Studies on Human Immunodeficiency Virus and Hepatitis C Virus Coinfection in the Gambia

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DEDICATION

This work is dedicated to:

The Vairagi Eck Masters for their infinite love;

My Dad whose sudden transition gave me a better understanding of man;

Imelda and my boys, Ibi and Ayezele, whose sacrifice for this work has impoverished them.

ABSTRACT

Co-infection with Hepatitis C Virus (HCV) is a common occurrence in Human Immunodeficiency Virus (HIV)-positive patients and an increasing cause of morbidity and mortality. Little is known however of the burden or the natural history of these infections or their interactions in most parts of sub-Saharan Africa, where both viruses are endemic. In this study a total of 1500 people aged 11 months to 76 years referred to the serology unit of Royal Victoria Teaching Hospital between the months of July to December 2003 were evaluated for anti-HIV, anti-HCV and CD4+ T-cell count and compared with the subjects' socio-demographic and risk factors. HIV and HIV/ HCV seropositive persons who consented to a follow-up study were age and sex matched with HIV and HCV seronegative control subjects and followed for 18 months with biannual monitoring of trends in CD4 count against a possible HIV or HCV seroconversion of the seronegative control subjects. The overall prevalence of antibodies to HIV and HCV was 6.7% (101/1500) (CI, 5.6-8.2) and 2.1% (31/1500) (95 % CI, 1.4-2.9) respectively. HIV rates in asymptomatic adults were 3.6 %(43/1189) (OR: 0.16; CI: 0.13-0.28) and 1.0 %(12/1189 (OR: 0.16; CI: 0.08-0.34) for HCV. HIV/HCV co-infections rate was 0.6% among all the subjects sampled and 8.6% in HIV positive persons. The HIV rate in this study is twice the UNAIDS/WHO estimate for the country and twice the numbers of women than men were infected with HIV at a comparatively younger age, while males 55 years and over had higher HIV rates than those below 35. HCV and HIV/HCV co-infection was more commonly associated with males than females. This study showed that Hepatitis C serotype 2 is the most prevalent type in the country and was predominantly associated with HIV-1, and suggests that HCV serotype 2 spread earlier than serotypes 1 and 3. The mean CD4 count of apparently healthy males and females was 489/μl and 496/μl respectively, while the mean CD4 count at diagnosis (CD4dx) of HIV, and HIV/HCV persons was 310 cells/μl and 306 cells/μl respectively. Only about half of the apparently healthy population had CD4 counts of 500 cells and over (51%), while 1.1% (15/1377) had counts below 200 cells per microlitre for no explained reasons. HIV/HCV co-infected person recorded a lower CD4 count at diagnosis than HIV alone infected persons and also a more significant decline in CD4+ than HIV infected alone persons. The study shows that high HIV rates were independent of the educational status of the individual, while history of sexually transmitted diseases, high income earning and involvements in polygamous marriages were all significant risk factors for HIV, HCV and HIV/HCV co-infection. Female circumcision, knowledge and use of condoms, blood oath, histories of blood transfusion and wife inheritance were not associated with HIV or HCV transmission. The study found an HIV incidence rate of 1.4% (4/288) during the 18 months follow-up period and identified Sexually Transmitted Diseases (STDs) as the associated risk factor. There is need for a new CD4+ staging in the country based on the population within the country and the initiation of a large scale longitudinal study to elucidate the risk factors associated with HCV in the country. The study has provided baseline data on CD4 and its trends in co-infected persons and also a baseline on the distribution and epidemiological pattern and associated risk factors of co-infection between HIV and HCV in the country. It has also determined the incidence of HIV and its associated risk factors in the country. The study has therefore contributed to our understanding of the natural history of these infections and provided an important frame work for possible intervention.

ATTESTATION

This thesis titled "Studies on Human Immunodeficiency Virus and HCV coinfection in the Gambia" was carried out by Mr. Clement Mboto of the Royal Victoria Teaching Hospital, Banjul- the Gambia as a collaborative work between Royal Victoria Teaching Hospital, Banjul and Kingston University, London- United Kingdom.
All components of this work were carried out in the Pathology Department of the Royal Victoria Teaching Hospital, Banjul- the Gambia.

CERTIFICATION

I certify that this work titled "Studies on Human Immunodeficiency Virus and HCV Coinfection in the Gambia" is an independent work carried out by me at the Royal Victoria Teaching Hospital, Banjul- the Gambia. That the work has the approval of the Management of the Hospital and that of the Department of State for Health and all ethical rules were adhered to and that at stages patients consent and that of their parents in the case of minors was obtained for participation.

Name of Student:

I .D Number: 130355

Signature:

Date:

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ABBREVIATIONS

ADCC antibody- dependent cellular

AIDS acquired immunodeficiency syndrome

ARC Related Complex

ART antiretroviral therapy

bDNA branched-chain DNA

Bp base pair

C2 second constant region in env gene

CA cased

CCR5 CC-chemokine receptor-5 CCR5

CD4 cluster of differentiation 4

CD8 complement differentiation 8

CD4dx CD4 count at diagnosis

CI, confidence interval

Cpx complex

CTL cytotoxic T-cells

CRF circulating recombinant form

DNA deoxyribonucleic acid

EDTA ethylenediamine tetra-acetic acid

Env envelope gene

ER endoplasmic reticulum

ESLD end-stage liver disease

FHF fulminant hepatic failure

GAG group antigen gene

GBV/HGV- hepatitis G virus

Gp glycoprotein

HbsAg Hepatitis B surface antigen

HCV hepatitis C virus

HCC hepatocellular carcinoma

HIV human immunodeficiency virus

HIV D- Dual infection with human immunodeficiency virus type 1 and type 2

HVR hypervariable region

1^0 Primary education
2^0 Secondary education
3^0 Post Secondary education

IDU injecting drug use(r)

IFN interferon

IVDA Intravenous drug abuse

Kb kilo base

LIA line immunoassay

LP Liver associated problems
LTR long terminal repeat

mRNA messenger RNA

M group major group of HIV-1

MA matrix

N group non-M non-O group of HIV-1

NACP National AIDS Control Programme- NACP

NC nucleocapsid

NG None Gambians
NCp7 nucleocapsid protein 7

Nef negative regulator factor

0^C Celsius

Occ Occasionally
O group- outlier group of HIV-1

PBMC peripheral blood mononuclear cell

PCR polymerase chain reaction

PGL persistent generalized lymphadenopathy

PR protease

Pol polymerase gene

Rev regulator of virion proteins gene

RIBA recombinant immunoblot assay

RNA ribonucleic acid

RT reverse transcriptase

RVTH Royal Victoria Teaching Hospital

SIV simian immunodeficiency virus

S/N Serial number

ssRNA single stranded RNA

STI(s) sexually transmitted infection(s)

STD(s) sexually transmitted disease(s)

SU surface envelope

Tat viral transactivator gene

Taq Thermus aquaticus

TM transmembrane

TMA transcription-mediated amplification

TTV Transfusion transmitted virus

µl micro litre

V3 third variable domain in env gene

vif virus infectivity factor

vpr virus protein R gene

vpu virus protein U gene

WB Western blot

WHO World Health Organization

Number

CHAPTER ONE

1.0 INTRODUCTION

1.1 Co-infection with HIV: an overview

Since the emergence of the virus associated with Acquired Immune Deficiency Syndrome (AIDS), Human Immunodeficiency Virus (HIV) in 1981 (Montagnier *et al*, 1984), several studies have revealed the association with various other agents and clinical parameters, which may influence the clinical course of the disease (Selik *et al*, 1987; Monga *et al*, 2001; Udo *et al*, 2003). As the life expectancy of patients with HIV infection increases, greater attention is also being focussed on these concurrent illnesses. Increase in these associations has continued to be reported following highly improved molecular techniques which have facilitated the diagnosis, and even discovery, of novel viruses (Prescott and Simmonds 1998; Pray, 2001). Generally, HIV-infected persons have been reported to be susceptible to over a hundred opportunistic infections by viruses, bacteria, fungi and protozoa (El-Serag *et al*, 2003). In this regard, Weiss (2001) observed that the increased prevalence of these diseases in the HIV-infected population means that there is an increased possibility of these other diseases being spread to people who are not infected with HIV, thus making studies of these associations from several perspectives a necessity. With over 40 million global cumulative infections with HIV, and 70% of these in sub-Sahara Africa (UNAIDS/WHO, 2002), the threat posed to health by these associations cannot be over-estimated.

Like HIV, documented cases of Hepatitis C Virus (HCV) transmission have been traced back to the mid-1950s and were termed non-A non-B hepatitis by default (Choo *et al*, 1989; Purcell, 1994). It is estimated that more than 170 million people are globally infected (Barret and Grant, 2002). Similarly, like HIV, most infected individuals do not experience symptoms early in the course of HCV infection (Healey *et al,* 1995 and Hoofnagle. 1997). Like HIV, HCV is associated with a prolonged incubation period. Despite the severe consequences associated with HCV infection, several issues remain unknown about the virus and the infection it causes. Mbaye *et al*, (2000) observed that in black Africa the role of HCV

in patients to the onset of chronic hepatitis is unclear. Furthermore evidence abounds that several studies have failed to identify any clinical, serological or virological features that predict the outcome of the disease (Alter *et al*, 1989; Farci, *et al*, 2000). This has made necessary the investigation of routes of transmission such as sexual or household exposure (Alvarez-Munoz *et al*, 1997, Alter *et al*, 1989).

Evidence that HIV and HCV co-infection was common only became apparent following the development of diagnostic tests for HCV (Aach *et al*, 1991). Despite the fact that HCV was only isolated in 1989 (Dusheiko, 1997) the increasing association with HIV is probably the most predominant of all HIV associated diseases and is gradually becoming recognised in several parts of the world and a major public health concern globally. The predominant nature of this association among all others may be due to some epidemiological risk factors shared by these viruses (Yeo *et al*, 2003). Another significant reason for this importance is the highly variable clinical course of the infection that appears to be adversely affected in co-infection with HIV (Sabin, 1998; Tedaldi *et al* 2003a).

Studies of HIV/HCV can provide detailed information on the spread and variation of the infection (Marcellin *et al*, 1994; Bonaccini *et al*, 1999). They can also provide data to help in designing preventive measures; development of diagnostic test kits and in intervention programmes including vaccine trials and could be relevant for understanding of the biology of the virus. In countries in sub-Saharan Africa, studies of HIV and HCV co-infection is particularly important taking into consideration that the evolutionary routes of some subtypes or clades of both HIV (Lemey *et al*, 2003) and HCV (Candotti et *al*, 2003) have been traced to the continent. This is further enhanced by the fact that countries in sub-African Africa account for only 20% of the world population but 70 % of the world HIV/AIDS disease burden (UNAIDS 2003). Similarly several studies have shown that sub-Saharan Africa has the highest HCV prevalence globally (Simpore *et al*, 2005). Taking into consideration that these infections share some common routes of transmission and are perpetuated by practices which are endemic in the culture and behavioural pattern of the persons in the region, it may be envisaged that HIV/HCV co-infection may emerge as a third epidemic to the already existing HIV and HCV epidemic in the continent. Thus, as death from AIDS declines in the developed countries and increases in the developing ones, HIV/HCV co-infection may

become an increasingly important problem leading to significant morbidity and mortality in many sub-Saharan Africa countries.

Globally, infection with HIV and other diseases that are predominantly transmitted sexually are often associated with behavioural risk factors and are influenced by local taboos, cultural practice (Halperin 1999) and socio demographic variables (Atrah *et al.* 1994, Boerma *et al*, 2003). However, little is known about the frequency of these co-infections in many parts of the world, especially the third world, nor the contributory role of local practice, behavioural or socio-demographic factors in the perpetuation of the infections (Auvert *et al*, 2003).

In The Gambia few documented studies if any exist on HIV/HCV co-infection in the region. However in The Gambia, unlike most parts of Sub-Saharan Africa, the HIV epidemic has developed at a comparatively low pace (Schim van der Loeff, 2003). In recent years the number of diagnosed cases of HIV infection has increased (laboratory records held at Royal Victoria Teaching Hospital; unpublished material). It is not certain if this increase is due to increase in improved diagnostic facilities that have recently taken place or to a difference in behavioural change or a combination of the two. It is however known that injecting drug use (IDU) remains an uncommon practice in the country. However, Gambia is said to be in the early stages of the AIDS epidemic (Schim van der Loeff, 2003). Da Costa, (1994) had earlier observed that despite awareness of the facts about AIDS, "most Gambians are not motivated to change their behaviours. High-risk practices include circumcision rites, multi-partner sex, prostitution on the part of young rural wives to supplement family income, men having sex with their dead brothers' wives in keeping with the tradition of wife inheritance and polygamy".

1.2 History and origin of HIV

HIV is an enveloped RNA virus belonging to the lentivirus family of retroviruses. Two distinct types of HIV designated HIV-1 and HIV-2 has been identified on the basis of serologic properties, and sequence analysis of molecularly cloned viral genomes. Sequence variation is displayed by each isolate in the env gene, which encodes the glycoproteins in the virion membrane (Chen *et al*, 1997a). A classification scheme based on this recognises nine subtypes (Clades) of HIV-1 (A through I) and seven HIV-2 subtypes (Damond *et al*, 2001).

Only HIV-2 subtype A and B are prevalent, the other being considered self-limiting infections at the epidemiological level.

Both HIV-1 and HIV-2 share many characteristics of some other lentiviruses, including morphologic and structural features, tendency to cause slowly progressing often-lethal disease and ability to persist within cells such as macrophages for prolonged periods (Kedzierska and Crowe, 2002).

