57(62.64)	32(57.14)	25(71.43)		2(50)	55(63.22)	
History of Blood Transfusion							
No	79(86.81)	50(89.29)	29(52.86)	0.5256	3(75)	76(87.36)	0.4378
Yes	12(13.19)	6(10.72)	6(17.14)		1(25)	11(12.64)	
Sex with Multiple Partner							
No	24(26.37)	16(28.57)	8(22.86)	0.6296	1(25)	23(26.44)	1
Yes	67(73.63)	40(71.43)	27(77.14)		3(75)	64(73.56)	
Sex with Protection							
No	64(70.33)	36(64.29)	28(80.00)	0.0112	2(50)	62(71.26)	0.5791
Yes	27(29.67)	20(35.71)	7(20)		2(50)	25(28.74)	
Oral Sex							
No	64(70.33)	40(71.43)	24(68.57)	0.8162	1(25)	63(72.41)	0.0766
Yes	27(29.67)	16(28.57)	11(31.43)		3(75)	24(27.59)	

Percentage values in parenthesis represent respondents. P is significant at 0.05.

Table 6: Associated factors (characteristics) of respondents stratified by Rubella IgM and IgG reactivity profile

Parameter	Total	Rubella virus IgM			Rubella virus IgG		
		Negative (85)	Positive (6)	p-value	Negative (5)	Positive (84)	p-value
History of Miscarriage/Stillbirth							
No	47 (51.65)	44(51.76)	3(50)	1	2(40)	45(53.57)	0.6636
Yes	44(48.35)	41(48.24)	3(50)		3(60)	39(46.43)	
Exposure to Children							
No	33(36.67)	32(37.67)	1(16.67)	0.4086	3(60)	29(34.94)	0.349
Yes	57(63.33)	52(61.18)	5(83.33)		2(40)	54(65.06)	

Percentage values in parenthesis represent respondents. P is significant at 0.05

Table 7: Associated factors (characteristics) of respondents stratified by HSV-2 IgG reactivity profile

Parameter	Total	Negative (29)	Positive (62)	p-value
History of Miscarriage/Stillbirth				
No	47(51.65)	18(62.07)	29(46.77)	0.1865
Yes	44(48.35)	11(37.93)	33(53.23)	
History of Blood Transfusion				
No	79(86.81)	25(86.21)	54(87.10)	1
Yes	13(13.19)	4(13.79)	8(12.90)	
Sex with Multiple Partner				
No	24(26.37)	9(31.03)	15(24.19)	0.6104
Yes	67(73.63)	20(68.97)	47(75.80)	
Sex with Protection				
No	64(70.33)	19(65.52)	45(72.58)	0.6229
Yes	27(29.67)	10(34.48)	17(27.42)	
Oral Sex				
No	64(70.33)	21(72.41)	43(69.35)	0.8108
Yes	27(29.67)	8(27.59)	19(30.65)	

Percentage values in parenthesis represent respondents. P is significant at 0.05

CHAPTER FIVE

DISCUSSION

5.1 General Prevalence of CMV, Rubella and HSV-2

The high rate of morbidity and mortality caused by CMV, Rubella and HSV-2 especially to foetus of infected mothers due to congenital and neonatal infections with their consequent wide range of abnormalities and social as well as financial burdens to families and countries has made the screening of pregnant women important research activity. This would help to determine susceptible pregnant women for appropriate interventions. This notwithstanding, few studies have been carried out concerning the prevalence and other related epidemiological study of these viruses in Ghana. So far, the few studies on the prevalence of these viruses include a CMV study carried out among the general Ghanaian population (Adjei *et al.,* 2008) and among voluntary blood donors (Adjei *et al.,* 2006) and another on Rubella in pregnant women over a decade ago (Lawn *et al.,* 2000). The present thus extend these studies as well as providing information on some associated factors; this could be helpful in health promotion activities.

In this current study, 95.60% seropositivity of CMV IgG antibodies was observed among pregnant women and this is in line with findings in Nigeria (97.2%) seropositivity (Akinbami *et al.,* 2011), and in Turkey; 94.0% (Ocak *et al.,* 2007). This indicates susceptibility level of 4.40% among the study population which also confirms other studies carried out in other sub-Saharan African counties (Cannon *et al.,* 2010). In other studies, higher CMV seropositivity levels have been ascribed to overcrowded living conditions, increased breast feeding, sharing of utensils, and decreased hygiene (Ho, 1990). However, in this study, even though higher CMV seropositivity was found among subjects with multiple sexual partners, practised sex without protection and

those exposure to children; changing diapers, bathing, feeding and touching of children especially those under 3 years of age, there were no association observed since equally high seropositivity was seen among subjects who said no to these factors.

The IgM of CMV Seroprevalence was 38.46% in this study and the fact that all subject with IgM to CMV also had IgG antibodies is a possible indication of recurrent infection (reactivation or reinfection) and not necessarily a primary infection since the IgM antibodies could be present during recurrent infections and not in primary infection alone (Lazzarotto *et al.*, 2008). Even though results from the IgG CMV seropositivity indicate a low susceptibility levels (4.40%), appropriate preventive measures should be taken to curb the occurrence of primary infection which has a high potential of causing congenital infection and devastating consequences. These preventive measures as advised by scientist and health promoters include simple good hygiene practises such as washing of hands regularly especially after handling children among many other measures as mentioned in the literature review (Buxmann *et al.*, 2012; Onorato *et al.*, 1985) as well as education.

Only (7.69%) of respondents were observed to be susceptible or at risk of primary rubella infection since majority of respondents (92.31%) showed evidence of past infection (had the IgG antibodies to the rubella virus) in this study. This is actually a confirmation of a similar study carried out over a decade ago at the Komfo Anokye Teaching Hospital, Kumasi, where about 92.6% of the pregnant women showed evidence of past rubella infection (Lawn *et al.*, 2000). This finding is not startling at all as higher Seroprevalence and their corresponding low susceptible levels have been reported in pregnant women in other Africa countries such as

Mozambique, Cameroon, Nigeria and Western Sudan with prevalence of 95%, 88.6%, 76% and 65.3% respectively (Hamdan *et al.,* 2011; Fokunang *et al.,* 2010; Barreto *et al.,* 2006; Onyenekwe *et al.,* 2000). Most of these African countries with very high Seroprevalence have not yet included rubella vaccination into their national immunisation programmes as practised by many of the developed countries. According to Cutts and Vynnycky, as of 1997, less than one-third of developing countries had introduced the rubella vaccine in their national immunization programme (Cutts & Vynnycky, 1999). Some argue that since the disease is a childhood disease and the prevalence is high, it is better to allow it spread especially among children so that by the child-bearing age, most of them would have developed antibody that is capable of fully protecting them as well as reducing the number of babies born with CRS. This is a good measure for resource poor countries.

However, 6.59% of the respondents had IgM antibodies to the rubella virus but this may be a reinfection and not primary infection as all respondents with IgM also had IgG antibodies. This is less likely to result in congenital infection leading to congenital rubella syndrome (CRS) though some few cases of reinfection have been linked to CRS (Robinson *et al.,* 1994).

The prevalence of HSV-2 found in this study is comparable to that reported for sub-Saharan Africa; 30% to 80% (Weiss, 2004). In this study, HSV-2 seroprevalence was found to be 68.13% and the susceptibility levels to be 31.87%. Seroprevalence of HSV-2 in this study could be attributed to multiple sexual partners and sexual activities without protection as practiced by majority of the respondents, however this is not conclusive enough since no further analysis was done due to the limitation by sample size.

5.2 Socio-demographic characteristics and prevalence of CMV, Rubella and HSV-2

Generally, increased prevalence of CMV, Rubella and HSV-2 was recorded for age of respondents (Table 2) and significant difference exists between the IgG CMV prevalence among the age groups. As observed from the result (Table 2), all respondents of ages of 40 and above had IgG antibodies to CMV and this was not so different for the IgG antibodies to rubella. This is actually expected because exposure to CMV and rubella starts during early childhood stage and this is especially true for those in developing countries as several conditions expose the populace to these infections at early stages in life.

Several studies have shown that CMV Seroprevalence approaches 100% as young as 11 years in some developing countries while above 90% seropositivity usually occurs at age 80 in the advanced countries (Staras *et al.*, 2006; Stackhouse *et al.*, 1991; Ho, 1990). The pattern seen in the HSV-2 seroprevalence in this study among the age groups differs from that seen in CMV and Rubella. There was a gradual increase at the ages 16 – 25 and peaks at ages 31–35 and then gradually declines (ages above 36). This probably may be due the pattern of sexual activity among the age groups. Active sexual involvement begins early and gradually increases and then declines as one ages as reflective in this study.

The trend as observed with respect to educational background only had a significant difference between IgG CMV seropositivity among the group. The few individuals who had no indication of CMV past infection were all within the tertiary category. This probably imply that the higher the educational level, the less likely an individual will acquire the CMV probably due to knowledge of the consequences of the infection and practise of preventive measures. However

this is not conclusive enough because of the fewer numbers of participants recorded within this group. Majority of the respondents equally showed an evidence of past infection to Rubella and HSV-2 among every class of educational level. Nevertheless, there was no serious association between educational level and HSV-2 and Rubella infectivity and therefore suggests that ones' educational background of did not significantly influence her seropositivity as far as this study is concerned. The high past infectivity of CMV and Rubella seen in all the groups may be attributed to equal exposure to these viruses through contact with people both young and old in our Ghanaian setting. The occupations of most of the subjects were primarily trading with a large number of them being market women. Their regular interaction and contacts with a lot of people as part of their business activities may probably be a contributing factor to why majority have already been exposed to these viruses. They may have come into contact with CMV through contact with contaminated body fluids such as saliva and rubella through contaminated aerosol. Again, HSV-2 was also high among all the educational groups and the possible reason to be ascribed to this trend is that equally higher number of subjects regardless of their educational background have had multiple sexual partners with only few using protection (condom) during sexual intercourse as seen in other studies (Jonsson & Wahren, 2004).