The origins of HIV-1 and HIV-2 remains controversial, however, it has been suggested that the viruses arose from zoonotic transmissions between non-human primates and humans (Sharp *et al*, 2001; Lemey *et al*, 2003) and that the virus was introduced into humans around 1930 (Murray, 2000). HIV type 2 was first isolated in 1986 from peripheral blood mononuclear cells (PBMC) from patients in the Cape Verde islands and Guinea Bissau (Clavel *et al*, 1986). Initially HIV-2 infection was mostly confined to West Africa (De Cock *et al* 1989, Da Costa, 1994). However, recent data shows that HIV-2 now has a global distribution (Lemey *et al*, 2003) and subtype A strains is estimated to have been in existence since 1940, while type B strain is estimated to have equally been in existence since 1945 (Lemey *et al*, 2003). A study reported by Lemey *et al*, (2003) provides evidence for a zoonotic transfer of HIV-2 during the first half of the 20th century. It also had evidence of an epidemic initiating in Guinea-Bissau that coincides with the independence war (1963-1974), suggesting that war-related changes in socio-cultural patterns had a major impact on the HIV-2 epidemic.

Some studies have found that cross-reactivity between HIV-2, subtype B and HIV-1 may lead to HIV-2 subtype being misidentified as HIV-1, leading to an underestimate of HIV-2 prevalence (Boerma *et al*, 2003). Also studies have shown that some HIV screening assays may not detect certain HIV-2 subtypes (Damond *et al*, 2001). Similarly, some studies have reported variation in the heterosexual and vertical transmission rates of HIV-2 (Kanki *et al*, 1994; Berry *et al*, 1998) with HIV-2 reported to be more difficult to transmit than HIV-1 and less pathogenic than HIV-1, with lower rates of replication, cell killing, and syncytium formation (Berry *et al*, 1998). Similarly, response to antivirals also differs between HIV-1 and HIV-2, with HIV-2 being significantly less responsive to non-nucleoside reverse transcriptase inhibitors (Damond *et al*, 2001).

1.3 HIV virion

The mature HIV virion is a roughly spherical (actually icosahedral) particle with a diameter of approximately 110 nanometres (Hirsch and Curran, 1996). The outer envelope, which is acquired during virion budding, is studded with 72 spikes formed by the two major viral envelope glycoproteins gp120 and gp41 (Fig. 1, page 6). Four viral proteins (p24-the major core protein, p17- a matrix protein, p9, and p7) are contained in the central core, two copies of the HIV RNA genomic three viral enzymes (reverse transcriptase, integrase, and protease) essential for viral replication (Hirsch and Curran, 1996).

1.3.1 HIV genome

The HIV genome is a 9-kilo base single-stranded RNA genome containing three genes *gag, env* and *pol* essential for retroviral replication but also has at least six additional genes that mediate regulatory or other functions in the cycle of HIV (Baraz and Kotler, 2004). *gag* is the group antigen gene and is found in all retroviruses. It makes various proteins necessary to protect the virus. In HIV, it has three parts: MA (matrix), CA (cased), and NC (nucleocapsid). *pol* is the polymerase gene found in all retroviruses. It makes enzymes necessary for virus replication. In HIV, it also has three parts: PR (protease), IN (endonuclease), and RT (reverse transcriptase). The *env* gene is the envelope gene and is also found in all retroviruses. It makes proteins for the envelope to the virus. In HIV, it has two parts, SU (surface envelope, gp120) and TM (transmembrane envelope, gp41). Other gene proteins include the transactivator (*tat*) gene, which influences the function of genes some distance away. It controls transactivation of all HIV proteins (Hirsch and Curran, 1996). The differential regulator of expression of virus protein (*rev*) genes, the virus infectivity factor (*vif*) gene are required for infectivity as cell-free virus The negative regulator factor (*nef*), retards HIV replication and the virus protein R gene has an undetermined function (*vpr*); the virus protein U *(vpu)* gene is required for efficient viral replication and release. It is found only in HIV-1 and the virus protein X *(vpx)* gene also has an undetermined function. It is found only in HIV-2 and SIV (Levesque *et al*, 2003).

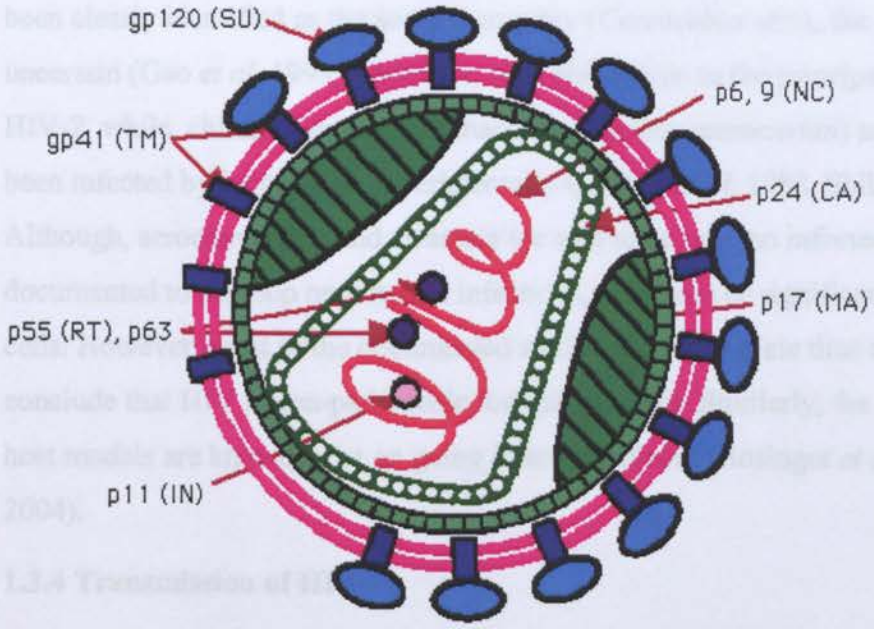

Fig1: Schematic representation of the structure of HIV (Source: http://www.tulane.edu/~dmsander/WWW/335/335Structure.html).

1.3.2 HIV receptors

The principal receptor for the attachments of HIV-1 and HIV-2 has long been established to be CD4, present on most T-helper cells and many cells of the monocyte-macrophage lineage. A study reported by Sattentau *et al.*, (1986) early in the AIDS epidemic indicated that purified CD4 receptor bearing peripheral blood lymphocytes preferentially support the replication of the virus. LaBonte *et al.*, (2002) have shown that the blockade of CD4+ receptors by certain monoclonal antibodies prevents infection of most cell types, while Smith *et al* (1987) confirmed that soluble CD4+ molecules inhibit replication of both HIV-1 and HIV-2 in vitro. Prior to this, Robinson *et al* (1988) had showed that under certain laboratory conditions, low concentrations of antibody might enhance HIV-1 entry into monocyte-macrophages via the Fc receptor. The ability to demonstrate either CD4+ protein or its messenger RNA in some cells that appear infected in *vivo* (such as endothelial cell and certain astrocyte cell lines) is suggestive that other receptors or mechanisms of entry exist.

1.3.3 Host Range

The human AIDS viruses, human immunodeficiency virus type 1 (HIV-1) and type 2 (HIV-2) represent cross-species (zoonotic) infections. Although the primate reservoir of HIV-2 has

been clearly identified as the sooty mangabey (*Cercocebus atys*), the origin of HIV-1 remains uncertain (Gao *et al*, 1999). However, man also serves as the principal host for HIV-1 and HIV-2, while, chimpanzees, pigtail macaque (*Macaca memestrian*) and baboons are have also been infected by inoculation experimentally Gardner *et al*, 1988, Shibata *et al*, 1999. Although, seroconversion and viraemia are said to be rare, no infected chimpanzee has been documented to develop opportunist infections, neoplasm or significant diminution of CD4+ cells. However, most of the documented studies are incomplete thus making it premature to conclude that HIV is non-pathogenic for chimpanzees. Similarly, the developments of several host models are known to be on going in several places (Bosinger *et al*, 2004, Buch *et al*, 2004).

1.3.4 Transmission of HIV

HIV is predominantly transmitted globally through heterosexual sex (Kreiss *et al*, 1986, Moss *et al* 1987, UNAIDS/WHO, 2003). Other modes of transmission includes from mother to child (Chama *et al*, 2004), intravenous injection, blood transfusion of infected blood or blood products and transplant of infected organs. However, these latter modes of transmission have drastically reduced especially in the developed countries. The efficiency of transmission of HIV is also associated with the disease stage of the infection, which determines the amount of infectious virus that is present in body fluid at a particular time (Shattock and Moore, 2003). Variation also exists in the viral trace found in different body fluids (Chiodi *et al*, 1992)

Several studies have shown that there is no difference in the routes of transmission of both HIV-1 and HIV-2 (Vittinghoff et *al*, 1999, Tatt *et al*, 2001, Shattock and Moore, 2003). However, variations in the efficiency of transmission as per the route of contact are well established. The risk of acquiring HIV depends upon viral shedding which is markedly affected by the presence of other sexually transmitted diseases (STDs) (Shattock and Moore, 2003). Similarly, Shattock and Moore, (2003) have observed that all genetic sub types or clades of HIV-1 are transmissible in contrast to some studies that revealed that people are more susceptible to the heterosexual transmission of sub type C and Env-E than B and D (Tatt *et al*, 2001)

Several controversies and problems surround accurate determination of the rate of HIV infection (Eyer-Silva, 2003; Abrams, 2004). Furthermore, most reports are on HIV – I and on discordant couples as a model (Shattock and Moore, 2003). While these parameters are ideal for the developed countries, they are either inadequate and or not applicable in many countries in the western Saharan region where HIV-2 is most prevalent and polygamous relationships and other culture-related high-risk sexual practices are inherent. In a similar vein, several mechanisms have been proposed for the in *vivo* transmission of HIV, which may vary according to the tissues involved (Shattock and Moore, 2003). Most of these proposals are based on studies on HIV-1, and it is not yet certain if they can be applied to infection with HIV-2.

1.3.4.1 Sexual transmission of HIV

Several studies have shown that anal sex is the most efficient means of transmitting HIV sexually (Bautista *et al,* 2004; Coates, 2004), while HIV can be transmitted by oral sex but at a lower degree of efficiency than anal or vaginal sex (Rozenbaum *et al*, 1988). Independent studies conducted on homosexuals early in the HIV/AIDS epidemics by Moss *et al* (1987), Winkelstein *et al*, (1987) and Detels *et al*, (1989) revealed that the receptive partner in anal intercourse is at a higher risk of HIV infection. This because the rectal mucous membranes have more receptors to bind HIV and the tissue is more easily traumatized thus enhancing the transmission of the virus (Zuckerman *et al*, 2004). The risk of transmission is also affected by several other factors including the presence of other sexually transmitted diseases (STDs) (Nusbaum *et al*, 2004), viral load (Kamara *et al*, 2005), condom use (de Visser 2005), female circumcision (Klouman *et al*, 2005) and douching. (Cottrell, 2003, Myer *et al*, 2004)

In male to female sexual transmission, semen-containing HIV infects the female partner through the lining of the vagina, cervix and uterus (Kalichman *et al*, 2001). Studies have also shown that seminal fluid on the average harbours more virus than the female vaginal fluid thus enhancing the risk of male to female transmission ((Tachet *et al*, 1999) Nusbaum *et al*, 2004). Similarly it has been shown that in the absence of other risk factors (like STDs) men are two to three times more likely to transmit HIV to women than *vice versa* (Mastro *et al*, 1994). Cold and Taylor, (1999) and Van Howe (1999), have shown the large

surface of the foreskin increases the potential for micro trauma in uncircumcised men, thus suggesting a reduced risk for circumcised men. Hence since female genitalia have a larger surface area, such as the vagina, cervix and uterus, it implies that there are more predisposed to a higher risk for HIV infection than men in which only penis head and urethra are exposed. Furthermore, women are exposed to a larger quantity of infectious semen for longer time than men are to vaginal fluids (Shattock and Moore, 2003).

Generally, the sexual transmission of HIV is physically hindered by the multiple layers of stratified squamous epithelium which lines the exposed regions of the vagina and ectocervix in women, inner foreskin, penile glans and *fossa navicularis* in men (Shattock and Moore, 2003). This barrier is further enforced by its limited permeability to particles with a diameter greater than 30nm. This implies that the barrier is impermeable to HIV-1 (Shattock *et al*, 2000), and HIV-2 viruses with diameters of 80 –100 nm (Shattock and Moore, 2003). The physical integrity of this epithelial barrier is sustained by the presence of desmosomes and amorphous lipoidal material, which are in abundance (Coombs *et al*, 2003). The outmost apical surfaces of the genital epithelial cells are made of dead epithelial cells that are impermeable to the virus and are renewed every three days (Shattock and Moore, 2003). Independent studies conducted by Marx *et al* (1996) and Smith *et al* (2000) using rhesus macaques demonstrated a dramatic increase of susceptibility to vaginal Simian immunodeficiency virus (SIV) transmission when the epithelial layer is thinned by progesterone treatment. Miller and Shattock, (2003) had also shown that stratified genital epithelial cells are not susceptible to HIV-1 infection and do not transcytose viral particles, an assertion that supports two earlier independent reports by Greenhead *et al* (2000 and Dezzutti *et al* (2001, Thus it has been established that a minimal breakdown of the integrity in the epithelial layer in *vivo* is necessary for the transmission of HIV infection. These cells are however capable of binding to viral particles on the surface and thus facilitate the infection of other cells. Thus some studies have reported the detection of microorganisms in 60% of women involved in consensual sexual intercourse (Novell *et al*, 1984) and (Van Howe, 1999). Similar observations have also been made on the inner foreskin and penile glans (Novell *et al*, 1984 and Van Howe, 1999).