5.3 Associated factors and the prevalence of CMV, Rubella and HSV-2

The associated factors considered in this study includes parity, history of miscarriage/stillbirth, exposure to children, blood transfusion, number of sexual partners, the use of protection (condom) and practice of oral sex. The factors considered for a particular viral infection was based on epidemiological data or information obtained from literature. Some of these factors have already been considered in other studies where in some studies they were considered as risk

factors while others only considered them as merely associated factors. In this study, the significance of these factors in the acquisition or susceptibility to these infections was assessed. In other words, how significant are these viral infections associated with these factors.

From the results (Tables 5, 6 & 7), most of the factors were not significantly linked to susceptibility to the infections. A study by Hamdan *et al.* (2011), reported high parity as a risk factor for CMV, however in this study such conclusion could not be drawn. There were equally high seroprevalence among all the groups even though it was more in Multiparity group. Children are known to shed the CMV and rubella and this may explain why most infected individuals had high parity (multi parity and grand parity) as they may have had exposure to children of their own, neighbouring household or work place. Touching, bathing, changing of diapers and feeding (Stadler *et al.,* 2012) potentially expose people to CMV infection.

Majority of the respondents who had ever experienced either miscarriage or stillbirth or both had antibodies to the IgG of CMV, rubella and HSV-2. These viruses may have played a role in the cause of miscarriage and or stillbirth in these pregnant women because they are known to cause miscarriage and or stillbirth (Cengiz *et al.,* 1993). However, it cannot be said they were the cause of the miscarriage and stillbirth as seen among respondents.

Most of the individuals who with history of more than one sexual partner (multiple sexual partners) had higher seropositivity of CMV and HSV-2. This is only a confirmation of findings seen in other research where having multiple partners was significantly associated with the acquisition of CMV and HSV-2 (Dipankar *et al.,* 2011; Bucher *et al.,* 2004; Jonsson & Wahren, 2004; Robain *et al.* 1998; Collier *et al*. 1990) even though in this study it such conclusion could not be made.

Again, out the few participants who had received blood transfusion before, almost all were seropositive to CMV (IgG). The same have been reported in other studies where individuals with history of blood transfusion showed higher indication of past CMV infection. However it is worth noting that only about 3-5% CMV infected blood is usually transmitted to blood recipient. Statistically there was no significant difference between the proportions. Rubella and HSV-2 were not considered in this study to be associated with blood transfusion because they are no evident of their transmission through the blood even though one study revealed HSV-2 DNAemia but this only occurs in primary herpes infection and not recurrent herpes infection (Juhl *et al.,* 2010).

CHAPTER SIX

CONCLUSIONS, LIMITATIONS AND RECOMMENDATIONS

6.1 Conclusions

This study was important in generating current information on susceptibility levels of CMV, Rubella and HSV-2 in pregnant women as well as some factors influencing their susceptibility. The following conclusions can be drawn from the analyses and discussions of results obtained in this study;

- Though there are very high seroprevalence of CMV, Rubella and HSV-2 among pregnant women, there are significantly low levels of susceptibility to these infections among the study subjects (pregnant women). This implies that only few of these subjects are at risk of primary infection that may lead to possible abnormalities in newborns.
- Most of the considered associated factors were not significantly associated with the acquisition of these infections. In other words in study, it was observed that these associated factors may not have significantly influenced the infectivity among individual with evidence of past infection to these viruses.

6.2 Recommendation

The current study revealed a very low susceptibility levels among pregnant women and this is good news. However, this study should be replicated in other parts of Ghana so as to determine the overall susceptibility levels in the entire country. Again, a critical study in newborns would be necessary to estimate the numbers of newborns who suffer abnormalities as a result of maternal primary infection among the few susceptible individuals.

6.3 Limitation of the study

The only limitation to this study was the fact that a convenience sample size was used. This limitation was not only due to resource constraints but also limited period within which the study was carried out. This limitation should therefore be considered as such when interpreting these data.

6.4 REFERENCE

1. Abdel-Fattah, S. A., A. Bhat, S. Illanes, J. L. Bartha, and D. Carrington. (2005). TORCH test for fetal medicine indications: only CMV is necessary in the United Kingdom. Prenat. Diagn. 25:1028-1031
2. Ackerknecht, Erwin Heinz (1982). A short history of medicine. Baltimore: Johns Hopkins University Press. p. 129. ISBN 0-8018-2726-4.
3. Adjei AA, HB Armah, and EG Narter-Olaga. (2006). Seroprevalence of Cytomegalovirus among Some Voluntary Blood Donors at the 37 Military Hospital, Accra, Ghana. Ghana Med J.: 40(3): 99–104.
4. Adjei AA, Henry B Armah, Foster Gbagbo, Isaac Boamah, Clement Adu-Gyamfi and Isaac Asare. (2008). Seroprevalence of HHV-8, CMV, and EBV among the general population in Ghana, West Africa. BMC Infectious Diseases, 8:111. doi:10.1186/1471-2334-8-111
5. Adler, S. P. and G. Nigro (1999). "The importance of cytomegalovirus-specific antibodies for the prevention of fetal cytomegalovirus infection or disease." Herpes 15(2): 24-27
6. Agabi YA, Banwat EB, Mawak JD, Lar PM, Dashe N, Dashen MM, Adoga MP, Agabi FY, Zakari H (2010). Seroprevalence of herpes simplex virus type-2 among patients attending the Sexually Transmitted Infections Clinic in Jos, Nigeria. J Infect Dev Ctries.; 4(9):572-5.
7. Ahmed A. (2011). Antiviral treatment of cytomegalovirus infection. Infect Disord Drug Targets.: 11(5):475-503.

8. Akinbami, A. A., K. A. Rabiu, et al. (2011). "Seroprevalence of cytomegalovirus antibodies amongst normal pregnant women in Nigeria." Int J Womens Health 3: 423-428.
9. American Academy of Pediatrics. Red Book: (2003). 2003 Report of the Committee of Infectious Diseases. 26th. Edited by: Pickering LK. Elk Grove Village, IL; 2003:309-318.
10. Andrews WW, Kimberlin DF, Whitley R, Cliver S, Ramsey PS, Deeter R: Valacyclovir therapy to reduce recurrent genital herpes in pregnant women. Am J Obstet Gynecol 2006, 194:774-781.
11. Andria G.M. Langenberg, M.D., Lawrence Corey, M.D., Rhoda L. Ashley, Ph.D., Wai Ping Leong, M.S., and Stephen E. Straus, M.D. (1999). for the Chiron HSV Vaccine Study Group A Prospective Study of New Infections with Herpes Simplex Virus Type 1 and Type 2 N Engl J Med; 341:1432-1438 DOI: 10.1056/ NEJM 199911043411904
12. Anzivino E, Fioriti D, Mischitelli M, Bellizzi A, Barucca V, Chiarini F, et al. (2009). Herpes simplex virus in pregnancy and in neonate: status of art of epidemiology, diagnosis, therapy and prevention. Virol J:6:40
13. Arabpou M, Kariyanee K, Jankhah A, et al. (2007). Human cytomegalovirus in women of child bearing age throughout Fars province-Iran: A population based cohort study. Malaysian Journal of Microbiology: 3(2):23–28.
14. Arthurs, S., Eid, A., Pedersen, R., Kremers, W., Cosio, F., Patel, R. & Razonable, husbands and primary cytomegalovirus infection during pregnancy. J Infect Chemother; 6 (2):104-6.

15. Arvaja M, M. Lehtinen, P. Koskela, M. Lappalainen, J. Paavonen, and T. Vesikari, (1999). "Serological evaluation of herpes simplex virus type 1 and type 2 infections in pregnancy," Sexually Transmitted Infections, vol. 75, no. 3, pp. 168–171.
16. Arvin A M, Fast P, Myers M, Plotkin S, Rabinovich R. (2004). Vaccine development to prevent cytomegalovirus disease: report from the National Vaccine Advisory Committee. Clin Infect Dis; 39(2):233-9.
17. Arvin A, Whitley R, Gutierres K. (2006). Herpes simplex virus infections. In infectious diseases of the foetus and newborn infant. 6th edition eds. Remmington J. O. Klein C.B. Wilson & C. J. Baker. Philadelphia. W.B. Saunders Company; 845-865
18. Atreya CD, Mohan KV, Kulkarni S (2004). ''Rubella virus and birth defects:molecular insight into the viral teratogenesis at the cellular level.'' Birth Defects Res. Part a Clin. Mol. Terato. 70 (7)
19. Ashraf SJ, Parande CM, Arya SC. (1985). Cytomegalovirus antibodies of patients in the Gizan area of Saudi Arabia. J of Inf Dis; 152(6):1351.
20. Aurelius E, Johansson B, Skoldenberg B, Staland A, Forsgren M. (1991). Rapid diagnosis of herpes simplex encephalitis by nested polymerase chain reaction assay of cerebrospinal fluid. Lacent; 337189-92
21. Baldanti, F., Sarasini, A., Furione, M., Gatti, M., Comolli, G., Revello, M. G. & Gerna, G. (1998a). Coinfection of the immunocompromised but not the immunocompetent host by multiple human cytomegalovirus strains. *Archives of Virology* 143, 1701-1709.

22. Banatvala JE, Best JM. (1990). Rubella. In: Collier L, Timbury M, editors. Topley and Wilson's principles of bacteriology, virology and immunity. London: Edward Arnold; p. 501–31.

23. Barreto J, Sacramento I, Robertson SE et al., (2006). Antenatal rubella serosurvey in Maputo, Mozambique. Trop Med Int Health; 11:559-64.

24. Berardi A, L. Lugli, C. Rossi et al., "Neonatal herpes simplex virus," *Journal of Maternal-Fetal and Neonatal Medicine*, vol. 24, supplement 1, pp. 88–90, 2011.

25. Best, J.M., Cooray, S., Banatvala J.E. (2005). Rubella in Topley and Wilson's Microbiology and Microbial Infections, Vol. 2, Virology, Chapter 45, p.960-92, ISBN 0-340-88562-9,

26. Betts RR. (1983). Cytomegalovirus infection epidemiology and biology in adults. Semin Perinatol 7: 22, 1983

27. Beyrer C, Kriangsak Jitwatcharanan, Chawalit Natpratan, Rassamee Kaewvichit, Kenrad E Nelson, Cheng-Yen Chen, Judith B Weiss, Morse Stephen. (1999). Molecular methods for the diagnosis of genital ulcer disease in a sexually transmitted disease clinic population in northern Thailand: predominance of herpes simplex virus infection. J Infect Dis, 178:243-246.

28. Boeckh M, Huang M, Ferrenberg J, Stevens-Ayers T, Stensland L, Nichols WG, et al. (2004). Optimization of quantitative detection of cytomegalovirus DNA in plasma by real-time PCR. J Clin Microbiol; 42:1142–8.

29. Boppana SB, Pass RF, Britt WJ, Stagno S, Alford CA. (1992). Symptomatic congenital cytomegalovirus infection: neonatal morbidity and mortality. Pediatr Infect Dis J;11:93–9.

30. Boppana, S. B., Fowler, K. B., Britt, W. J., Stagno, S. & Pass, F. P. (1999). Symptomatic congenital cytomegalovirus infection in infants born to mothers with preexisting immunity to cytomegalovirus. *Pediatrics* 104, 55-64.

31. Boppana SB, Rivera LB, Fowler KB et al. (2001). Intrauterine transmission of cytomegalovirus to infants of women with preconceptual immunity. N Engl J Med; 344: 1366–71.

32. Bowden RA. (1991). Cytomegalovirus infection in transplant patients: methods of prevention of primary cytomegalovirus. Transplant Proc; 23: 136–8.

33. Brooks GF, Butel JS. and Morse S. A. (2010). Jawerts, Melnick & Alberg's Medical Microbiology 25th ed. International Edition, McGraw-Hill Companies, Inc.; Chapter 40.

34. Brown HL, Abernathy MP. (1998). Cytomegalovirus infection. Semin Perinatol; 22: 260-266.

35. Brown EL, Garella C, Malm G, Prober CG, Forsgren M, Krantz Em, et al. (2007). Effect of maternal herpes simplex virus (HSV) serostatus and HSV type on risk of neonatal herpes. Acta Obstet Gynecol Scand; 86:523-9

36. Brown ZA, Selke S, Zeh J, Kopelman J, Maslow A, Ashley RL, Watts H, Berry A, Herd M, Corey L. (1997). The Aquisition Of Herpes Simplex Virus During Pregnancy. The New England Journal of Medicine, 337:509-515.

37. Brugha R, Keersmaekers K, Renton A, Meheus A. (1997). Genital herpes infection: a review. International Journal of Epidemiology, 26:698-709.

38. Bullens D, Smets K, Vanhaesebrouck P. (2000). Congenital rubella syndrome after maternal reinfection. Clin Pediatr (Phila); 39:113–6.

39. Buxmann H, Stackelberg OM, Schlößer RL, Enders G, Gonser M, Meyer-Wittkopf M, Hamprecht K, Enders M. (2012). Use of cytomegalovirus hyperimmunoglobulin for prevention of congenital cytomegalovirus disease: a retrospective analysis. J Perinat Med.; (4):439-46. doi: 10.1515/jpm-2011-0257.

40. Cannon MJ, Schmid DS, Hyde TB (2010). Review of cytomegalovirus seroprevalence and demographic characteristics associated with infection. Rev Med Virol. ;20(4):202-13. doi: 10.1002/rmv.655.

41. Caviness AC, Gail J. Demmler, and Beatrice J. Selwyn. (2008). Clinical and Laboratory Features of Neonatal Herpes Simplex Virus Infection: A Case-Control Study. Pediatr Infect Dis J: 27: 425–430

42. Center for Disease Control (2010). Seroprevalence of Herpes Simplex Virus Type 2 among Persons Aged 14-49 Years - United States, 2005—2008. Morbidity and mortality weekly report. April 23, 2010 / 59(15); 456-459 (Accessed July, 2013) **http://www.cdc.gov/mmwr/preview/mmwrhtml/mm5915a3.htm**

43. Cengiz L, Kiyan M, Cengiz AT, Kara F, Uğurel MS. (1993). Detection of herpes simplex virus 1 and 2 (HSV-1 and HSV-2) IgG and IgM by ELISA in cord blood and sera of mothers with pregnancy complications. Mikrobiyol Bul. ;27(4):299-307.

44. Chandler SH, Alexander ER, Holmes KK. (1985). Epidemiology of cytomegaloviral infection in a heterogeneous population of pregnant women. J of Infect Dis; 152 (2):249-56.

45. Chen Cheng Y, Ballard Ronald C, Beck-Sague Consuelo M, Yusuf Dangor, Frans Radebe, Scott Schimd, Weiss Judith B, Vanessa Tshabalala, Glenda Fehler, Ye Htun, Morse Stephen A. (2000). Human immunodeficiency virus infection and genital ulcer disease in South Africa: the herpetic connection. Sexually Transmmitted Diseases, 27:21-29.

46. Cherpes T. L, L. A. Meyn, M. A. Krohn, J. G. Lurie, and S. L. Hillier. (2003). "Association between acquisition of herpes simplex virus type 2 in women and bacterial vaginosis," Clinical Infectious *Diseases,* vol. 37, no. 3, pp. 319–325.

47. Chin J, (2000). Control of Communicable Diseases Manual. 17th edn. Washington, DC: American Public Health Association, 2000.

48. Ciavattini A, M. Vichi, A. Rinci, and D. Tsiroglou, (2007). "Infezioni virali in gravidanza: gestione e raccomandazioni," La Colposcopia in Italia, vol. 2, pp. 11–16,

49. Collier AC, Handsfield HH, Roberts PL, et al. (1990). Cytomegalovirus infection in women attending a sexually transmitted disease clinic. J of Inf Dis;162 (1):46-51.

50. Compton, T. (1995). Towards a definition of the HCMV entry pathway. Scandinavian Journal of Infectious Diseases. Supplementum; 99:30-32.

51. Coulter C, Wood R, Robson J. (1999). Rubella infection in pregnancy. Commun Dis Intell; 23:936.

52. Cusi MG, Valensin PE, Cellesi C. (1993). Possibility of reinfection after immunisation with RA27/3 live attenuated rubella virus. Arch Virol. ;129(1-4):337-40.

53. Cusini M, Ghislanzoni M. (2001). The importance of diagnosing genital herpes. J Antimicrob Chemother, 47:9-16.

54. Cutts FT, Vynnycky E. (1999). Modelling the incidence of congenital rubella syndrome in developing countries. Int J Epidemiol; 28(6):1176-84.

55. D'Alton ME, DeCherney AH. (1993). Prenatal diagnosis. N Engl J Med; 328:114–20.

56. Davison AJ, Bhella D. (2007). Comparative genome and virion structure, Human Herpesviruses: Biology, Therapy, and Immunoprophylaxis. Cambridge: Cambridge University Press; 2007. Chapter 14

57. De Jong, M., Galasso, G., Gazzard, B., Griffiths, P. D., Jabs, D. A., Kern, E. R. & Spector, S. A. (1998). Summary of the II International Symposium on Cytomegalovirus. *Antiviral Research* 39, 141-162.

58. Demmler GJ. (1991). Infectious Diseases Society of America and Centers for Disease Control. Summary of a workshop on surveillance for congenital cytomegalovirus disease. Rev Infect Dis. ;(2):315-29.

59. Desselberger U. (1998). "Herpes simplex virus infection in pregnancy: diagnosis and significance," *Intervirology*, vol. 41, no. 4-5, pp. 185–190, 1998.

60. Deyi YM, Goubau P, Bodeus M. (2000). False-positive. IgM antibody tests for cytomegalovirus in patients with acute Epstein-Barr virus infection. Eur J Clin Microbiol Infect Dis; 19:557–60.

61. Diawara S, Toure Kane C, Legoff J, Gaye AG, Mboup S, Bélec L. (2008). Low seroprevalence of herpes simplex virus type 2 among pregnant women in Senegal. Int J STD AIDS. ;19 (3):159-60. doi: 10.1258/ijsa.2007.007139.

62. Dipankar Biswas, Biswajyoti Borkakoty, Jagadish Mahanta, Kamini Walia, Lahari Saikia, Brogen S Akoijam, Lobsang Jampa, Alia Kharkongar, and Eric Zomawia (2011). Seroprevalence and risk factors of herpes simplex virus type-2 infection among pregnant women in Northeast India. BMC Infect Dis. 2011; 11: 325. doi: 10.1186/1471-2334-11-325

63. Edlich RF, Winters KL, Long WB 3rd, Gubler KD (2005). Rubella and congenital rubella (German measles). J Long Term Eff Med Implants. 2005; 15(3):319-28.

64. Emery, V. C. (2001b). Investigation of CMV disease in immunocompromised patients. Journal of Clinical Pathology 54, 84-88.

65. Enders G, Bader U, Lindemann L, Schalasta G, Daiminger A. (2001). Prenatal diagnosis of congenital cytomegalovirus infection in 189 pregnancies with known outcome. Prenat Diagn; 21:362–77.

66. Eskild A, Stig Jeansson, Jenum Pål A. (1999). Antibodies against herpes simples virus type 2 among pregnant women in Norway. Tidsskr Nor Laegeforen, 119:2323-2326.

67. Eskild A, Stig Jeansson, Stray-Pederson Babill, Jenum Pål A. (2002). Herpes simplex virus type 2 infection in pregnancy: no risk of fetal death: results from a nested case-control study within 35,950 women. BJOG, 109:1030-1035.