Several studies have shown that anything that can cause abrasion or inflammation of the epithelial barrier, alteration in the hormonal status or vaginal preparations for dry sex can

enhance the transmissibility of HIV infection, (Baleta, 1998, Novell *et al*, 1984, Strathdee *et al*, 1996). Similarly, viral load has been shown to play a significant role in the transmission of HIV (Quinn *et al*, 2000). It is highest during both early and late stages of HIV infection, thus the risk of transmission is highest during these phases (Laga *et al* 1989, Clark *et al* 1991). Studies have shown that antiretroviral therapy usually decreases the amount of HIV in the genital tract as well as the blood. However, some studies have also shown that in some persons even when the virus has attained undetectable levels in the blood they remained in the genital tract (Rasheed *et al,* 1996; Spinillo *et al*, 1999)

Some practices by women such as douching have been shown to destroy the commensal bacteria that protect the lining of the vagina and anus. Douching also dries out and traumatises rectal and vaginal mucosa and renders them more susceptible to HIV infection among others (La Ruche *et al*, 1999). In a similar consideration, some traditional and cultural practice such as female circumcision, tattoos, tribal marks, rituals and blood oaths have been shown to be effective means of HIV transmission (Hardy, 1987).

Distinct geographical variations in the sexual transmission rate of HIV-1 in Africa have been reported (de Vincenzi, 1994). Similarly, reports of a comparatively higher female to male transmission have been reported in some developing countries compared with developed countries (Nicolosi *et al*, 1994, Mastro *et al*, 1994, Padian *et al*, 1997, Gray *et al*, 2001). The major impediment to our understanding of the existence of such differences is our poor knowledge of the beliefs and cultural practice that enhance the risks associated with sex in many parts of the world. This problem is further compounded by a difference in the geographical distribution of other STDs in areas that may facilitate the transmission of HIV.

1.3.4.2 Other modes of HIV transmission

Other non-sexual cultural practices that may enhance HIV transmission include practices resulting in exposure to blood and other fluids such as medicinal bloodletting (Boerma *et al*, 2003), blood oaths, rituals and medicinal enemas, the use of shared instruments such as injection of medicines, ritual scarification, group circumcision (Carballo, 1988; Brady, 1999), tattooing (Van de Perre *et al*, 1984*)*, shaving of body hair (Desrosiers, 1986) and wife inheritance (Schoofs, 1999). The contributory roles of these however vary from one

society to another. Because these transmission modes are considered non-conventional, accurate data does not exist on their contribution to HIV epidemiology.

1.3.4.3 Gender differences in the transmission of HIV infection

Differences in the anatomy and physiology of men and women as enumerated in 1.3.4.1 above have resulted in gender-based differences in the transmission of HIV. A study by Miller and Lu (2003), have shown that the cervicovaginal mucosa contains a complete set of immune cells, including antigen-presenting cells, CD4+ and CD8+ T cells, and B cells. This study also revealed that the cervix of HIV-infected women and SIV-infected female rhesus macaques contain variable levels of antiviral antibodies. They attributed some of this variation to the effects of female ovarian hormone cycles. IgG antibodies were reported to make up the bulk of the antiviral antibody response. IgA antibodies were found at lower levels, while HIV/SIV-specific CD8+ cytotoxic T lymphocytes were found in the cervicovaginal mucosa of infected women and rhesus macaques. Prior to this, Sterling *et al*, (1999) in a longitudinal study of HIV-1 infected persons found that women have comparatively lower plasma RNA levels at the time of seroconversion. Similarly, Ashton *et al*, (1998) had earlier observed that women with HIV infection progress to AIDS at approximately the same rate with men but at a lower RNA concentration. Other studies of the sexual transmission of HIV- 1 have suggested that there is a selective barrier or "filter" that results in differences between the viral variant found in the donor and the recipient (Zhu et al, 1993; Zhu *et al*, 1996). These reports are strengthened by the finding of a relatively homogeneous population of variants in men infected by men and a diverse population of variants in heterosexually infected women (Wolfs *et al*, 1992 and Poss *et al*, 1995). Related studies reported by Long *et al*, (2000) also suggest that the genetic diversity of HIV-1 during acute infection may differ between women and men. The significance of this in HIV-1 pathogenesis cannot be underestimated, since genetic diversification is known to be an important mechanism for HIV-1 pathogenesis and most probably also for HIV- 2. Since the efficiency of HIV transmission is dependent on the route of transmission, a gender factor has generally been accepted as a significant factor in the risk of the transmission of the virus. Shattock and Moore (2003) gave the probability of male to female transmission of HIV as1/200-1/2000, female to male as 1/200-1/10,000 and male to male as /1/10- 1/1600 per coital act.

1.3.5 Pathogenesis of HIV and AIDS

Patients in the early stages of HIV infection have been found to posses a relatively high level of T-helper cells, which do not function normally (Eales and Parkins 1988). The hypothesis advanced for this is that integration of the viral genome in the host cell may affect function either by switching the cell to producing viral proteins rather that those needed for cellular activities or inserting at an important area of the chromosome. Alternatively, intracellular viral proteins may directly interfere with cellular functions. An example of this was shown by Hoxie *et al*, (1986), in which they found the complexing of HIV envelope protein with the CD4 molecule in the cytoplasm of infected cells leading to a decreased expression of CD4 on the cell surface. This severely affects the function of T-helper cells, which uses the CD4 molecule with the specific antigen receptor site to recognize antigen-presenting cells (Lifeson *et al*, 1986). It has also been suggested that extra cellular viral proteins or products of virally infected cells may directly suppress the function of uninfected cells. Schnittman *et al*, (1986) gave an example of this when they demonstrated the activation of B cells to polyclonal immunoglobulin by HIV without infecting such cells. They concluded that this might be the cause of the gammaglobulinaemia observed in patients with HIV infection. In the early stages of the infection and in patients with persistent generalized lymphadenopathy (PGL) the ratio of T helper to suppressor cells (CD4+/CD8 ratio) may be inverted owing to elevated CD8+ positive T cell number and normal or reduced CD4+ cell number (Colebunders, 1987). However, as the disease progresses, the CD8 lymphocytes are lost and a progressive CD4+ lymphopenia develops (CDC, 1986). This depletion has been reported to become more marked as the disease manifests itself clinically and patients with "full blown" AIDS usually exhibit a severe generalized lymphopenia (Colebunders, 1987). Eales and Parkin (1988) observed that the functional abnormalities in individuals infected with HIV cannot be fully explained by the numerical loss of CD4+ positive cells

The production of soluble factors, such as interleukin-2 (IL2) and gamma interferon by both types of lymphocytes also has profound effects on numerous immunological reactions (Hofman *et al,* 1985). It has also been shown that individuals infected with HIV are deficient in the production of both IL2 and gamma interferon, the effect being most profound in patients with full-blown AIDS (Murray *et al*, 1984). In contrast to this, the cellular receptors

for IL2 appear to be normally expressed suggesting that the proliferative abnormalities observed in HIV infection are due to a block in cell stage progression.

It has been recognized that HIV infections result in the emergence of opportunist infection, which is indicative of a defect in cell-mediated immunity. Cells of the monocyte/macrophage lineage in co-operation with T-cells mediate control of such infections. These T-cells are required to produce specific monocyte/macrophage activation factors. It has been established that in HIV infection, the T-cells become defective (Murray *et al*, 1984). Studies of the microbicidal /fungicidal activity of monocyte/macrophages with recombinant interleukins appear to indicate that it was the inability of T cells to produce these lymphokines that allowed the survival of intracellular pathogens (Olafsson *et al*, 1991, Martinez-Maza and Breen 2002). However, studies of the microbicidal/fungicidal activity of cells from some patients with persistent generalized lymphadenopathy (PGL) have shown defective monocyte/macrophage function (Washburn *et al*, 1985 and Kryworuchko *et al*, 2003). Similarly, monocyte related Fc receptor function has been shown to be defective in persons infected with HIV (Kryworuchko *et al*, 2003). This may be due to the blocking of the receptor by immune complexes or decreased receptor recycling due to an inhibitory effect of HIV on intracellular biochemistry. The later hypothesis may also explain the decreased surface expression of HLA-DR antigen on monocyte/macrophages earlier observed by Heagy *et al*, (1984) and the reported lack of normal inflammatory response in AIDS, which may complicate diagnosis since many signs and symptoms usually associated with infection arise directly as a result of inflammatory response (Kryworuchko *et al*, 2003).

Since monocytes express the CD4+ antigen, it seemed likely that the reported abnormalities of monocyte/macrophage phenotype and function might reflect direct infection with HIV. Indeed Rieber and Reithmuller as far back as 1986 demonstrated the selective loss of CD4+ cells in patients infected with HIV. Similarly, Kazanjian *et al*, (2002) isolated HIV from the peripheral blood monocytes of infected individuals, while Olafsson *et al*, (1991) had earlier found HIV in macrophages including those from the lungs. Martinez-Maza and Breen (2002) found the most striking B cell related changes in HIV infection to be the non-specific (polyclonal) activation that gives rise to the characteristic hypergammagobulinaemia. They concluded that there appears to be a progressive increase in immunoglobulin from a symptomatic HIV infection through the persistent lymphadenopathy stage with the highest

levels in AIDS Related Complex (ARC). The mechanism for this activation appears to be unclear. However, Procaccia *et al* (1987) had earlier in an unrelated work suggested that this may be due to the reactivation of latent B cells and high proportion of opportunist infections including cell transforming viruses. Although, total levels of all immunoglobulin classes are raised in individuals with HIV infection, the analysis of IgG subclasses by Eriksson *et al*, (1995) revealed a selective elevation of IgG1 and IgG3 and depression of IgG2 and IgG4. The progressive loss of IgG2 with disease has been associated with encapsulated bacteria such as *Haemophilus sp, Pneumococcus sp and Staphylococcus aureus*, in such patients (Martinez-Maza and Breen (2002).

Measurement of HIV specific cytotoxicity in infected individuals have also been reported to prove extremely difficult owing to the lytic action of the virus and the low expression of virus related antigens on the target cell surface (Sharma and Gupta, 1985). Thus HIV seropositive individuals appear to have a disturbance of their Cytotoxic T-cell (CTL) activity towards certain viruses, which becomes worse with progression of the disease. These observations are in line with the conclusion of Gourevitch *et al*, (1993) that HIV infection directly or indirectly affects all the major immunological pathways resulting in severe complications.

1.3.6 Complications of HIV infection

Acquired Immunodeficiency Syndrome (AIDS) is the most documented complication of HIV infection (Laga *et al*, 1997). It is the most severe end of the clinical spectrum of HIV infection. Studies have shown that almost 95 percent of infected persons become seroconverted within six months of infection (Busch and Satten, 1997). However, remote cases of individuals with long-term HIV infection who appear clinically and immunologically healthy 10-15 years after HIV seroconversion, with stable CD4+ counts have been documented (Mhalu *et al*, 1987, Buchbinder *et al*, 1994). Progression from an HIV carrier status to a full blown AIDS case is known to be due primarily to the critical injury to the immune system caused by the selective infection of CD4+ cells which results in functional defect in virtually every aspect of the immune system including humoural and cellular immunity (Phillips *et al*, 1994, Barret and Grant, 2002).

Infected macrophages have been reported to play a significant role in spreading HIV all over the body (Popovic *et al*, 1984) and have also been reported to be responsible for directly infecting the brain cells. The functional loss of body immunity results in the development of long-term clinical illness due to HIV infection or to opportunistic agents and progressing to life threatening infections and, in most cases, death. The rate of progression also seems to depend on virus type (Ashton *et al*, 1998).

The rate of progression from an HIV infected status to a full blown AIDS case and the types of opportunistic infections or associated diseases has been documented to vary from one environment to another (Selik *et al*, 1987 and Webber *et al*, 1999). It is also influenced by a number of socio-demographic factors. Studies carried out on apparently healthy HIV seropositive individuals in Zaire (Mann *et al*, 1986) and prostitutes in Kenya (Cameron *et al*, 1989) revealed that progression to AIDS occurred in 2.3-5 percent in one year. Although survival after AIDS diagnosis varies it has been attributed with a mean time of 12-18 months even in the developed countries (Mocroft *et al*, 1996). Since the emergence of effective antiretroviral therapies the survival times for HIV-infected individuals have drastically improved (Tedaldi *et al*, 2003b, Sani *et al*, 2005). A recent study by Zhang *et al*, (2005) has shown that there is no uniformity in the survival time of HIV infected persons. The study revealed that the survival times of infected persons obviously is influenced by the age as per the time of infection.

No difference has been reported in the clinical manifestation of infections or disease due to HIV-1 or HIV-2 (De Cock *et al*, 1989) other than HIV-2 is slower acting (Sarr *et al*, 1999). Complications due to HIV infection are often severe, persistent, and relapsing most especially when appropriate treatment is terminated. Most of such complications are due to opportunistic infections (Albini and Rao 2003). Warner and Fisher (1986) in a study conducted early in the AIDS epidemic found opportunistic infection in AIDS to generally result from reactivation rather than from primary infections. Most complications or diseases due to HIV infection (AIDS) are treatable or suppressible (Kashiyama *et al*, 2003). Death however, eventually occurs due to HIV wasting as a result of total functional loss of the immune system (Katzenstein *et al*, 1996). Patients with AIDS have a high incidence of common malignancy often associated with the diseases. These include Kaposi's sarcoma (KS), non-Hodgkin's lymphoma, most often of B-cell type and extra nodal origin, as in brain

and gastrointestinal tract, and squamous-cell carcinoma of cervix and anus. The role of cytomegalovirus (Reuter, 2005), human papilloma virus, (Fernandes *et al*, 2005), and hepatitis B virus, (Burnett *et al,* 2005) that are often found in AIDS-associated KS cells is still not clear. However the human herpes virus, HHV type 8 or KSHV, Kaposi Sarcoma-associated Herpes Virus, is implicated as a candidate aetiologic agent in AIDS-associated KS, the most common malignant tumour in patients with AIDS, and also in much rarer sporadic (classic) KS unrelated to HIV infection. Similarly, EBV (Epstein-Barr virus) is frequently found in AIDS-associated B-cell lymphoma and is associated with both Burkitt's lymphoma and nasopharyngeal carcinoma independent of AIDS. Also, HPV (human papilloma virus), particularly strains 16 and 18, is associated with the development of cervical squamous-cell carcinoma in patients with HIV infection (Burnett *et al,* 2005).