68. Fabbri E, Revello MG, Furione M, Zavattoni M, Lilleri D, Tassis B, Quarenghi A, Rustico M, Nicolini U, Ferrazzi E, Gerna G. (2011). Prognostic markers of symptomatic congenital human cytomegalovirus infection in fetal blood. BJOG.118(4):448-56. doi: 10.1111/j.1471-0528.2010.02822.x. Epub 2010 Dec 24

69. Fleming DT, McQuillan GM, Johnson RE, Nahmias AJ, Aral SO, Lee FK, St Louis ME. (1997). Herpes simplex virus type 2 in the United States, 1976 to 1994. *N Engl J Med,* 337:1105-1111

70. Fernando AB Colugnati, Stephanie AS Staras, Sheila C Dollard2 and Michael J Cannon. (2007). Incidence of cytomegalovirus infection among the general population and pregnant women in the United States. BMC Infectious Diseases, 7:71 doi:10.1186/1471-2334-7-71

71. Fowler KB, McCollister FP, Dahle AJ, Boppana S, Britt WJ, Pass RF. (1997). Progressive and fluctuating sensorineural hearing loss in children with asymptomatic congenital cytomegalovirus infection. J Pediatr; 130(4):624-30.

72. Fowler KB, Pass RF. (1991). Sexually transmitted diseases in mothers of neonates with congenital cytomegalovirus infection. J of Inf Dis; 164 (2):259-64.

73. Fowler KB, Stagno S, Pass RF, Britt WJ, Boll TJ, Alford CA. (1992). The outcome of congenital cytomegalovirus infection in relation to maternal antibody status. N Engl J Med; 326(10):663-7.

74. Frey TK, Abernathy ES. (1993). Identification of strain-specific neucleotide sequences in the RA 27/3 rubella vaccine. J Infect Dis; 168:854–64.

75. Gabbe SG, Niebyl JR, Simpson JL, eds. (2002). Obstetrics-normal and problem pregnancies. 4th ed. New York: Churchill Livingstone, Inc: 1328–30.

76. Garland, S. M., and G. L. Gilbert. (1993). Investigation of congenital infection—the TORCH screen is not a legitimate test. Paediatric Infectious Diseases Group of the Australasian Society for Infectious Diseases. Med. J. Aust. 159:346-348.

77. Giessen, M. van der, A.P. van den Berg, W. van der Bij, S. Postma, W.J. van Son, T.H. (1990). The. Quantitative measurement of cytomegalovirus-specific IgG and IgM antibodies in relation to cytomegalovirus antigenaemia and disease activity in kidney recipients with an active cytomegalovirus infection. Clinical and Experimental Immunology; 80:56-61

78. Gindes, L., Teperberg-Oikawa, M., Sherman, D., Pardo, J. & Rahav, G. (2008). Congenital cytomegalovirus infection following primary maternal infection in the third trimester. British Journal of Obstetrics and Gynaecology 115, 830-835.

79. Gomwalk NE, Ahmad AA. (1989). Prevalence of rubella antibodies on the African continent. Rev Infect Dis.; 11(1):116-21. (http://www.ncbi.nlm.nih.gov/pubmed/2783785)

80. Goodpasture E, Talbot F (1921). Concerning the nature of "protozoan-like" cells in certain lesions of infancy. Am J Dis Child 21:415–421

81. Gottlieb S. L, J. M. Douglas Jr., D. S. Schmid et al., (2002). "Seroprevalence and correlates of herpes simplex virus type 2 infection in five sexually transmitted-disease clinics," Journal of Infectious Diseases, vol. 186, no. 10, pp. 1381–1389.

82. Gouarin S, Vabret A, Gault E, Petitjean J, Regeasse A, Hurault de Ligny B, et al. (2004). Quantitative analysis of HCMV DNA load in whole blood of renal transplant patients using real-time PCR assay. J Clin Virol; 29:194–201.

83. Green Manfred S, Cohen Dani, Slepon Raphael, Robin Guy and Wiener Michael. (1993). Ethnic and Gender Differences in the Prevalence of Anti-Cytomegalovirus Antibodies among Young Adults in Israel. International Journal of Epidemiology Volume 22, Issue 4 Pp. 720-723

84. Grefte, A., M.C. Harmsen, M. van der Giessen, S. Knollema, W.J. van Son, (1994). T.H. The. Presence of human cytomegalovirus (HCMV) immediate early mRNA but not ppUL83 (lower matrix protein pp65) mRNA in polymorphonuclear and mononuclear leukocytes during active HCMV infection. The Journal of General Virology; 75:1989-1998.

85. Grundy, J.E., J.A. McKeating, P.J. Ward, A.R. Sanderson, P.D. Griffith. (1987). Beta 2-Microglobulin enhances the infectivity of cytomegalovirus and when bound to virus enables class I HLA molecules to be used as a virus receptor. The Journal of General Virology; 68:793-803.

86. Guerra B, Simonazzi G, Puccetti C, Lanari M, Farina A, Lazzarotto T, Rizzo N. (2008). Ultrasound prediction of symptomatic congenital cytomegalovirus infection. Am J Obstet Gynecol. 198(4):380.e1-7. doi: 10.1016/j.ajog.2007.09.052. Epub 2008 Jan 14.

87. Gupta R, T. Warren, and A. Wald. (2007). "Genital herpes," The Lancet, vol. 370, no. 9605, pp. 2127–2137.

88. Hamdan Z Hamdan, Ismail E Abdelbagi, Nasser M Nasser and Ishag Adam. (2011). Seroprevalence of cytomegalovirus and rubella among pregnant women in western Sudan Virology Journal, 8:217

89. Harter, G. and D. Michel (2012). "Antiviral treatment of cytomegalovirus infection: an update." Expert Opin Pharmacother 13(5): 623-627.

90. Hecker M, Qiu D, Marquardt K, Bein G, Hackstein H. (2004). Continuous cytomegalovirus seroconversion in a large group of healthy blood donors. Vox Sang;86 (1):41-4.

91. Hayes K, Danks DM, Gibas H et al. (1972). Cytomegalovirus in human milk. N Engl J Med; 287: 177–8.

92. Hess, Alfred Fabian (1914). "German measles (rubella): an experimental study". The Archives of Internal Medicine (Chicago) 13: 913–916. as cited by Enersen, Ole Daniel. "Alfred Fabian Hess". Who Named It. Retrieved 2007-07-03.

93. Hicks T, Fowler K, Richardson M, et al. (1993). Congenital cytomegalovirus infection and neonatal auditory screening. J Pediatr; 123: 779-782.

94. Ho M (2008). The history of cytomegalovirus and its diseases. Med Microbiol Immunol 197:65– 73

95. Ho M. (1990). Epidemiology of cytomegalovirus infections. Rev Infect Dis; 12 Suppl 7:S701-10.

96. Hodinka RL. Human cytomegalovirus. In: Murphy PR, Baron EJ, Pfaller MA, Tenover FC, Yolken RH (1999). Manual of Clinical microbiology. p. 889–991.

97. Hummel, M. & Abecassis, M. A. (2002). A model for reactivation of CMV from latency. *Journal of Clinical Virology* 25, S123-136.

98. Jamison DT, Feachem RG, Makgoba MW, et al., (2006). Disease and Mortality in sub-Saharan Africa. 2nd edition. Washington (DC): World Bank

99. Jesionek A, Kiolemenoglou R (1904) Uber einen Befund von protoenartigan.Muench Med Wochenschr 51:1905–1907

100. Jones CA (2003). Congenital cytomegalovirus infection. Curr Probl Pediatr Adolesc Health care; 23:70-93

101. Jonsson MK, Wahren B. (2004). Sexually transmitted herpes simplex viruses. Scand J Infect Dis. ; 36(2):93-101.

102. Juhl D, Mosel C, Nawroth F, Funke AM, Dadgar SM, Hagenström H, Kirchner H, Hennig H. (2010). Detection of herpes simplex virus DNA in plasma of patients with primary but not with recurrent infection: implications for transfusion medicine? Transfus Med. ;20(1):38-47. doi: 10.1111/j.1365-3148.2009.00951.x. Epub 2009 Aug 25.

103. Joseph SA, Béliveau C, Muecke CJ et al. (2006). Cytomegalovirusas an occupational risk in daycare educators. Paediatr Child Health; 11:401-7.

104. Kas-Deelen, A.M., T.H. The, N. Blom, B.W. van der Strate, E.F. de Maar, J. Smit, W.J. van Son, M.C. Harmsen. (2001). Uptake of pp65 in in vitro generated pp65- positive polymorphonuclear cells mediated by phagocytosis and cell fusion? Intervirology: 44:8-13.

105. Kaur, R., N. Gupta, D. Nair, M. Kakkar, and M. D. Mathur. (1999). Screening for TORCH infections in pregnant women: a report from Delhi. Southeast Asian J. Trop. Med Public Health 30:284-286.

106. Kenneson A, Cannon MJ (2007). Review and Meta-analysis of the epidemiology of congenital cytomegalovirus (CMV) infection. Rev Med Virol 17 (4): 253-76

107. Khan, N. A., and S. N. Kazzi. (2000). Yield and costs of screening growth-retarded infants for torch infections. Am. J. Perinatol. 17:131-135.

108. Kimberlin DW, Lin CY, Jacobs RF, et al. (2001). Natural history of neonatal herpes simplex virus infections in the acyclovir era. *Pediatrics*; 108:223–229.

109. Kimberlin DW. (2007). Herpes simplex virus infections of the newborn. Semin Perinatol; 31:19-25.

110. Lagasse N, Dhooge I, Govaert P. (2000). Congenital CMV-infection and hearing loss. Acta Otorhinolaryngol Belg ; 54: 431-436

111. Lawn JE, Reef S, Baffoe-Bonnie B, Adadevoh S, Caul EO, Griffin GE. (2000). Unseen blindness, unheard deafness, and unrecorded death and disability: congenital rubella in Kumasi, Ghana. Am J Public Health. (10):1555-61.

112. Lazzarotto T, Guerra B, Lanari M, Gabrielli L, Landini MP. (2008). New advances in the diagnosis of congenital cytomegalovirus infection. J Clin Virol.41(3):192-7.

113. Lee Jia-Yee and D. Scott Bowden. Rubella Virus Replication and Links to Teratogenicity. (2000) *Clin. Microbiol. Rev.* vol. 13 no. 4 571-58. doi: 10.1128/CMR.13.4.571-587.2000

114. Leland Diane S. and Christine C. Ginocchio (2007). Role of Cell Culture for Virus Detection in the Age of Technology. Clin Microbiol Rev. 2007 January; 20(1): 49–78. doi: 10.1128/CMR.00002-06

115. Leventon-Kriss S, Rannon L, Smetana Z, Yoffe R. (1983). Increase in laboratory – confirmed cases of herpes genitalis and neonatal herpes infection in Israel. Isr J Med Sci; 19:946-9

116. Li, F., Kenyon, K. W., Kirby, K. A., Fishbein, D. P., Boeckh, M. & Limaye, A. P. (2007). Incidence and clinical features of ganciclovir-resistant cytomegalovirus disease in heart transplant recipients. *Clinical Infectious Diseases* 45, 439-447.

117. Mace M, Cointe D, Six C, et al. (2004). Diagnostic value of reverse transcription-PCR of amniotic fluid for prenatal diagnosis of congenital rubella infection in pregnant women with confirmed primary rubella infection. J Clin Microbiol; 42(10):4818–20.

118. Major CA, Towers CV, Lewis DF, Garite TJ. (2003). Expectant management of preterm premature rupture of membranes complicated by active recurrent genital herpes. Am J Obstet Gynecol, 188:1551-1555.

119. Malm G, Forsgren M. (1999). Neonatal herpes simplex virus infections: HSV DNA in cerebrospinal fluid and serum. Arch Dis Child Fetal Neonatal Ed; 81:24-9.

120. Mbizvo M, Msuya EM, Stray-Pedersen B, Chirenje M, Munjoma M, Hussain A. (2002). Association of herpes simplex virus type 2 with the human immunodeficiency virus among urban women in Zimbabwe. International Journal of STD & AIDS, 13:343-348.

121. Mendelson E, Yair Aboudy, Zahava Smetana , Michal Tepperberg , Zahava Grossman. (2006). Laboratory assessment and diagnosis of congenital viral infections: Rubella, cytomegalovirus (CMV), varicella-zoster virus (VZV), herpes simplex virus (HSV), parvovirus B19 and human immunodeficiency virus (HIV) Reproductive Toxicology 21; 350–382

122. Mettenleiter TC, Klupp BG, Granzow H (2006). "Herpesvirus assembly: a tale of two membranes". *Curr. Opin. Microbiol.* 9 (4): 423–9.

123. Mocarski, E. Cytomegaloviruses and their replication. In: B.N. Fields, D.M. Knipe, R.M. Chanock, et al. (1996). (eds.). Fields virology. 3 rd ed. Vol.2 New York: Raven Press; 3:2447-2492.

124. Munjoma Marshall W, Edith N Kurewa, Munyaradzi P Mapingure, Grace V Mashavave, Mike Z Chirenje, Simbarashe Rusakaniko, Akhtar Hussain, Babill Stray-Pedersen. (2010). The prevalence, incidence and risk factors of herpes simplex virus type 2 infection among

pregnant Zimbabwean women followed up nine months after childbirth BMC Women's Health, 10:2

125. Munro, S. C., Hall, B., Whybin, L. R., Leader, L., Robertston, P., Maine, G. T. & Rawlinson, W. D. (2005). Diagnosis of and screening for cytomegalovirus infection in pregnant women Journal of Clinical Microbiology 43, 4713-4718.
126. Nelson CT, Demmler GJ. (1997). Cytomegalovirus infection in the pregnant mother, fetus, and newborn infant. Clin Perinatol; 24: 151-160.
127. Nigro G, Adler SP, La Torre R, Best AM. Passive immunization during pregnancy for congenital cytomegalovirus infection. (2005). N Engl J Med; 353: 1350–1362.
128. Nilson A, Myrmel H. (2000). Changing trends in genital herpes simplex virus infection. Obstet Gynecol; 79:693–6.
129. Ocak S, Zeteroglu S, Ozer C, Dolapcıoglu K, Gungoren A, (2007). Seroprevalence of *Toxoplasma gondii*, rubella and cytomegalovirus among pregnant women in southern Turkey, Scan J of Infec Diseases, 2007; 39: 231-4.
130. Ojide C. K, E. A. Ophori, N. O. Eghafona and C. Omoti (2012). Seroprevalence of Cytomegalovirus (CMV) Amongst Voluntary Blood Donors in University of Benin Teaching Hospital (UBTH), Edo State, Nigeria. British Journal of Medicine & Medical Research 2(1): 15-20,
131. Onorato IM, Morens DM, Martone WJ, Stansfield SK. (1985). Epidemiology of cytomegaloviral infections: recommendations for prevention and control. Rev Infect Dis. ; 7(4):479-97. (http://www.ncbi.nlm.nih.gov/pubmed/2994198)

132. Onyenekwe CC, Kehinde-Agbeyangi TA, Ofor US, Arinola OG. (2000). Prevalence of rubella-IgG antibody in women of childbearing age in Lagos, Nigeria. West African Journal of Medicine [19(1):23-26]

133. Pass RF. (2002). Cytomegalovirus infection. Pediatr Rev: 23:163-169.

134. Pass RF. (2005). Congenital cytomegalovirus infection and hearing loss. Herpes ;12 (2):50-5.

135. Pass, R. F., Fowler, K. B., Boppana, S. B., Britt, W. J. & Stagno, S. (2006). Congenital cytomegalovirus infection following first trimester maternal infection: symptoms at birth and outcome. Journal of Clinical Virology 25, 216-220.

136. Patel R, Barton SE, Brown D, Cowan FM, Kinghorn GR, Munday PE, Scoular A, Timmins D, Whittaker M, Woolley P. (2001). Herpes Simplex Virus Special Interest Group of the Medical Society for the Study of Venereal Diseases, United Kingdom, European Branch of the International Union against Sexually Transmitted Infection and the European Office of the World Health Organization: European guideline for the management of genital herpes. Int J STD AIDS, 12:34-39.

137. Paz-Bailey G, M. Ramaswamy, S. J.Hawkes, and A. M. Ger . (2007). "Herpes simplex virus type 2: epidemiology and management options in developing countries," Sexually Transmitted Infections, vol. 83, no. 1, pp. 16–22.

138. Peckham C, Tookey P, Logan S, Giaquinto C. (2001). Screening option for prevention of congenital cytomegalovirus infection. J Med Screen; 8:119-24

139. Peltola H, Jokinen S, Paunio M, Hovi T, Davidkin I. (2008). Measles, mumps, and rubella in Finland: 25 years of a nationwide elimination programme. Lancet Infect Dis.;8(12):796-803.

140. Piiparinen H, Hockerstedt K, Gronhagen-Riska C, Lautenschlager I. (2004). Comparison of two quantitative CMV PCR tests, Cobas Amplicor CMV Monitor and TaqMan assay, and pp65-antigenemia assay in the determination of viral loads from peripheral blood of organ transplant patients. J Clin Virol;30:258–66.

141. Raynor BD. (1993). Cytomegalovirus infection in pregnancy. Semin Perinatol; 17:394–402.

142. Riley H Jr (1997) History of the cytomegalovirus. South Med J 90:184–190

143. Roback JD (2002). CMV and blood transfusions. Rev Med Virol.; 12(4):211-9.

144. Robain M, Carre N, Dussaix E, Salmon-Ceron D, Meyer L. (1998). Incidence and sexual risk factors of cytomegalovirus seroconversion in HIV-infected subjects. The SEROCO Study Group. Sexually transmitted diseases; 25(9):476-80.

145. Robinson J, Lemay M, Vaudry WL. (1994). Congenital rubella after anticipated immunity: two cases and a review of the literature. Pediatr Infect Dis; 13:812–5.

146. Ross, S. A. & Boppana, S. B. (2005). Congenital cytomegalovirus infection: outcomes and diagnosis. Seminars in Pediatric Infectious Disease 16, 44-49.

147. Rowe W, Hartley J, Waterman S, Al E (1956) Cytopathogenic agent resembling human salivary gland virus recovered from tissue cultures of human adenoids. Proc Soc Exp Biol Med 92:418–424

148. Ryan KJ, Ray CG (editors) (2004). *Sherris Medical Microbiology* (4th ed.). McGraw Hill. pp. 555–62.

149. Sauerbrei A, Wutzler P. (2007). Herpes simplex and varicella-zoster virus infections during pregnancy: current concepts of prevention, diagnosis and therapy. Part 1: herpes simplex virus infections. *Med Microbiol Immunol*, 196:89-94.

150. Sheevani, N Jindal, A Aggarwal. (2005). A pilot seroepidemiological study of cytomegalovirus infection in women of child bearing age. Indian Journal of Medical Microbiology, 23 (1):34-36

151. Sheffield JS, Hill JB, Hollier LM, Laibl VR, Roberts SW, Sanchez PJ, Wendel GD Jr. (2006). Valacyclovir prophylaxis to prevent recurrent herpes at delivery: a randomized clinical trial. *Obstet Gynecol*, 108:141-147.

152. Slobedman, B. & Mocarski, E. S. (1999). Quantitative analysis of latent human cytomegalovirus. *Journal of Virology* 73, 4806-4812.

153. Smith MG (1956). Propagation in tissue cultures of a cytopathogenic virus from human salivary gland virus (SGV) disease. Proc SocExp Biol Med, 92:424-430.