The complications of HIV infection are the primary criteria employed by WHO (1986) for the clinical staging of the disease. In this classification patients who are confirmed HIV-antibody positive are clinically staged (Category 1, 2, 3, or 4) on the basis of the clinical condition of performance scored, whichever is higher. The clinical staging of HIV infection and disease is also complemented by a clinical laboratory classification (Piot *et al*, 1992). The laboratory classification divides each clinical category into three strata (A, B, C) depending on the number of CD4+ lymphocytes per mm^3 (> 500, 200-500, <200). In the absence of CD4+ counts, the world health organisation (1993), recommended total lymphocytes count as an alternative means of staging. This is also divided into three different strata (> 2000, 1000-2000, <1000) with patients classified as 1A, 1B etc. They also recommended that a suffix be used to indicate if the laboratory classification is based on CD4+ numbers or lymphocytes counts (i.e. 1AC, 2BI etc and patients are classified as 1X, 2X, 3X, and 4X or simply 1, 2, 3, or 4). The major advantage of the system is that the clinical/laboratory classification can be done even in the absence of definitive information on the prognostic value of all possible combinations. This provides a useful framework for comparing results of investigation in different parts of the world. Another advantage of the system is that it allows a reclassification of the patients when additional laboratory information is obtained or when the prognostic significance of some of the clinical laboratory combinations is better understood.

1.3.7 Prognosis of HIV Infection: Plasma Viral Load and CD4+ cell count

Studies by Mellors *et al*, (1996) using quantitative assays for plasma HIV RNA, plasma "viral load" tests, indicate that a baseline measure of plasma viral load (number of copies of plasma HIV-1 RNA per ml) early in the course of HIV infection is a reliable predictor of prognosis. Secondly, they further showed that the rate of progression to AIDS and death, and viral load, is a CD4+ T-cell independent predictor of clinical outcome. Similarly, Katzenstein, (1996) showed that reduced levels of plasma HIV RNA in response to antiretroviral therapy are predictive of improved prognosis. Documented cases also exist of subjects who remain healthy, have stable CD4+ T-cell counts, and show no progression of disease after many years of HIV infection. Summaries of the reasons advanced for this disparity include the fact that the HIV burden in plasma and in peripheral blood mononuclear cells of these long-term non-progressors is less than typically found in patients with progressive disease (Brantley *et al*, 2003). This finding is consistent with the prevailing concept that HIV disease progression is driven by an increasing viral burden. Kirchhoff *et al.*, (1995) using molecular analysis of one of five long-term non-progressors found a partially defective or attenuated form of HIV with a deletion of portions of the HIV auxiliary gene *nef*. This unique finding in human beings may be in line with the observation that adult (but not neonatal) macaque monkeys infected with an attenuated simian immunodeficiency virus (SIV) having *nef* gene deletions also show non-progressive SIV infection (Chakrabarti *et al*, 2003)

Other reasons are that in infection with HIV, like in many other viral diseases, CD8+ T cells plays a critical function in the containment of the virus and disease progression, by cytolytic mechanisms. It has also been shown that the release of HIV-suppressive factors-RANTES, MIP-1 alpha & beta (beta chemokines) can achieve this containment by CD8+ T-cells (Cocchi *et al*, 1995). Deng *et al* (1996) have showed the natural receptor for these chemokines to be cell-surface glycoprotein called CC-chemokine receptor-5 (CCR5). CCR5 is also known to be a specific coreceptor for facilitating fusion and entry of macrophage (M)-tropic strains of HIV into CD4+ target cells. CXCR4 has also been identified as a specific receptor for late-stage T-lymphocyte (T)-tropic strains of HIV. Samson *et al.*, (1996) study of HIV infected individuals including some subjects with slow disease progression found inactive (functionless) CCR5 receptors and identical mutations in both alleles of the CCR5

gene suggesting a protective role of homozygous mutation of the CCR5 gene in HIV infection and disease progression. Similarly, Bonhoeffer *et al* (1997) has shown that patients with HIV infection treated with a potent antiprotease inhibitor of HIV replication can produce 10^9-10^{10} virions of plasma virus daily; while more than 10^9 of CD4+ cells can be destroyed daily. Similarly, they added that the life span of the plasma virus and of virus-producing cells is very short (with a half life of approximately two days). They showed that plasma virus must come mainly from recently infected cells and that in the absence of total virus suppression drug-resistant mutants can emerge rapidly. These findings are in support of the conclusion that the development of AIDS is primarily caused by continuous rounds of HIV infection and replication, resulting in virus- and immune-mediated killing of CD4+ cells (Pierson *et al,* 2000).

1.3.8 Global Epidemiology of Human Immunodeficiency virus (HIV)

The global distribution of HIV remains on the increase despite several concerted global efforts towards prevention and a possible cure. The World Health Organization (WHO) and Joint United Nations Programme on HIV/AIDS (UNAIDS) report indicates that, at the end of 2002, approximately 42 million people, acquired the human immunodeficiency virus (HIV). The report added that about half of them acquired it in 2002 alone, while 3 million died as a result of AIDS within the same period (UNAIDS/WHO, 2002). Like previous reports, many African countries still account for the bulk of the disease burden. Worst affected in the HIV/AIDS epidemic is sub-Sahara Africa, where a total of 3.5 million new infections were estimated to have occurred in 2002 bringing the total number of people estimated to be infected within the region to 29.4 million. This implies that sub-Saharan Africa, which accounts for less than 20 per cent of the world population, is saddled with more than 70 per cent of the global HIV infection; this notwithstanding, many epidemiologists are still of the opinion that a fully-fledged epidemic is only beginning to take hold in many African countries (Mwachari *et al,* 2004, Nalugoda *et al,* 2004), as a much as greater number of the people who acquired HIV over the past several years fall ill. This means that the worst of the epidemic impact in the society will be felt in the next decade and beyond. With no cure in sight, the significance of this in terms of public health and patient care may be better imagined. The UNAIDS/WHO report (2002) further showed that in some South African countries including Botswana (38.8%), Lesotho (31%), Swaziland (33.4) and Zimbabwe

(33.7%), an average of one third of the adult population is infected. While in the West Africa sub-region, eight of the countries including Cameroon (11.8%), Central Africa Republic (12.9%), Cote d' Ivoire (9.7%) and Nigeria (5.8%) are reported to have prevalences above 5%. Comparatively, lower prevalence of less than 2% was reported for Senegal (approx. 1%) and Mali (1.7%). A decline in HIV prevalence was also reported in some African countries like Ethiopia with functional intervention programmes. Unlike Africa, the report showed that the HIV epidemics in Asia are still recent with a total of 9 million people estimated to be infected. Similarly, the epidemiological trend of spread of the virus in Asia is slightly different from the situation in sub-Saharan Africa. The virus spread in Asia both sexually and through intravenous drug use (UNAIDS/WHO Report, 2002).

Unlike the United States, Europe and Asia, the epidemiological pattern of the HIV transmission in the West Africa sub-region remains predominantly through heterosexual contacts (Laga *et al,* 1997; UNAIDS/WHO Report, 2002). This trend makes it possible to find more women and children with HIV than in other regions (UNAIDS/WHO Report, 2002). De Cock (1993) and Laga *et al,* (1997) had observed that HIV-2 predominates HIV-1 in West African countries such as Senegal, Ivory Coast, Cape Verde, Gambia, Guinea-Bissau, Liberia, Ghana and Nigeria and that it spread mainly to countries with strong links to these West African countries – France, Portugal, Angola and Mozambique. De Cock (1993) further concluded that very few cases have been reported outside these countries. However, the trend as observed by De Cock, (1993) is gradually changing within increasing numbers of HIV-1 infection now found in the West African sub-region (Schim van der Loeff *et al,* 2003).

1.3.9 Epidemiology of HIV in the Gambia

Most available data on HIV distribution in the Gambia is mainly limited to surveillance on pregnant women and records held by individual hospitals, even though the disease is notifiable. A two–year nationwide survey of 29,670 pregnant women attending antenatal clinics in the Gambia between1993-1995 yielded an HIV-1 seroprevalence of 0.6%, and 1.1% for HIV-2 (Schim van der Loeff *et al,* 2003). According to Schim van der Loeff *et al,* (2003) a repeat survey carried out between May 2000 and August 2001 on over 8000 pregnant women gave a prevalence of 1% and 0.8% for HIV-1 and HIV-2 respectively. Prior to these surveys, the available data were records held by the individual hospitals with facilities

for HIV diagnosis. With the establishment of a national body (National AIDS Control Programme- NACP) for the coordination of HIV/AIDS affairs last year, compilation and collation of data on the disease in the country is gradually being put in place. However, this is still in its infancy. Of concern in the Gambia is a recent trend towards infection with human immunodeficiency virus type 1 (HIV-1) (Schim van der Loeff *et al*, 2003). In 1990, HIV-2 infections among commercial sex workers out numbered HIV-1 by a ratio of nine to one. According to the most recent data, however, HIV-1 was present in 42% of acquired immunodeficiency syndrome (AIDS) patients; 60% of AIDS cases were attributable to HIV-2 and 4% involved in infection with both HIV-1 and HIV-2 (Schim van der Loeff *et al*, 2003). In 1994, Da Costa estimated that about 2.2% of Gambia's 1 million residents are HIV carriers, compared to 1.7% in 1986. However, HIV is routinely screened for at the Royal Victoria Teaching Hospital (RVTH) the main referral hospital in the country and at Medical Research Council (MRC) laboratories at Fajara. Most other hospitals in the country employ rapid test kits and where necessary samples are sent to RVTH for confirmation. Unpublished data (Figure 2) extracted from the records of the Serology unit of the Royal Victoria Teaching Hospital showed that the prevalence of HIV among blood donors have maintained a steady trend of approximately one percent for over a period of five years (1996-2000). A close to 100% upsurge in prevalence rates began to emerge in 2001. Similarly, a steady prevalence of approximately 14 per cent was recorded among all patients referred for HIV screenings tests in the hospital between 2000 and 2002 (Figure 3). This figure apparently includes patients, antenatal cases and students among others referred for medical examination. However, these records are limited in the scope of information contained. In most cases patients are simply documented as adults with no appropriate information on their age. Similarly information on the patient's sex is often omitted. Similarly records of patients screened between 1996 and 1999 were not available for comparison.

The data also show a significant decline in the prevalence of HIV-2 over the years for both donors (Figures 2, page 21) and patients (Figures 3, page 22). In contrast, a hundred-fold increase in the prevalence of dual infection between HIV-1 and HIV-2 was recorded among all patients screened in year 2002 (Fig.2, 21). Although, the country has so far escaped the devastating HIV-1 epidemic typical of other countries in the east and south of Africa, and it has continued to maintain an almost steady prevalence of approximately 1% for over five

years among blood donors and 14.0% among all other groups of patients. However, the gradual decline in the prevalence of HIV-2 in comparison to HIV-1 and the increasing prevalence of dual infection between HIV-1 and HIV-2 does not seem to offer a favourable future. However, the general low prevalence of HIV found among blood donors in the Gambia is comparatively similar to the findings in Senegal, a country that shares an extensive border with the Gambia (UNAIDS/WHO, 2002).

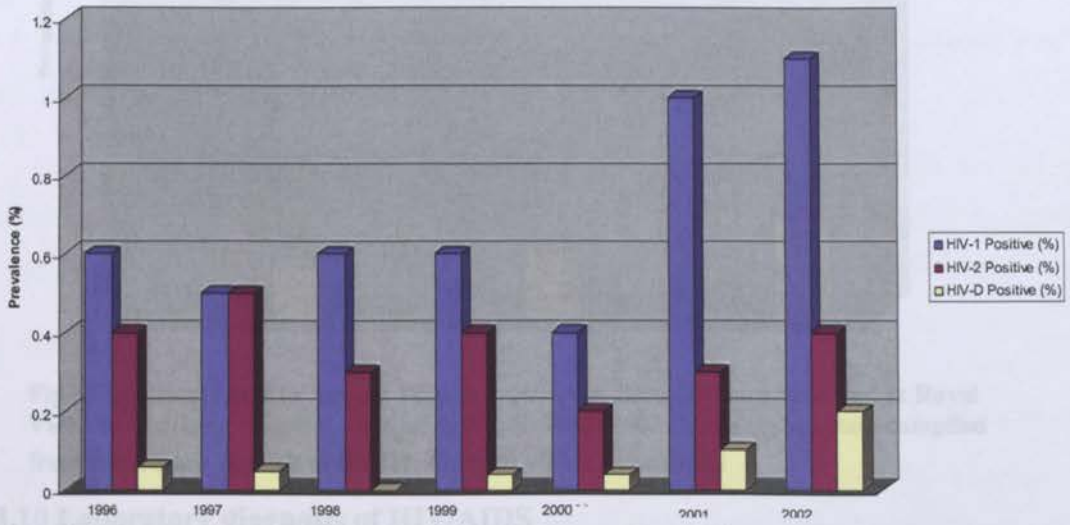

Fig 2: Prevalence of HIV among Blood Donors at Royal Victoria Teaching Hospital –Banjul-Gambia: 1996-2002(Unpublished data: compiled from laboratory records of RVTH, Banjul-The Gambia.)