154. Smith Jennifer S. and N. Jamie Robinson (2002). Age-Specific Prevalence of Infection with Herpes Simplex Virus Types 2 and 1: A Global Review. *J Infect Dis;* 186 (Supplement 1): S3-S28. doi: 10.1086/343739

155. Sohn YM, Oh MK, Balcarek KB, Cloud GA, Pass RF. (1991). Cytomegalovirus infection in sexually active adolescents. J of Inf Dis;163 (3):460-3.

156. Sia IG, Patel R. (2000). New strategies for prevention and therapy of CMV infection and disease in solid-organ transplant recipients. Clin Microbiol Rev: 13:83-121.

157. Stackhouse FA, Kumar A, Maier GA, Dyke JW (1991). Cytomegalovirus infection and excretion in residents of a nursing home. J Med Virol. ; 34(4):209-11. (http://www.ncbi.nlm.nih.gov/pubmed/1658211)

158. Stadler Laura Patricia, David I. Bernstein, S. Todd Callahan, Christine B. Turley, Flor M. Munoz, Jennifer Ferreira, Mekhala Acharya, Gina A. Gorgone Simone, Shital M. Patel,Kathryn M. Edwards, and Susan L. Rosenthal. (2012). Seroprevalence and Risk

Factors for Cytomegalovirus Infections in Adolescent Females Journal of the Pediatric Infectious Diseases Society pp. 1–8, DOI:10.1093/JPIDS/PIS076

159. Stagno S, Pass RF, Dworsky ME et al. (1982). Maternal cytomegalovirus infection and perinatal transmission. Clin Obstet Gynecol; 25: 563–76.
160. Stagno S. (1999). Cytomegalovirus. In: Behrman RE, Kliegman RM, Jenson HB, eds. Nelson Textbook of Pediatrics. Philadelphia: W.B. Saunders Co., p. 981-983.
161. Staras SA, Dollard SC, Radford KW, Flanders WD, Pass RF, Cannon MJ. (2006). Seroprevalence of cytomegalovirus infection in the United States, 1988- 1994. Clin Infect Dis; 43(9):1143-51.
162. Straface G, Alessia Selmin, Vincenzo Zanardo, Marco De Santis, Alfredo Ercoli, and Giovanni Scambia. (2012). Herpes Simplex Virus Infection in Pregnancy Infectious Diseases in Obstetrics and Gynecology Volume, Article ID 385697, 6 pages doi:10.1155/2012/385697
163. Soderberg, C., T.D. Giugni, J.A. Zaia, S. Larsson, J.M. Wahlberg, E. Moller. (1994). CD13 (human aminopeptidase) mediates human cytomegalovirus infection. Journal of Virology; 67: 6576-6585.
164. Stinski MF. (1990). Cytomegalovirus and its replication. In: Fields BN, Knipe DM, eds. Fields Virology. New York : Raven Press;1959-80.
165. Streblow, D. N. & Nelson, J. A. (2003). Models of HCMV latency and reactivation. *Trends in Microbiology* 11, 293-295.
166. Studhal, M., Ricksten, A., Sandberg, T. & Bergstrom, T. (1992). Cytomegalovirus encephalitis in four immunocompetent patients. *Lancet* 340, 1045-1046.

167. Suárez M, Briones H, Luchsinger V, Schultz R, Peña M, de Diego S, Grunberg A. (1994). Primary cytomegalovirus infection in pregnant women of different socioeconomic status. Rev Med Chil. ; 122(10):1153-7.

168. Suligoi B, M. Cusan, P. Santopadre et al., (2000). "HSV-2 specific seroprevalence among various populations in Rome, Italy. The Italian herpes management forum," Sexually Transmitted Infections, vol. 76, no. 3, pp. 213–214.

169. Sun, H., Wagener, M. M. & Singh, N. (2008). Prevention of posttransplant cytomegalovirus disease and related outcomes with valganciclovir: a systematic review. *American Journal of Transplantation* 8, 2111-2118.

170. Tamer GS, Devrım Dundar, Eray Calıskan (2009). Seroprevalence of *Toxoplasma gondii*, rubella andcytomegalovirus among pregnant women in western region of Turkey. *Clin Invest Med*; 32 (1): E43-E47.

171. Tang JW, Aarons E, Hesketh LM, Strobel S, Schalasta G, Jauniaux E, *et al.* (2003). Prenatal diagnosis of congenital rubella infection in the second trimester of pregnancy. Prenat Diagn; 23:509–12.

172. Tookey PA, Ades AE, Peckham CS. (1992). Cytomegalovirus prevalence in pregnant women: the influence of parity. *Arch Dis Child*; 67(7): 779–783.

173. Wang A, Tank H, Tee CS, et al. (2000). Seroprevalence of Cytomegalovirus, Toxoplasmosis, and Parvovirus in pregnancy. Singapore Med J; 41(4):151–155.

174. Watts DH, Brown ZA, Money D, Selke S, Huang ML, Sacks SL, Corey L. (2003). A double-blind, randomized, placebo-controlled trial of acyclovir in late pregnancy for the reduction of herpes simplex virus shedding and cesarean delivery. Am J Obstet Gynecol, 188:836-843.

175. Weiss H. (2004). Epidemiology of herpes simplex virus type 2 infection in the developing world. *Herpes*, 11:24A-35A. (http://www.ncbi.nlm.nih.gov/pubmed/15115627)

176. Weller T, Macauley J, Craig J, Wirth P (1957) Isolation of intranuclearinclusion producing agents from infants with illnesses resembling cytomegalic inclusion disease. Proc Soc Exp Biol Med 94:4–12

177. Weller, T. H. 1971. The Cytomegalovirus: Ubiquitous agents with protean clinical manifestations. N. Eng. J. Med. 285:203-214, 267-274.

178. Wesselhoeft C (1949). "Rubella and congenital deformities". N. Engl. J. Med. 240 (7): 258–61. doi:10.1056/NEJM194902172400706. PMID 18109609.

179. Whitley, R. J., Cloud, G., Gruber, W., Storch, G. A., Demmler, G. J., Jacobs, R. F., Dankner, W., Spector, S. A., Starr, S., Pass, R. F., Stagno, S., Britt, W. J., Alford, C., Jr. , Soong, S., Zhou, X., Sherrill, L., FitzGerald, J. M. & Sommadossi, J. (1997). Ganciclovir treatment of symptomatic congenital cytomegalovirus infection: results of a Phase II study. *The Journal of Infectious Diseases* 175, 1080-1086.

180. Wingard JR. (1990). Opportunistic infections after blood and marrow transplantation. Transpl Infect Dis;1:3-20.

181. World Health Organization. (2000). Weekly epidemiological record: preventing congenital rubella syndrome; 75:289–96.

182. World Health Organization. (2012). Weekly epidemiological record: Immunization, Vaccines and Biologicals: Rubella (Accessed on April, 2012) http://www.who.int/immunization/topics/rubella/en/

183. Williamson, W., Percy, A., Yow, M., Gerson, P., Catlin, F., Koppelman, M. & Thurber, S. (1990). Asymptomatic congenital cytomegalovirus infection. Audiologic,

neuroradiologic, and neurodevelopmental abnormalities during the first year. *American Journal of Diseases in Children* 144, 1365-1368.

184. Wyatt J, Saxton J, Lee R, Al E (1950) Generalized cytomegalic inclusion disease. J Pediat 36:271–294

185. Yip L, McCluskey J, Sinclair R (2006). Immunological aspects of pregnancy. Clin Dermatol, 24:84-87.

186. Yamanishi Koichi; Arvin, Ann M.; Gabriella Campadelli-Fiume; Edward Mocarski; Moore, Patrick; Roizman, Bernard; Whitley, Richard (2007). *Human* herpesviruses: biology, therapy, and immunoprophylaxis. Cambridge, UK: Cambridge University Press. ISBN 0-521-82714-0.

187. Yow MD, Williamson DW, Leeds LF et al: (1988). Epidemiologic characteristics of cytomegalovirus infection in mothers and their infants. Am J Obstet Gynecol 158: 1189,

188. Zanghellini F, Boppana SB, Emery VC, Griffiths PD, Pass RF. (1999). Asymptomatic primary cytomegalovirus infection: virologic and immunologic features. J of Inf Dis ;180(3):702-7

6.5 APPENDIX

APPENDIX 1**: Copy of Questionnaire**

KWAME NKRUMAH UNIVERSITY OF SCIENCE AND TECHNOLOGY AND THE KOMFO ANOKYE TEACHING HOSPITAL, SCHOOL OF MEDICAL SCIENCES DEPARTMENT OF CLINICAL MICROBIOLOGY

QUESTIONNAIRE ON THE PREVALENCE OF CYTOMEGALOVIRUS, RUBELLA VIRUS AND HERPES SIMPLEX -2 VIRUS INFECTIONS AND ASSOCIATED RISK FACTORS AMONG THE PREGNANT WOMEN WHO VISIT KATH FOR ANTENATAL CARE (ANC)

Participants are required to answer all the questions and they are to be sincere in answering these questions. All information collected is solely for the purposes of research and shall not be used for other purposes, all participants information shall be kept safe. No name will be recorded.

SECTION A

1. Sample ID:_____

2. Age: ☐ 18 – 24 ☐ 25 – 30 ☐ 31 – 35 ☐ 36 – 40 ☐ Above 41

3. Place of residence: _____

4. Marital status: ☐ Single ☐ Married ☐ Divorced

5. Gestational Age: ☐ 1st trimester (1st 3 months) ☐ 2nd trimester (Next 3 months)
   ☐ 3rd trimester (Last 3 months)

6. Educational level: ☐ Primary ☐ Secondary ☐ Tertiary ☐ None

7. Occupation: ☐ House wife/Unemployed ☐ Farmer ☐ Self-employed ☐ Other _____

SECTION B

8a. Is this your first pregnancy? ☐ YES ☐ NO

8b. IF NO how many children do you have? _____

8c. what are their ages? _____

8d. Do you have any child with birth defect? ☐YES ☐NO

8e. IF YES state the birth defect. _____

9. Have you had miscarriage and or stillbirth before? ☐YES ☐NO

10a. In your daily activities do you interact with children ≤ 3 years of age? ☐NO ☐YES

10b. If YES to Q 10a, In what way? ☐Bathing/changing diapers ☐Feeding ☐Touching

☐Other _____

10b. If YES to Q 10a, where? ☐Home ☐Work ☐Church ☐Other_____

11a. Before your present relationship have you had any other sexual relationship before?

☐ YES ☐ NO

11b. IF YES how many people? _____

11c. During sexual relations (intercourse) with partner(s) former or present do you use any Protective measures?

☐ YES ☐ NO

11d. IF YES what protective measure(s) are used? _____

11e. How often? ☐ Sometimes ☐ Most times ☐ Rarely

11f. Have you ever engaged (practiced) oral-genital sex with your partner (s)? ☐ YES ☐ NO

11g. IF YES, how often? ☐ Sometimes ☐ Most times ☐ All the time

1B: Key of Questionnaire

Below are the keys or explanations to some of the technical or scientific terminologies used in the questionnaire

- Miscarriage: The spontaneous expulsion of the products of pregnancy before the middle of the second trimester.
- Stillbirth: The birth of an infant who has died prior to delivery especially after 22 weeks of conception.
- Birth defect: Any malformation or abnormalities seen in newborns be it physical, psychological or mental.
- Protective measure: Any means of protection from sexually transmitted disease such as the use of male and female condoms

Appendix 2: ASSAY VALIDATION

CMV and HSV-2: The test run was considered valid provided that the following criteria are met

- Substrate blank in well A1 is less than 0.150
- MNC ≤0.250
- MPC ≥0.750

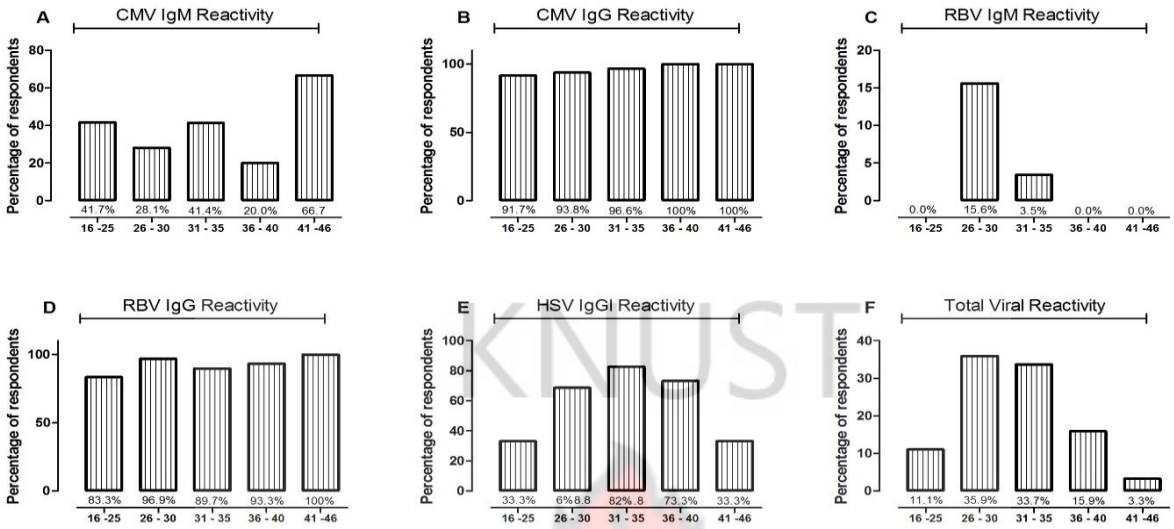
- MPC : MNC is ≥ 5

Rubella: The test run was considered valid provided that the following criteria were met

- Substrate blank in well A1 is less than 0.150
- MNC ≤ MCC
- MPC ≥ 0.750
- MPC : MNC ≥ 2.5

Appendix 3: Arranging serum samples to begin testing

Appendix 4: Graph showing the trend of CMV, Rubella and HSV-2 among the age groups



Appendix 5: The principle underlining ELISA test (Classic EIA)

The HUMAN viral (CMV, HSV-2 & Rubella) IgM & IgG ELISA is based on the classical ELISA technique. The microtitre strip wells as a solid phase are coated with cell culture derived viral antigens (CMV Ag, HSV-2 Ag or Rubella Ag). In the first incubation step corresponding specific antibodies (eg. CMV-IgM-Ab) present in patient specimens or controls bind to the antigens at the solid phase. The sample dilution buffer contains anti-human IgG to prevent rheumatoid factor (RF) interference and competition from specific IgG present in the specimen. At the end of the incubation unbound components are washed out. For the second incubation step, ant-IgM or anti-IgG conjugate; anti-human IgM antibodies, peroxidase conjugated) is added which binds specifically to the IgM class antibodies resulting in the formation of typical immunocomplexes. After a second washing step to remove excess conjugate, TMB/Substrate is added. A blue colour develops changing to yellow after stopping the reaction. The intensity of

the colour is directly proportional to the concentration of the antibodies (eg. CMV-IgM-Ab) in the specimen.

Appendix 6: Tabulation of the assessed associated factors (characteristic) and test results – See below

Patient Code	Age	Marital Status	Education	Occupation	Gesta-tion	No. Child	Child Defect	Miscar/ Stilbirth	Exposure Children	Blood Trans	Multi Sex P	Sex Protection	Oral Sex	CMV IgM	CMV IgG	RUB IgM	RUB IgG	HSV2 IgG
1	16	S	Sec	Student	3rd	0			No		No	No	Yes	Pos	Pos	Neg	Pos	Pos
2	25	M	Sec	Trader	3rd	2			Y, Home		Y, 1	No	No	Pos	Pos	Neg	Neg	Neg
3	29	M	JHS	Trader	2nd	2	Blind		No		Y, 2	Y, Condom	No	Neg	Pos	Neg	Pos	Pos
4	27	M	JHS	Trader	2nd	2			Y, Home		No	No	Yes	Pos	Pos	Pos	Pos	Pos
5	26	M	Sec	Trader	3rd	1		Yes	Y, Home		Y, 2	No	No	Pos	Pos	Neg	Pos	Pos
6	35	M	JHS	Trader	3rd	1			No		No	No	Yes	Neg	Pos	Neg	Pos	Pos
7	39	M	Tertiary	Teacher	2nd	3			Y, Work		Y, 2	Y, Condom	No	Neg	Pos	Neg	Pos	Neg
8	32	M	Sec	Hair dreser	3rd	2			Y, Home	Yes	Y, 2	No	No	Pos	Pos	Neg	Pos	Pos
9	34	M	Pri	Trader	3rd	0		Yes	Y, Home		Y, 1	No	Yes	Pos	Pos	Neg	Pos	Pos
10	32	M	JHS	Trader	2nd	2		Yes	No		Y, 1	No	No	Neg	Pos	Neg	Pos	Pos
11	38	M	JHS	Trader	3rd	3		Yes	No	Yes	Y, 2	Y, Condom	Yes	Neg	Pos	Neg	Pos	Pos
12	31	C	Sec	Trader	1st	0	Deaf		No		Y, 1	Y, Condom	Yes	Pos	Pos	Neg	Pos	Pos
13	40	M	JHS	Trader	2nd	4			Y, Home	Yes	Y, 3	No	No	Pos	Pos	Neg	Pos	Pos
14	25	M	None	Hair dreser	1st	2			Y, Home		Y, 1	No	Yes	Pos	Pos	Neg	Pos	Neg

15	28	M	Tertiary	Teacher	2nd	1			Y, Home		Y, 1	Y, Condom	Yes	Neg	Pos	Neg	Pos	Neg
16	25	C	JHS	Hair dreser	3rd	0			Y, Home		No	No	No	Pos	Pos	Neg	Pos	Neg
17	28	C	Pri	Simstress	2nd	1		Yes	Y, Home	Yes	Y, 1	No	No	Pos	Pos	Pos	Pos	Neg
18	27	M	Pri	Hair dresser	3rd	1		Yes	No		No	No	No	Pos	Pos	Neg	Pos	Pos
19	28	M	Tertiary	Teacher	3rd	1			Y, Home		Y, 1	Y, Condom	Yes	Neg	Pos	Neg	Pos	Pos
20	24	C	Tertiary	Student	3rd	0			Y, Home		Y, 2	No	Yes	Neg	Pos	Neg	Pos	Pos
21	31	M	JHS	Trader	2nd	0		Yes	No		Y, 1	No	No	Pos	Pos	Pos	Pos	Neg
22	32	M	JHS	Trader	3rd	2		Yes	Y, Home		Y, 2	No	No	Pos	Pos	Neg	Pos	Pos
23	23	M	Tertiary	Student	2nd	0			Y, Home		No	Y, Condom	No	Neg	Pos	Neg	Pos	Pos
24	30	M	Tertiary	Adminstrtor	3rd	0		Yes	Y, Home		No	No	No	Pos	Pos	Pos	Pos	Neg
25	26	M	Pri	House Wife	3rd	2			Y, Home		Y, 1	No	No	Pos	Pos	Pos	Pos	Pos
26	29	M	None	Simstress	3rd	0		Yes	Y, Home		Y, 1	No	No	Neg	Pos	Neg	Pos	Pos
27	33	M	JHS	Simstress	2nd	2			Y, Home		No	No	No	Pos	Pos	Neg	Pos	Pos
28	30	M	Tertiary	Teacher	1st	2			Y, Home	Yes	Y, 1	Y, Condom	No	Pos	Pos	Pos	Pos	Neg
29	27	M	None	Trader	2nd	1		Yes	No		No	No	No	Pos	Pos	Neg	Pos	Pos
30	34	M	Sec	Ward Assist	3rd	0			Y, Work		Y, 2	Y, Condom	No	Pos	Pos	Neg	Pos	Neg