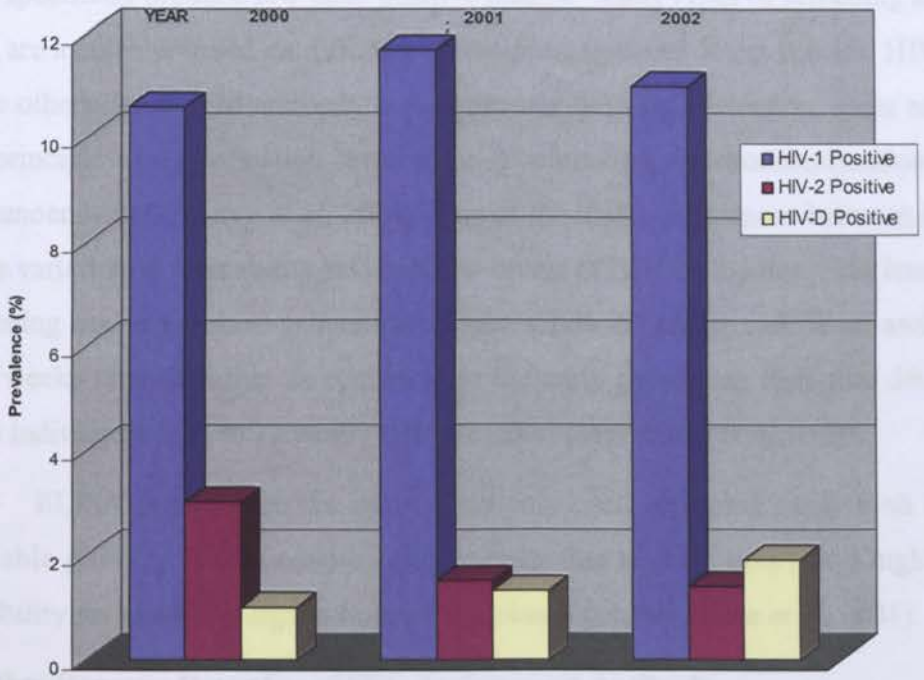

Fig 3: Prevalence of HIV among Persons other than Blood Donors Screened at Royal Victoria Teaching Hospital –Banjul-Gambia: 2000 -2002 (Unpublished data compiled from laboratory records of RVTH, Banjul –The Gambia.)

1.3.10 Laboratory diagnosis of HIV/AIDS

Several laboratory methods are available for the diagnosis of HIV. The test of choice however, depends on many parameters ranging from the purpose of test, budget, and available manpower among other criteria. For the ease of description these tests may be classified into three groups: Qualitative and quantitative antibody detection tests and nucleic acid detection assays (Sickinger *et al*, 2004).

1.3.10.1 Screening assays

a) Qualitative antibody detection tests

The qualitative antibody detection tests for HIV can be further divided into two broad groups namely screening assays and confirmatory or supplemental assays. (Sickinger *et al,* 2004). Screening assays usually have a high degree of sensitivity facilitating the easy identification of individuals who are not infected but who have reactive screening test results. Unlike screening tests, confirmatory assays are designed to have a high specificity.

Generally, tests with high sensitivity produce few false-negative results, whereas tests with high specificity produce few false-positive results. Many types of screening and confirmatory tests are available, based on different principles, eg some detect specific HIV IgM antibody, some others detect IgM antibody simultaneously with IgG detection, some are also based on the principle of agglutination tests, some precipitation, electrochemiluminescence or lineal immunoenzymatic (Levy *et al*, 2004). One of the major differences between screening assays is the variation in their ability to detect low levels of HIV antibodies. The latest version of the screening assays the third-generation antigen sandwich assays can detect antibody at about 3 to 4 weeks after infection as compared to the early generation tests that detects antibody in most individuals by 6 to 12 weeks after infection (McFarland *et al*, 1999).

ELISA test kits are the most commonly used screening assay with several versions available globally. Their extensive use may be due to their simplicity, high sensitivity, and suitability for handling large volume of samples. (Koblavi-Dème *et al*, 2001).

(b) Simultaneous Detection of HIV Antigen and Antibody

The fourth generation assays that emerged in the late 1990s allow for the simultaneous detection of HIV antigen and antibody. Tests based on the simultaneous detection of both HIV antibody and HIV p24 antigen further reduced the window period to about two weeks and also has the advantage of eliminating the need to perform separate assays. The tests are available in several formats including ELISA and rapid kits (Ly *et al*, 2004).

(c) Rapid Tests

Rapid tests for the detection of HIV antibodies are defined as tests that can be performed in less than 30 minutes. They were developed in the late 1980s and became popular when they proved to be as accurate as the ELISA (Meda *et al*, 1999) Most of these rapid assays incorporates an anti-human immunoglobulin that binds any immunoglobulin in the sample and produces a separate indicator when all reagents are added appropriately. This built-in control has added more confidence to their use. In addition some rapid kits allow the differentiation of HIV-1 and HIV-2 infection (Rouet *et al*, 2004). The disadvantages include the possibility of subjective interpretation and comparatively higher cost than the ELISA. (Owusu-Ofori *et al*, 2005).

(d) Simple Tests

Simple tests are defined as those HIV tests, which require more than 30 minutes; can be accomplished and can be performed easily without instrumentation. Versions based on varied principles are available, but common among these are agglutination assays, passive haemagglutination (PHA) and latex agglutination. The major disadvantage of many simple tests is that they often have to be performed under temperature-controlled conditions (Aghokeng *et al*, .2004).

1.3.10.2 Confirmatory tests for HIV Infection

The World Health Organisation (1986) and the Centre for Disease control (CDC) have a laid down algorithms for HIV confirmatory tests (Constantine *et al*, 1992). Prior to the advent of the newer technologies most testing algorithms require the use of very specific assays, such as the Western blot, indirect fluorescent antibody assay (IFA), or the radioimmunoprecipitation assay (RIPA) as a verification tool for reactive screening test results. These are specific tests and do not produce biologic false-positive results. However, they are labour intensive and comparatively more expensive than screening assays. They are gradually being replaced by molecular assays.

(a) Western Blot Test

Until the emergence of molecular assays, Western blot (WB) was probably the most widely accepted confirmatory assay for the detection of antibodies to the retroviruses. The Western Blot test is based on the use of strips of nitrocellulose paper blotted with the full bands of HIV protein antigens, which have been separated by electrophoretic procedures (Constantine *et al*, 1992). The HIV-1 viral antigens separated are gp160, gp120, p66, p55, p51, gp41, p31, p24, p17, and p15 .A single trip is incubated with a 1:50 dilution of each serum sample to be tested, washed and re-incubated with horseradish peroxidase or alkaline phosphatase tagged antihuman globulin. Reaction with a positive serum sample produces a characteristic band. The CDC (1996) guidelines require reactivity to any two or more of the following antigens: p24, gp41, gp120/160 for a positive classification, while a negative result is the absence of all bands. WHO(1986) requirements include reporting a result as negative if there is only a very weak p17 band and as indeterminate when the actual criteria for a positive reaction is not fulfilled but there is there is reaction to one or more antigens.

The major disadvantage of the Western blot is the possible presence of non-specific reaction bands in response to the presence of non-viral proteins derived from the host cells in which the virus was grown and are present in the nitrocellulose strip. Up to 15% of normal non-infected persons who may even be nonreactive by screening assays show some reactivity to one or more antigens if tested by Western blot (CDC 1998). Many of the initially indeterminate western blots results especially with reactivity to p24 and p55 have been shown to be indicative of early infection in most circumstance, while other indeterminate reactivity to other proteins that remains indeterminate have been shown to probably be the result of non-specific reactions, hypergammagobulinaemia, the presence of cross-reactive antibodies, infection by HIV-2, infection by an unknown, but related retrovirus or autoimmune diseases such as systemic lupus erythematosus (Jindal *et al*, 1993).

The modern Western blot assays incorporate the use of viral lysates from HIV-1 and synthetic peptides artificially applied from HIV-2 on the same nitrocellulose strip to identify and differentiate infections by HIV-1 and HIV-2. Thus multiple HIV-1 antigens and one HIV-2-specific band such as gp36 or gp41 are present on the strip. Acceptable HIV-1 positive reaction includes reactions to one gene product from each of the three major groups (gag, pol, and env) for positivity for HIV-1, while an HIV-2 positive reaction must show reactions to the HIV-2-specific antigen plus a reaction to HIV-1-specific antigens (Truong and Klausner, 2004).

(b) Line Immunoassay

The Line Immunoassay (LIA) was a comparative alternative to Western blot as a confirmatory test. In this technique recombinant or synthetic peptide antigens are applied on a nitrocellulose strip in place of the viral natural antigens. This method reduces the contamination sometimes associated substances derived from cell culture, which is encountered in Western blot assays. Like Western blot, Line Immunoassay is gradually being replaced by molecular assays.

(c) Qualitative and Quantitative Assays of HIV viral load

The presence of HIV RNA in the blood can be detected (qualitative) or quantitated using molecular techniques. These tests are therefore suitable for the confirmation of HIV infection. They are also often used for pre-treatment evaluation and for monitoring patients' responses to therapy (Roland *et al*, 2004).

Tests based on Polymerase Chain Reaction (PCR) or Branched-chain DNA (bDNA) or HIV-RNA-PCR and Transcription-mediated amplification (TMA) can thus be employed for the qualitative and quantitative assays of HIV viral load. PCR tests can detect as few as 50 IU/μl of HIV RNA in the blood, while the bDNA method for quantitative viral load testing can only measure viral loads greater than 500 IU/μl and The TMA is reported to be able to measure 5-10 IU/μl (Roland *et al*, 2004).

1.3.10.3 Alternative HIV confirmation Algorithm

The ability to obtain similar predictive values by using two screening assays in tandem has been universally accepted most especially in as an alternative algorithm for HIV confirmation. This alternative algorithm is particularly useful in developing countries where facilities for expensive confirmatory tests are often not available (Tamashiro *et al*, (1993). Using this method the most sensitive screening test available is employed initially e.g. ELISA or a rapid/simple assay. This is usually followed by a second ELISA or rapid/simple assay ensuring that the initial and second tests are of different principle (eg bead versus microtitre) and/or use a different antigen source (eg lysates versus recombinant or synthetic peptide) (Tamashiro *et al,* 1993).

1.3.11 HIV diagnosis in infants

The major difference between HIV diagnosis in adults and infants is due to transplacental passage of IgG antibody, which may continue to give a false positive test in infants up to the age of 18 months. Viral assays preferably HIV DNA PCR or HIV RNA PCR, or viral culture on samples obtained within 48 hours of birth, and again during the fourth and eight weeks to twelve weeks of life are particularly important especially when clinical evidence of the infection is not apparent. However, the p24 antigen test is less sensitive than other virological tests in infants less than one month of age with a high frequency of false-positive results (Rich

et al, 1997). HIV DNA PCR is preferred as the virological assay for diagnosis because of the high sensitivity by age 14 days (Rouet *et al*, 2001).

Another alternative is HIV culture, which is also sensitive for early diagnosis (Lambert *et al*, 2003). The major disadvantage of the latter is that it is more expensive, more complex with a longer turnaround time for results. Studies have, however, shown that using these tests approximately 40% of infected infants can be identified by age 48 hr and are considered to have early or intrauterine infection; infants with initial negative testing during the first week of life and subsequent positive tests are considered to have intrapartum infection. Almost all infected infants can now be diagnosed by the age of 6 months (Lambert *et al*, 2003).

1.3.12 HIV diagnoses using other body fluids.

The occasional difficulty encountered in collecting blood and the need for easy compliance (Frerichs *et al,* (1994) has made necessary the search for alternatives to blood for HIV diagnosis. In this regard, tests involving the use of saliva and or urine for the diagnosis of HIV infection have gained global acceptability. Some of these are available as ELISA or Rapid kits. Antibody concentration in saliva has been estimated to be 1/400 of that in plasma (Tamashiro *et al*, 1993, Saville *et al*, 1997) hence the tests that employs saliva as a specimen for HIV diagnosis are extremely sensitive to enable the detection of such small quantities.

Unlike saliva, intact IgG antibodies are found in urine facilitating the adoption of urine for the diagnosis of HIV (Saville *et al*, 1997). However, these kits have not gained much popularity.

1.3.13 HIV disease staging

(a) Classification of HIV disease

Staging of HIV disease is a classification scheme aimed primarily to facilitate easy recognition of groups that have different prognoses and can be used in guiding treatment decisions. The classification is based on categorizing the progressive sequence of prognosis from least to most severe, each higher stage having a poorer prognosis or different medical management than the preceding stage. To this end, several classification and staging systems for HIV disease are in use, most based on a combination of CD4+ lymphocyte count and

symptoms, however, the classification scheme constructed by the Centres for Disease Control and Prevention (CDC) has a greater acceptability.

(b) CDC Classification Scheme for HIV Disease

The revised CDC (1993a) classification of HIV disease established three levels of CD4+ lymphocyte count (0-199, 200-499, 500 and greater). Treatment recommendations to initiate antiretroviral therapy at the time of the revision in 1993 influenced the choice of these categories. The first CDC classification scheme for HIV published in 1986 was aimed as a means of categorizing HIV-related symptoms into four groups specifically for public health purposes and was not intended for staging purposes (CDC, 1986). The current scheme that was revised in 1993 was aimed as a guide for clinical management of HIV-infected adolescents and adults (CDC 1993). This scheme combines three categories of the CD4+ count with three symptom categories. Table 1 below shows a summary of the CDC (1993a) classification scheme.

Table 1: Classification of CD4+ T-lymphocyte counts per microlitre of blood

Category 1:	> 500	cells/mm^3 (or CD4+% > 28%)
Category 2:	200-499	cells/mm^3 (or CD4+% 14% - 28%)
Category 3:	< 200	cells/mm^3 (or CD4+% < 14%)

In this classification these categories correspond to CD4+ T-lymphocyte counts per microlitre of blood and the percentage of CD4+ T cells can be substituted for the count as indicated in parentheses. The lowest accurate, but not necessarily the most recent, CD4+ T-lymphocyte counts or percentage is employed used for classification purposes.

(c) Classification of symptoms (CDC 1993)

Category A

This consists of one or more of the conditions listed below in an adolescent or adult (>13years) with documented HIV infection. Thus the conditions listed in Categories B and C must not have occurred.

-Asymptomatic HIV infection

-Persistent generalized lymphadenopathy

-Acute (primary) HIV infection with accompanying illness or history of acute HIV infection.

Category B

This category consists of symptomatic conditions in an HIV-infected adolescent or adult that are not included among conditions listed in clinical Category C and that meet at least one of the following criteria: (a) the conditions are attributed to HIV infection or are indicative of a defect in cell-mediated immunity; or (b) the conditions are considered by physicians to have a clinical course or to require management that is complicated by HIV infection. Examples of conditions in clinical category B include but are not limited to:

-Bacillary angiomatosis

-Candidiasis, oropharyngeal (thrush)

-Candidiasis, vulvovaginal; persistent, frequent, or poorly responsive to therapy

-Cervical dysplasia (moderate or severe)/cervical carcinoma in situ

-Constitutional symptoms, such as fever (38.5 degrees centigrade) or diarrhoea lasting greater than 1 month

-Hairy leukoplakia, oral

-Herpes zoster (shingles), involving at least two distinct episodes or more than one dermatome

-Idiopathic thrombocytopaenic purpura

-Listeriosis

-Pelvic inflammatory disease, particularly if complicated by tubo-ovarian abscess

-Peripheral neuropathy

For classification purposes, Category B conditions take precedence over those in Category A. Thus, someone previously treated for oral or persistent vaginal candidiasis (and who has not developed a Category C disease) but who is now asymptomatic will be classified in Category B.

Category C

This includes the clinical conditions listed in the 1993 AIDS surveillance case definition (CDC, 1993a) such as all human immunodeficiency virus (HIV)-infected adolescents and adults aged greater than or equal to 13 years who have either a) less than 200 CD4 T-lymphocytes/μl; (b) a CD4+ T-lymphocyte percentage of total lymphocytes of less than 14%; or c) any of the following three clinical conditions: pulmonary tuberculosis, recurrent pneumonia, or invasive cervical cancer. CDC (1993 a) recommends that once a Category C condition has occurred, there should be no reclassification implying that the person shall remain in Category C.

(d) Other Staging Classifications

Unlike the CDC (1993a) classification most other classification schemes aimed at HIV disease staging employs stratification at 400 CD4+ T-cells and includes Walter Reed staging classification by the U.S. Army (Redfield, 1986). The scheme employs CD4+ lymphocyte count above or below 400/mm3 and the presence or absence of oral candidiasis, chronic lymphadenopathy, and delayed hypersensitivity skin test reaction. The Walter Reed staging classification has the disadvantage that a large proportion of infected persons cannot be placed in a stage. Secondly, an infected person can move in either direction through the stages (Royce *et al*, 1991).

Other classifications includes Zolla-Pazner *et al* (1987) classification based on immunologic staging system using the CD4+ lymphocyte count, the CD4+ to CD8 ratio, and the total lymphocyte count. While the Royce *et al* (1991) reported a four-stage scheme using only CD4+ counts above and below 400/mm3 and the presence of either oral candidiasis or hairy leukoplakia. Rabeneck *et al* (1993) amended this by adding night sweats to the CD4+ lymphocyte count and oral candidiasis. However none of these staging systems has been widely adopted due to the inherent setback associated with each.

The development of techniques for viral load assays in peripheral blood has significantly facilitated HIV staging. The level of HIV detectable in the peripheral blood has been accepted as a major predictor of the probability of developing AIDS among asymptomatic individuals. Thus a combination of CD4+ lymphocyte count, viral load and clinical symptoms provides an effective staging of HIV disease.

1.4 Biology of HCV

Hepatitis C virus belongs to a separate novel genus, Hepacivirus of the flaviviridae family (Wengler, 1991). The virus, which was discovered in 1989, is spherical in shape, enveloped and approximately 50nm in diameter (Fig: 4, page 32). The genome of the virus is a positive sense, single stranded RNA molecule of approximately 9.7-kilobases in length (Kamoshita *et al*, 1997). There are three functional regions of the genome, the 5' untranslated region (5'UTR), the coding region encoding the structural and non-structural viral proteins, and the 3'UTR (Robertson *et al*, 1998). The RNA of the virus has been elucidated to contain a single large (approximately 3000 amino acids) open reading frame linked by a highly conserved 5' and 3' untranslated regions (UTRs) (Tai *et al*, 1996). The 5' UTR is approximately 44 nucleotides in length with an extensive secondary and tertiary RNA structure. It contains approximately 300 nucleotide segments, which acts as an internal ribosomal entry site directing the independent translation of the viral open reading frame (Kamoshita *et al*, 1997). The genomes have high levels of secondary structure that controls the expressions of the genes (Kolykhalov *et al*, 1994).

Generally, higher rates of mutations and fragmentation occur when dealing with RNA genomes because errors in single strands generally do not get corrected. In the case of HCV, the open reading frame of approximately 9-kilobases encodes a polyprotein that is co-transitionally processed into at least 10 proteins. Four of these are structural proteins from the amino terminus (i.e. the viral nucleocapsid or core protein); the envelope proteins E1 and E2 and a transmembrane protein P7 or NS2A. The other six are non-structural proteins that are involved in replication of the viral RNA. Cellular and viral protein cases direct the processing of the polyprotein. Within the amino third of the polyprotein are four distinct signal sequences, which direct the translocation of the nascent protein into the endoplasmic reticulum. This results in the cleavage of the polyprotein at the C-E1, E1-E2, E2-NS2A and NS2A-NS2B junctions (Kolykhalov *et al*, 1994).

The highly basic core proteins of HCV polyproteins are made up of the first 191 amino acids cleaved from the nascent polypeptide by signal peptidases (Grakoui *et al*, 1993). The core proteins have RNA- binding activity and may undergo a subsequent internal cleavage by an unspecified proteinase. The large amino-terminal product of this cleavage is

then translocated to the nucleus. The core protein has been associated with several biologic activities including alterations in regulation of the cell cycle and transcription of cellular protoncogenes either induction or suppression of apoptosis and transformation of rat embryo fibroblasts (Chang *et al*, 1994). The core protein has also been reported to bind the cytoplasmic tail of several cellular receptors belonging to the tumour necrosis factor receptor-1 and the lymphotoxin-B receptor. Evidence that it may modulate signal transduction through these receptors has been shown (Chen *et al*, 1997b, Matsumoto *et al*, 1997, and Zhu *et al*, 1998). The HCV core protein is immunogenic and antibody to it is typically present in infected individuals.

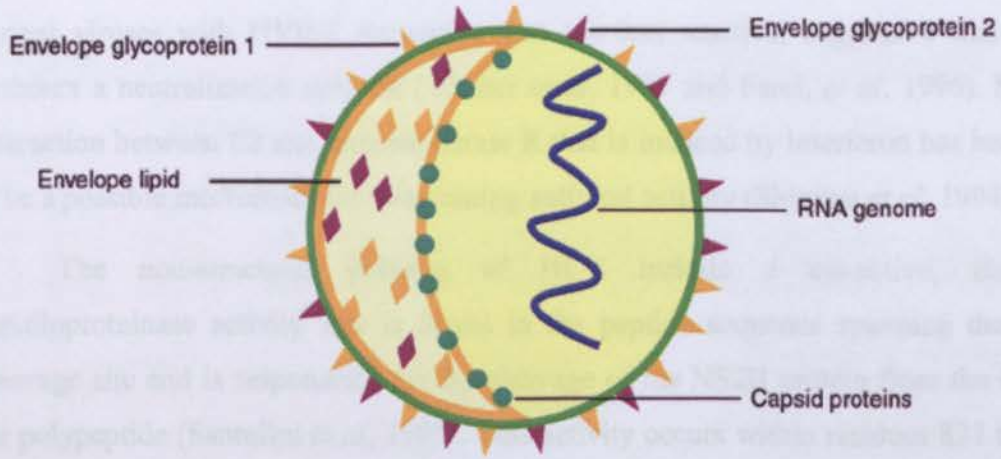

Fig 4: Diagram illustrating a model structure of Hepatitis C virus and it genomic Organisation [Source: Anzola M and Burgos JJ. (2003) Hepatocellular carcinoma: molecular interactions between hepatitis C virus and p53 in hepatocarcinogenesis. *Expert Reviews in Molecular Medicine 5*: 1-16].

Unlike other members of the flavivirus family, which have a single envelope protein and a glycosylated, cell-associated NS1 protein that can elicit neutralizing antibodies, HCV has two major envelope glycoproteins tagged E1 and E2 and no comparable NS1 (Zhu *et al*, 1998). The E1 and E2 proteins are produced by direct cleavage of the HCV polyprotein at amino acid residues 383 and 746 under the direction of signal peptidases (Hijikata *et al*, 1991). The E1 and E2 proteins are secreted into the endoplasmic reticulum (ER) as type 1-membrane proteins. The putative NS2A (p7) protein is produced, as the result of an additional cleavage. This short polypeptide has been associated with the membrane; however, its true function appears uncertain (Lin *et al*, 1994 and Mizushima *et al*, 1994). Yi *et al*, (1997)

showed that the E1 and E2 proteins are heavily glycosylated with sugar moieties representing about 50% of the mature mass of each (Fig: 5, page 34).

The localization of E1 and E2 when expressed from recombinant heterologous viruses suggests that during viral replication the HCV particles assemble and exit the cell by budding from the ER (Ralston *et al*, 1993 and Dubuisson *et al*, 1994). Near the amino terminus of E2 are a most variable segment of the envelope proteins called the hypervariable region 1 (HVR-1). This region contains approximately 30 amino acids residues in length and it exists as a polypeptide loop (Weiner *et al*, 1991). The demonstration of antibodies in infected persons against synthetic peptides representing the HVR-1 sequence has resulted in the selection of variant viruses with HVR-1 sequences that are less reactive, suggestive that the HVR-1 harbours a neutralization epitome (Weiner *et al*, 1995 and Farci, *et al*, 1996). Similarly the interaction between E2 and, protein kinase R that is induced by interferon has been suggested to be a possible mechanism for overcoming antiviral activity (Shimizu *et al*, 1994).

The non-structural proteins of HCV include a cis-active, zinc-dependent metalloproteinase activity that is found in the peptide sequence spanning the NS2B-NS3 cleavage site and is responsible for the cleavage of the NS2B protein from the remainder of the polypeptide (Santolini *et al*, 1995). This activity occurs within residues 827 through 1207 polyprotein. Unlike the NS2B, the NS3 protein has serine proteinase activity localized in the amino-terminal third and an RNA helicase with nucleoside triphosphatase activity in its carboxyl-terminal domain (Santolini *et al*, 1995).

The NS3 serine proteinase is located within the amino-terminal 181 amino acid residues of the NS3 protein and includes the catalytic triad, His-1083, Asp-1107, and Ser-1165. Its activity is responsible for the NS3-NS4, NS4A-NS4B, NS4A-NS5A and NS5A-NS5B cleavages (Tomei *et al*, 1993; Kolykhalov *et al*, 1994). Similarly, nucleotide triphosphatase and RNA helicase activity have been found to be one of the properties of the carboxyl-terminal 465 amino acids of NS3 (Kim *et al*, 1997). The RNA helicase has 3' to 5' directionality and binds to the 3' poly (U) sequence. Its primary role is the unwinding of duplex RNA molecule during the replication of viral genome (Kim *et al*, 1997). NS4A is thought to be a complement of the NS3 serine proteinase; it has a hydrophobic extension that is believed to anchor the proteinase-helicase to intracellular membranes during the replication

of the virus. Unlike NS4A, the functional role of NS4B remains controversial. The speculation however, is that it may be part of the viral RNA replicase complex. A similar view is also held for NS5A protein, however NS5A phosphorylation depends on NS4A (Kaneko *et al*, 1994 and Asabe *et al*, 1997). Unlike NS5A protein, the NS5B protein is highly conserved and contains a Gly – Asp – Asp motif that is characteristic of RNA–dependent RNA polymerase (Weiner *et al*, 1992). There are speculations that the NS5B RNA polymerase may serve as a suitable target for antiviral drug development (Blight *et al*, 1998).

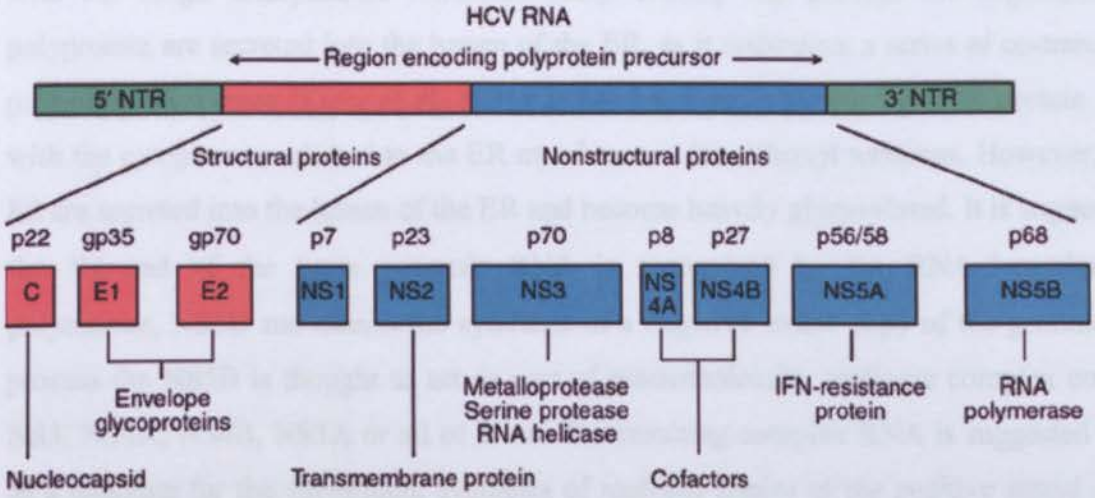

Fig 5: Diagram illustrating Proteins encoded by Hepatitis C virus genome. [Source: Anzola M and Burgos JJ. (2003) Hepatocellular carcinoma: molecular interactions between hepatitis C virus and p53 in hepatocarcinogenesis. Expert Reviews in Molecular Medicine 5: 1-16].