Patient Code	Age	Marital Status	Education	Occupation	Gesta -tion	No. Child	Child Defect	Miscar/ Stilbirth	Exposure Children	Blood Trans	Multi Sex P	Sex Protection	Oral Sex	CMV IgM	CMV IgG	RUB IgM	RUB IgG	HSV2 IgG
31	33	M	Tertiary	Civil serv	3rd	1		Yes	Y, Home		Y, 3	No	Yes	Pos	Pos	Neg	Pos	Pos
32	42	M	JHS	Simstress	3rd	3			No		Y, 1	No	No	Pos	Pos	Neg	Pos	Neg
33	44	M	JHS	Trader	3rd	7			Y, Home		Y, 1	No	No	Pos	Pos	Neg	Pos	Neg
34	23	C	JHS	Hair dresser	2nd	0		Yes	Y, Home		Y, 3	No	Yes	Neg	Pos	Neg	Pos	Neg
35	33	M	JHS	Trader	2nd	1		Yes	No		Y, 1	No	No	Pos	Pos	Neg	Pos	Pos
36	39	C	JHS	Trader	3rd	0		Yes	Y, Home	Yes	Y, 1	No	No	Pos	Pos	Neg	Pos	Pos
37	32	M	Sec	Trader	3rd	1			Y, Home		Y, 2	Y, Condom	Yes	Neg	Pos	Neg	Pos	Pos
38	35	M	JHS	Simstress	3rd	2		Yes	No		Y, 2	Y, Condom	No	Neg	Pos	Neg	Pos	Neg
39	32	M	JHS	Trader	2nd	5		Yes	Y, Home		Y, 1	No	No	Neg	Pos	Neg	Pos	Pos
40	32	M	JHS	Trader	3rd	2		Yes	No		Y, 1	No	No	Neg	Pos	Neg	Pos	Pos
41	40	M	JHS	Simstress	3rd	3			Y, Home		No	No	No	Neg	Pos	Neg	Pos	Pos
42	29	M	Pri	Hair dresser	3rd	0			Y, Home		No	No	No	Neg	Pos	Neg	Pos	Neg
43	30	M	JHS	Trader	3rd	2			No		Y, 2	No	No	Pos	Pos	Neg	Pos	Pos
44	31	M	Sec	Nurse	3rd	0		Yes	Y, Home		Y, 10	Y, Condom	Yes	Neg	Pos	Neg	Pos	Pos

45	26	M	Tertiary	Health Wk	3rd	0		Yes	No		Y, 1	Y, Condom	Yes	Neg	Pos	Neg	Pos	Neg
46	37	M	JHS	Trader	3rd	4			Y, Work		Y, 1	Y, Condom	No	Pos	Pos	Neg	Pos	Neg
47	28	M	JHS	Hair dresser	3rd	0			Y, Home		Y, 2	No	Yes	Pos	Pos	Neg	Pos	Pos
48	36	M	None	Trader	3rd	5		Yes	Y, Home		Y, 2	Y, Condom	Yes	Pos	Pos	Neg	Pos	Pos
49	27	M	Pri	Trader	2nd	1			Y, Home		Y, 1	Y, Condom	No	Neg	Pos	Neg	Pos	Pos
50	29	M	Sec	Trader	2nd	3			Y, Home		No	No	No	Neg	Pos	Neg	Pos	Neg
51	29	M	JHS	Hair dresser	3rd	1		Yes	Y, Home		Y, 2	Y, Condom	No	Neg	Pos	Neg	Pos	Pos
52	26	C	Tertiary	Nurse	3rd	0		Yes	Y, Home		Y, 1	No	No	Neg	Pos	Neg	Pos	Pos
53	22	C	Sec	House Wife	3rd	0		Yes	Y, Home		No	No	No	Neg	Pos	Neg	Pos	Neg
54	38	M	None	Trader	3rd	4			No		No	No	No	Neg	Pos	Neg	Pos	Pos
55	32	M	Sec	Hair dresser	3rd	3		Yes	Y, Home		Y, 2	No	No	Pos	Pos	Neg	Pos	Pos
56	36	M	JHS	Trader	3rd	4		Yes	No		No	No	No	Neg	Pos	Neg	Pos	Pos
57	28	M	Sec	Trader	3rd	0			No		Y, 2	Y, Condom	Yes	Pos	Pos	Neg	Pos	Pos
58	23	M	JHS	Hair dresser	3rd	0		Yes	No		No	No	No	Neg	Pos	Neg	Pos	Neg
59	26	M	JHS	House wife	2nd	1			Y, Home		Y, 1	No	Yes	Neg	Pos	Neg	Pos	Pos
60	34	M	JHS	Trader	3rd	3		Yes	No		Y, 1	No	No	Pos	Pos	Neg	Pos	Neg

Patient Code	Age	Marital Status	Education	Occupation	Gesta -tion	No. Child	Child Defect	Miscar/ Stilbirth	Exposure Children	Blood Trans	Multi Sex P	Sex Protection	Oral Sex	CMV IgM	CMV IgG	RUB IgM		HSV2 IgG
61	30	M	Pri	Hair dresser	3rd	0		Yes	No	Yes	Y, 4	Y, Condom	No	Neg	Pos	Neg	Pos	Pos
62	39	M	Pri	Trader	3rd	4		Yes	No		No	No	No	Neg	Pos	Neg	Pos	Neg
63	24	M	Tertiary	Civil serv	3rd	0		Yes	Y, Home	Yes	Y, 2	Y, Condom	Yes	Neg	Neg	Neg	Pos	Neg
64	25	M	JHS	House wife	3rd	2		Yes	Y, Home		No	No	No	Pos	Pos	Neg	Neg	Pos
65	34	M	Tertiary	Teacher	3rd	1			No		Y, 1	No	No	Neg	Neg	Neg	Pos	Pos
66	31	M	JHS	Trader	2nd	2			Y, Home	Yes	Y, 2	No	Yes	Pos	Pos	Neg	Pos	Pos
67	35	M	Tertiary	Teacher	1st	2		Yes	Y, Work	Yes	Y, 1	No	No	Neg	Pos	Neg	Pos	Pos
68	37	M	Pri	Hair dresser	3rd	4			Y, Home		Y, 1	No	No	Neg	Pos	Neg	Pos	Pos
69	33	M	None	Trader	3rd	2		Yes	No		Y, 1	No	No	Neg	Pos	Neg	Pos	Pos
70	28	M	Sec	Civil serv	3rd	0			Y, Home		Y, 2	No	No	Neg	Pos	Neg	Pos	Pos
71	31	M	JHS	Hair dresser	3rd	2			No		No	No	No	Neg	Pos	Neg	Neg	Pos
72	30	M	Pri	Trader	3rd	4			Y, Home		Y, 2	No	No	Neg	Pos	Neg	Pos	Pos
73	36	M	Tertiary	Teacher	2nd	0			No	Yes	No	No	No	Neg	Pos	Neg	Pos	Neg
74	28	M	JHS	House wife	3rd	2		Yes	No		Y, 1	No	No	Neg	Pos	Neg	Neg	Pos

75	46	M	JHS	Trader	3rd	2			Y, Home		Y, 1	No	No	Neg	Pos	Neg	Pos	Pos
76	29	M	JHS	Hair dresser	3rd	2			Y, Home		Y, 1	No	No	Neg	Pos	Neg	Pos	Neg
77	24	M	Tertiary	Teacher	3rd	0			Y, Home		Y, 2	No	Yes	Neg	Pos	Neg	Pos	Neg
78	30	M	JHS	Trader	3rd	1			Y, Home		Y, 4	No	Yes	Neg	Pos	Neg	Pos	Pos
79	29	M	Tertiary	Simstress	3rd	1		Yes	Y, Home		Y, 1	Y, Condom	Yes	Pos	Pos	Neg	Pos	Pos
80	40	M	Pri	Trader	2nd	0		Yes	Y, Home		No	No	No	Neg	Pos	Neg	Pos	Pos
81	34	M	Pri	Trader	3rd	3		Yes	No		Y, 2	Y, Condom	No	Neg	Pos	Neg	Pos	Pos
82	34	M	Sec	Civil serv	3rd	2		Yes	No	Yes	Y, 1	No	No	Neg	Pos	Neg	Pos	Pos
83	36	C	JHS	Trader	3rd	0		Yes	Y, Home		No	No	No	Neg	Pos	Neg	Pos	Pos
84	35	M	Tertiary	Teacher	3rd	2		Yes	Y, Home	Yes	Y, 2	Y, Condom	No	Neg	Pos	Neg	Pos	Pos
85	28	M	Tertiary	Teacher	2nd	0			No		No	No	Yes	Neg	Neg	Neg	Pos	Pos
86	26	M	Tertiary	Nurse	2nd	0			Y, Home		Y, 1	Y, Condom	Yes	Neg	Neg	Neg	Pos	Neg
87	31	M	JHS	Simstress	3rd	2			No		No	No	No	Neg	Pos	Neg	Pos	Neg
88	28	M	Sec	Trader	3rd	0			No		Y, 1	Y, Condom	Yes	Neg	Pos	Neg	Pos	Neg
89	31	M	Sec	Simstress	3rd	2		Yes	No		Y, 1	No	No	Neg	Pos	Neg	Neg	Pos
90	35	M	Sec	Simstress	3rd	1		Yes	No		Y, 3	Y, Condom	No	Neg	Pos	Neg	N/T	Pos

91	39	M	Tertiary	Teacher	3rd	2		Yes	Y, Home		Y, 2	Y, Condom	No	Neg	Pos	Neg	N/T	Pos