1.4.1 Replication of HCV

The non-availability of robust cell culture systems and small-animal model for the propagation of HCV has been a major handicap in the elucidation of the replication cycle of the virus. However, the recent development of sub genomic selectable HCV replicons (Lohmann *et al*, 1999) and the adaptation of some small animal models such as those in mice (Pietschmann and Bartenschlager, 2003) have facilitated some form of culture studies on the life cycle of the virus. However, most of these developments are still in their infancy and much more work will be required to optimise both systems. Thus the bulk of acceptable information is vastly still based on analogies deduced from studies on other positive – strand

RNA viruses. Based on the later premises, it is speculated that the virus enters the cell through an interaction with a specific cell surface receptor molecule. It is further speculated that E2 binds specifically to CD81, a cell surface molecule that is widely expressed in many different tissues including the liver (Pileri *et al*, 1998). An acceptable assumption is that the alteration of the conformation of the envelope proteins by acidic pH, which fuses with the endosomal membrane, occurs after attachment, penetration, and uptake into the cellular endosome. The viral RNA when released into the cytoplasm acts as messenger RNA directing the cap-independent translation of the viral polyprotein, while viral translation occurs in association with the rough endoplasmic reticulum (ER). During this process the segments of the polyprotein are secreted into the lumen of the ER, as it undergoes a series of co-translational proteolytic cleavages (Farci *et al*, 1991). It has been deduced that the core protein remains with the cytoplasm anchored to the ER membrane at its carboxyl terminus. However, E1 and E2 are secreted into the lumen of the ER and become heavily glycosylated. It is suggested that the 3N end of the input genomic RNA is recognized by the RNA-dependent RNA polymerase, NS5B and directs the synthesis of a negative strand copy of the genome. In the process the NS5B is thought to act as part of macromolecular replicase complex containing NS3, NS4A, NS4B, NS5A or all of these. The resulting complex RNA is suggested to serve as a template for the subsequent synthesis of multiple copies of the positive strand genomic RNA. Specific RNA structure at the 5N and 3N ends of the viral RNA in association with intracellular membranes is thought to serve as promoters for these series of transcription events. It is logically assumed that the genomic RNA is packaged into new viral particles, and is extruded into the ER leading to the release of the virus via the vesicular secretary pathway (Kolykhalov *et al*, 1996). Thus the virus initiates the translation of its protein in the absence of any protein translating initiation factors. In this unique prokaryotic like mechanism the 5N UTR binds directly to the 40-s ribosome subunit to form a binary complex. This involves specific macro molecular interactions around the initiator AUG codon of HCV (Honda *et al*, 1996). Unlike the 5N UTR the 3N UTR consists of a relatively variable 30-nucleotide segment downstream of the termination codon that is followed by a highly variable poly U (C) tract of approximately 100 nucleotides. A highly conserved 98-base sequence is found downstream of the poly U (tract). This segment of the HCV genome is required for the

replication of the virus and also serves as a replicase recognition site during the initiation of minus- strand RNA synthesis (Kolykhalov *et al*, 1996 and Tanaka *et al*, 1996)

The identification of HCV specific antigens, including both negative and positive strand HCV RNA with hepatocytes suggests that the replication does not occur within the cell type, via a negative strand intermediate (Negro *et al*, 1994 and Shimizu *et al*, (1996). Similarly, the works of Lerat *et al*, (1996) provided evidence of the replication of the virus within peripheral mononuclear cell of lymphoid or perhaps, bone marrow origin. Neumann *et al*, (1998) in mathematical models of viral kinetics, suggest a half-life of approximately 2.5 hours for virions in the blood stream. Further deductions made from the mathematical models; indicate that up to 1.0×10^{12} virions may be produced each day in a clinically infected human, a rate far in excess of the estimate of the production of HIV. Persistent infection appears to rely upon rapid production of virus and continuous cell-to-cell spread along with a lack of vigorous T cell immune response to HCV antigens.

1.4.2 HCV Genotypes, Disease Association and Distribution

Eleven HCV types and over 80 subtypes have already been described and shown to have diverse geographical distribution (Martins *et al*, 1998), while several studies have shown the importance of HCV genotypes and subtypes in the epidemiology and pathogenesis of HCV-related disease (McOmish *et al*, 1994). Even though there are clear global differences in the distribution of HCV subtypes the full significance of these subtypes are unknown (Laskus *et al*, 2004; Herring *et al*, 2005). Subtypes 1a-b are found in North and South America, Europe, Asia and the Far East; while 1c is common in Lebanon and Indonesia. Similarly, 2a-b is prevalent in America, Europe, Asia and the Far East; 2c in Argentina and Scotland; 2e-f in Indonesia; 3a in Europe, America, Asia and the Far East; 3b-f in Nepal, India, Singapore, Bangladesh; 4a-f in the Middle East (Egypt, Saudi Arabia and Jordan) and in Central and Northern Africa; 5a is found in South Africa; 6a in Vietnam, 6b in Thailand.1-3(McHutchinson *et al.*, 1998). There is however, a paucity of information on the genotypic distribution of HCV in most sub-Saharan African countries. Independent small scale surveys conducted in some West African countries including Burkina-Faso (Mellor *et al.*, 1995), Gambia (Ruggieri *et al.*, 1996) Ghana (Wansbrough-Jones *et al.*, 1998) Benin and Guinea (Jeannel *et al.*, 1998) have shown the predominance of type 2 in the region. Furthermore,

considerable sequence diversity has been associated with the type 2 infection reported in this region. Some studies have shown that in most instances each individual is infected with different subtypes, each in turn distinct from the 2a, 2b and 2c subtypes found in the Western countries. Similar extreme subtype diversity also characterised genotype 4 infection found in some Central Africa countries including Congo, Gabon and Central African Republic (Xu *et al.*, 1994 Menendez *et al.*, 1999; Stuyver *et al.*, 1993; Fretz *et al.*, 1995; Bukh *et al.*, 1993).

Pawlotsky *et al*, (1995) and Feray *et al*, (1995) about a decade ago had shown that HCV genotype 1b was more associated with severe liver disease than other types. Similarly it has been shown that HCV subtypes 1a and 1b do not respond actively in treatment with Interferon alpha (IFN-α), (Mahaney *et al*, 1994, Nousbaum *et al*, 1995 Pawlotsky *et al*, 1996; Nolte, 2001) often typically requiring twice the dose and duration of treatment; whereas sub-types 2a, 2b and 3a are three fold more receptive to treatment with IFN-α (Nolte, 2001)

This disparity in the distribution, pathogenicity and response to therapy has prompted some to advocate routine determination of HCV genotype to allow the tailoring of therapeutic schedules to specific genotypes as a necessary requisite for the effective management of HCV patients (De Cock and Vranckx, 2003). The implication of these is that a vaccine against multiple HCV genotypes is overdue, making an understanding of the geographical distribution of HCV genotypes necessary. The geographical distribution of HCV genotypes is influenced by biological characteristics, which include pathogenicity, sensitivity to therapy and prognosis in chronic active hepatitis and prevailing risk factors.

1.4.3 Epidemiology of HCV

HCV was first identified in the blood of chimpanzees and to date humans and chimpanzees remain the only host of the virus (Yen *et al,* 2003). Approximately 200 million HCV carriers exist globally. North and South America with and an estimated population of 786 million people is estimated to have 3.1 million people infected giving her a prevalence rate of 1. 7%, while the Eastern Mediterranean countries with a population of 466 million have an estimated prevalence of 4.6% with a total of 21.3 million infected. South East Asia and Western Pacific countries with populations of 1.5 billion and 1.6 billion respectively have a total of 94.5 million people infected giving the mean prevalence of the of the virus for the two regions as 3.0% (Zein *et al* 1996) Europe with population of 858 million is estimated to

have the worlds' lowest prevalence of 1.03 giving the total number of people harbouring the virus in the region to be 8.9 million. The highest global prevalence of 5.3% has been reported for the continent of Africa with a population of 602 million people giving her a total of 31.9 million infected people (WHO Weekly Epidemiological Record, 1999)

In all parts of the world the high risk groups for HCV infection includes injecting drug users, recipients of unscreened blood, haemophiliacs, dialysis patients and persons with multiple sex partners who engage in unprotected sex (Weekly Epidemiological Record, 1999). Some reports have shown that in developed countries up to 90% of persons with chronic HCV infection are either current and or former injecting drug users and those with a history of transfusion of unscreened blood or blood products (WHO Weekly Epidemiological Record 1999). In many developing countries where unscreened blood and blood products are still being used, the major means of transmission are unsterilized injection equipment and unscreened blood transfusions (Gisselquist *et al*, 2004) In addition, people who use traditional scarification, communal barbing devices (Zahraoui-Mehadji *et al*, 2004) and circumcision practices are at risk if they use or re-use unsterilized tools.

1.4.4 Transmission of HCV

The transmission of HCV primarily requires that infectious virions make contact with susceptible cells that are permissive for replication (CDC, 1993b; Thomas, 2000), thus, making blood transfusion, tissue and organ transplants (Fabrizi *et al*, 2001) the most efficient means of transmission of the virus. However, improved laboratory diagnostic facilities for the virus have made transmission through these means minimal in developed countries. Although cases of transmission by various means have been reported, some are still controversial. They include sexual transmission, maternal to infant transmission (Zanetti *et al*, 1995) and through saliva (Abe *et al*, 1991). Other documented cases include the simultaneous transmission of HCV and HIV from a needle-stick injury (Ridzon *et al* 1997). Available data indicates that seroconversion occurs in 0 to 10% of non-immune health care workers (HCWs) who sustain needle-stick injury from a source case with hepatitis C (Bolyard *et al*, 1998). However, no correlation between the HCV generation level in the source person and the risk of transmission has been established in any of the documented cases. Contact with a wound or splashes of blood may also result in rare transmission. It has been documented that in the

United States 1-2% of health care workers are HCV-positive, however this is lower than the prevalence in the general population (CDC, 1993b).

Studies have also shown that about 6% of babies born to HCV infected mothers become HCV-infected and that the figure may rise to 17% if the mother is also HIV-infected (Ruiz-Extremera. *et al*, 2000) Evidence available also supports the transmission of HCV through breast milk. However, this has not been accepted as a transmission mode for HCV (Mast, 2004; Mast *et al*, 2005).

1.4.4.1 Sexual transmission

The detection of HCV RNA in semen and saliva (Liou *et al*, 1992, Wang *et al*, 1992 and Fiore *et al*, 1995) has been extensively documented. However, among the early studies, there has been no general acceptance of the transmission of HCV during sexual intercourse. The controversies may be due to cases of HCV infection in instances where sexual, but not other, exposures were recognized (Healey *et al* 1995 and Capelli *et al*, 1997). Independent studies of long-term sexual partners of HCV infected haemophiliac patients and transmission recipient generally found no evidence of HCV transmission, even though there had been unprotected sexual intercourse (Everhart *et al*, 1990, Gordon *et al* 1992 and Brettler *et al*, 1992). However, early evidence in favour of sexual transmission of HCV but at a comparatively lower level of efficiency was reported by Donahue *et al*, (1991), Hallam *et al*, (1993) and Bodsworth *et al*, (1996). They found comparatively lower HCV prevalence among homosexuals than for other infections such as HIV, hepatitis B (HBV) infection, and syphilis, which share similar routes of transmissions. Similarly, in a study reported by Melbye *et al*, (1999) only 4.6% of cohorts of homosexual men were infected with HCV whereas 58% had been infected with HBV. Thomas *et al*, (1995) had advocated for further studies to determine several of the possible reasons for the relative infrequency of transmission which may include paucity of infectious virions in sexual or vaginal fluids or insufficient number of susceptible cells within the genital mucosa.

Despite these controversies, some studies have reported higher prevalence of HCV in female sex workers compared with the general population (Ndumbe and Skalsky 1993; Nakashima *et al*, 1992; Lissen *et al*, 1993). Similarly, it has been shown that in the United States the sexual transmission of HCV from a male to a female is more efficient than male to

male and that sexually transmitted HCV hepatitis constitutes about 20% of acute and chronic HCV infections in the United States (Alter, 1997). Ndumbe and Skalsky (1993) reported a prevalence of 15 percent in Cameroon, while Nakashima *et al*, (1992) found a prevalence of 6.2 percent in Japan. In Spain, Lissen *et al* (1993) reported a prevalence of 6.4 per cent in female Commercial Sex Workers (CSWs) and 6.8 per cent in their male clients in contrast to a significantly lower prevalence in the general population. In contrast, rates of seropositivity not higher than in the background population was reported by Chan *et al*, (1992) in Hong Kong and Watts *et al*, (1994) in Somalia. Generally, higher prevalence of the virus has been reported by several authors in persons with multiple sexual partners including commercial sex workers (Van Doornum *et al* 1991, Thomas *et al* 1994, Utsumi *et al* 1995). Similarly, some studies of patients attending clinics for sexually transmitted infections and others for commercial sex workers have confirmed that sexual behaviour and number of partners may influence the risk of acquiring HCV infection (Wu *et al* 1993 and Nakashima *et al*, 1996).

1.4.4.2 Co-factors for Transmission of HCV

The presence of a detectable level of HCV RNA in blood seems to be a factor in favour of HCV transmission (Thomas *et al*, 1998). Dore *et al* (1997) reviewed 2022 parenteral, sexual and perinatal exposures and found that only individuals with detectable viraemia transmitted HCV. Similarly, Thomas *et al*, (1998) showed that perinatal transmissions of HCV are rare when the level of viraemia is low. Some studies have also indicated that HIV-related immunosuppression may be closely associated with an increase in the level of HCV viraemia (Telfer *et al*, 1994, Eyster *et al*, 1994 and Darby *et al*, 1997), thus making infection with HIV an important co-factor for HCV transmission (Lam *et al*, 1993, and Gerberding *et al*, 1994).

Many host factors have been found to increase the risk of HCV transmission (Healey *et al*, 2000). These include old age, male gender, and immune-suppressed state, such as HIV co-infection (Hayashi *et al*, 1995). While documented environmental factors that may complicate or worsen the course of chronic hepatitis C include alcohol, iron overload, obesity, non-alcoholic fatty liver disease, schistosomal co-infection, hepatotoxic medications, and possibly environmental contaminants (Villa *et al* 1991, Yu and Yuan, 2004).

1.4.5 Risk factors for HCV transmission

It has been established that the prevalence of HCV in a population can be predicted by the risk factors associated with the transmission of infection (Strasser *et al*, 1995). These risk factors include drug use, blood product transfusion, organ transplantation, haemodialysis, and occupational injury, sexual and vertical transmissions (Vanderschuerea *et al*, 1991).

Risk factors for seropositivity to HCV have also been studied in sex workers. In Spain Lissen *et al*, (1993) found that the number of sexual partners was not a risk factor. However, in Japan, sex workers who had worked for more than one-year had a higher prevalence than those who had not been involved for more than one year (8.1%. v. 1.4%). Sex workers in Ghent, Belgium who reported a history of at least one episode of syphilis had a significantly higher prevalence of HCV. However, no data were available on intravenous drug use or a history of blood transfusion in these sex workers (Vanderschuerea *et al*, 1991).

Due to the increasing population of people infected with HCV who had no identifiable parenteral risk factors, the existence of nonparenteral routes of transmission of the virus has been suggested. Strasser *et al*, (1995) reported that 27 per cent of 342 consecutive anti-HCV positive patients presenting in a liver clinic in a major Australian metropolitan general hospital had no definite percutaneous risk factor. However, there have been a number of controversies particularly as regards the existence of a household route of transmission of the virus (Honda *et al*, (1993; Nakashima *et al*, 1996). Some of the problems associated with studies on household transmission are that members of a household often share behavioural characteristic that put them at risk of HCV, particularly intravenous drug use and that they may be reluctant to admit this. Important however, is that many studies have found a higher than background prevalence of HCV. Most studies have found higher prevalence in older household infants, and rates in children, which are similar to background rates (Hayashi *et al*, 1995). The reason advanced for this is that the shared behavioural factors, which led to transmission, were no longer relevant. In Japan, two independent studies (Nakashima *et al*, (1995) and Hayashi *et al*, (1995)) found higher rates of HCV in older people, and very low rates in children without other risk factors. However, the uses of injecting equipment in medical care were implicated as a possible cause of the high HCV rates in these areas.

Studies of people infected with HCV through medical procedures (eg people with haemophilia, dialysis patients) found no evidence of household transmission (Brackmann *et al*, 1993 and Hou *et al*, 1995). Also in Japan, Honda *et al*, (1993), found a greater degree of HCV nucleotide homology among family members with HCV than with infected non-family members suggestive of intrafamilial transmission. However, this is yet to be confirmed by others, who rather have found no clustering of genotype between spouses (Nakashima *et al*, 1996).

Other documented routes of possible household transmission of HCV include ritual blood exchange (Atrah *et al*, 1994), horizontal transmission due to sharing of non-sterile infecting equipment within families as part of medical care (Frosi *et al*, 1995), sharing of shaving equipment (Tumminelli *et al*, 1995) and tattoos. Reports also exist of the detection of HCV RNA in saliva, although a lower titre than in serum (Wang *et al*, 1991), although the contributory role of this a risk factor for the transmission of HCV is still unsure.

1.4. 6 Pathogenesis of HCV

When HCV gains access into the blood stream it infects the hepatocytes. In more than 80% of the cases the infection is self-limiting without symptoms (Falcon *et al,* 2005). In other instances, this is not the case resulting in a persistent infection leading to chronic disease. These symptoms, which occur, are due to the effect of the immune response, most especially the cytotoxic T cell response to the invading virus. This may include viraemia, which is detected one to three weeks after infection. Other symptoms include jaundice, abdominal pain, nausea, loss of appetite and pronounced dark urine. This may occur after the prodromal phase of two to seven weeks, sometimes extending to twenty-six weeks. This phase is referred to as the acute form. Even though at this stage the symptoms presented by HCV are milder than that of HBV and is very often asymptomatic, the infection cannot be clinically distinguished from acute cases of Hepaptitis A Virus and Hepatitis B Virus (Villamil *et al*, 1995; Memon and Memon (2002). By extrapolation from transfusion studies, Alter and Bradley (1995) observed that clinically apparent illness occurs in no more than 25 per cent of HCV infections.

HCV pathogenicity is enhanced by various defined and unknown conditions including co-infection with HIV or chronic HBV and alcohol consumption (Alter *et al*, 1989; Jarvis *et*

al, 1994). It is also greater in older people (more than 40 years) and in males (Yu and Yuan, 2005). HCV infected infants have been reported to do well with rare cases of severe HCV hepatitis.

Seroconversion to HCV has been documented by Alter *et al*, (1989), to take as long as 26 weeks following infection, although with second and third-generation assays the mean time between infection and the development of detectable antibody response is more often about two to three weeks (Aach *et al*, 1991; Mattson *et al*, 1992). However, it has been observed that HCV RNA could be detected in the serum even earlier, as soon as one week after infection (Farci *et al*, (1991). Brunetto *et al*, (1994) observed that anti-HCV detected during the convalescent phase of an acute self-limited HCV hepatitis becomes negative after one to four years, and its persistence indicates a chronic HCV infection. They also observed that post transfusion hepatitis proceeds to a higher number of cases than community-acquired sporadic hepatitis. Persistent HCV infection may last for many years.

Unlike Hepaptitis A Virus and Hepatitis B Virus, HCV appears to be the causative agent of a substantial number of fulminant hepatic failures (FHF) previously classified as indeterminate. However, documented cases of occurrence are extremely rare, (Viladomiu *et al*, 1992). This probably because the mechanism by which it occurs is obscure. However, the role of HCV in FHF is controversial. Reported series of FHF of indeterminate cause have shown a prevalence of HCV RNA by PCR of from 0 to 12 per cent in the USA and Europe, but it is substantially higher in Asia (Villamil *et al*, 1995). However, Villamil *et al*, (1995) observed that HCV appears to be the causative agent of a substantial number of cases of FHF classified as indeterminate in the Los Angeles area in the United States. They suggested that differences in patient populations or risk factors might explain the discordant incidences of HCV infection in FHF observed among different programs. In many patients with FHF, detection of anti-HCV antibodies remains negative although HCV RNA is detected; some of these patients go on to develop histological hepatitis after liver transplantation, sometimes severe and sufficient to require retransplantation (Villamil *et al*, 1995).

1.4.7 Clinical Diagnosis of HCV

Symptoms are the first aspect of diagnosis of HCV however, these do not occur in all cases (Villamil *et al*, 1995 Falcon *et al*, 2005). When they occur they includes jaundice,

nausea and fatigue accompanied by at least a ten-fold rise in alanine aminotransferase (Villamil *et al*, 1995) Antibodies against HCV are also clearly indicative but they do not appear until after the end of the prodromal phase (i.e. eight to twenty weeks after infection), throughout this period the virus is detectable in the bloodstream, while anti-HCV is detectable for several years if the patient is chronically infected. Thus, antibody is not a reliable indicator of acute infection (Leruez-Ville *et al*, 1998).

1.4.8 Laboratory diagnosis of HCV

HCV can be diagnosed using the markers of liver disease such as serum alanine aminotransferase (ALT) and aspartate aminotransferase (AST) activity (Jandreski, 1999). However, these are not specific and have no prognostic value. The specific test for the diagnosis of HCV is therefore the detection of antibodies to the virus or detection of the virus RNA. Several tests based on these have emerged since Choo and his colleagues identified and characterized non-A, non-B hepatitis virus in 1989. These may be classified into three broad groups for the ease of description.

1.4.8.1 Screening tests for hepatitis C infection

Most of the screening tests for hepatitis C infection are based on the detection of antibodies to HCV. These are mainly ELISA based and rapid test kits (Leruez-Ville *et al*, 1998). Most of these test have been undergone several modifications following improved technology. ELISA, which was the first commercially available anti-HCV test and the most widely used, has witnessed three generations of modifications that have made it more sensitive. Each generation has emerged as an improvement on the sensitivity of the other. The first generation test (ELISA-1) had c100-3 epitope from the nonstructural NS4 region of the viral genome incorporated. The second generation (ELISA-2) had NS5 protein incorporated, while the third generation (ELISA-3) detects antibodies to four recombinant HCV proteins and has the NS5 protein substituted by the 5-1-1 antigen (Gretch, 1997). Each generation has also shortened the average period for HCV seroconversion after blood transfusion. Gretch (1997) had reported HCV seroconversion after blood transfusion as 7-8 weeks if tested with ELISA-3, 10 weeks with ELISA-2 and 16 weeks with ELISA-1. ELISA tests also have the advantage of automation and ease of use. The major disadvantages however, include poor

sensitivity in the profoundly immunosuppressed and false-positives in low-prevalence populations (Leon *et al* 1998).

1.4.8.2 Supplemental or confirmatory tests for HCV

These are mainly tests employed to supplement or as a means of confirming the results of HCV screening tests. These include tests that identify antibodies to individual HCV antigens such as recombinant immunoblot assay (RIBA) (Lok 1997). Others are the third-generation RIBA (RIBA-3), RIBA HCV 2.0 (Damen *et al*, 1995) and INNO-LIA HCV Ab III and RIBA-3. INNO-LIA HCV Ab III and RIBA-3 have the advantage of not only serving as a confirmatory test, but as a good assessor of the possible response of a patient to interferon (INF).

This is possible because the INNO-LIA HCV Ab III and RIBA-3 tests expose several specific HCV peptides from two non-overlapping core regions (C1 and C2), E2, NS4 and NS5A regions, and recombinant NS3 of the HCV polyprotein. Thus the HCV non-structural 5A (NS5A) protein contributes to the interferon-resistant phenotype of HCV by repressing the action of PKR, a protein activated by interferon that shuts down viral protein synthesis thereby inhibiting replication of the HCV. These mechanisms have made both RIBA-3 and INNO-LIA HCV Ab III predictive markers for interferon (INF) resistance (Frangeul *et al*, 1998).

1.4.8.3 Molecular detection and quantitation of HCV RNA

Molecular assays for the diagnosis of HCV infection address the lapses of screening and some supplemental tests, thus while ELISA cannot detect early phases of HCV infection and cannot differentiate between active infection and disease resolution, nor can it be reliable in profoundly immunocompromised or haemodialysis patients, these problems do not exist if molecular assays are employed. Hence, the direct molecular qualitative detection of HCV RNA by reverse transcription (RT) and PCR is considered the gold standard for the diagnosis of HCV infection. It is also the tool of choice for the assessment of antiviral response to INF therapy (Lu *et al*, 1998). Thus these tools are very important in the detection of the extreme lower limit of the viral RNA. Particularly important is Transcription-mediated amplification (TMA), like the Versant HCV RNA Qualitative Assay (Bayer), which has a lower limit of

detection of 10 IU/ml and Amplicor HCV v2.0 (Roche), HCV v2.0 (Roche) with lower limit of detection of 50 IU/ml.

The quantitative assay of HCV can also be carried out using PCR techniques and its modification. Molecular quantitation of HCV RNA is particular very important in the clinical evaluation of patients before, during and after therapy. A handful of PCR assays for the quantitative assessment of HCV RNA levels, via signal amplification and quantitative PCR (Q-PCR) are today available among a host of PCR assays. The ability to detect minute amounts of HCV RNA in serum or plasma helps to resolve weakly positive or negative ELISA results when clinical signs and or risk factors are compatible with HCV infection. Commonly in use are the Cobas Amplicor HCV Monitor v2.0 (Roche) which has a range of 600 to 500,000 IU/ml, the branched DNA signal amplification technique such as the Versant HCV RNA Quantitative Assay (Bayer) has a quantification range of 615 to 7,700,000 IU/ml. Others include the SuperQuant (NGI) LCx (Abbott), and some real-time PCR techniques with a much higher range of quantitification.

1.4.8.4 Detection and quantitation of HCV quasispecies

All HCV isolates separate into phylogenetically related clusters called subtypes (Simmonds *et al,* 1993). Eleven HCV genomes have been documented (Maertens and Stuyver, 1997) so far as well as more than 80 subtypes (Stuyver *et al*, 1996) with more subtypes being discovered (Maertens and Stuyver, 1997). One or several subtypes can be classified into several major types that show similarities of 65 percent to 75 percent over the total genome (Simmonds *et al*, 1994, Ross *et al*, 2000.) The term genotype is used generically to refer to subtypes, types or both but not for quasispecies variants (Simmonds *et al*, 1994)

The quasispecies nature of HCV has been identified as an important factor in the inability of acutely infected individuals to clear the infection (Simmonds, 1999) and in the transmission of multiple infectious particles especially in communally community-acquired infection (Herring *et al*, 2005). It has been reported that additionally, mutations in the viral populations contributes to drug "resistance" during INF treatment and to the ineffectiveness of isolate-specific vaccines. Thus, quasispecies measurement can be used to predict INF responsiveness in patients infected with virus with mutations in HCV genotype.

Direct and indirect methods of detecting and quantifying quasispecies within an individual infected with HCV are available. However such studies are rare due to the difficulty of collection of samples during the early phase of the infection. Early studies were based on the use of cloned PCR products, a reliable but labour-intensive procedure. The modern methods include single-strand conformation polymorphism (SSCP), an indirect method involving the measurement of the number of different viral populations within an individual. In this technique PCR products are electrophoretically analysed under denaturing conditions to obtain single-stranded RNA (Laskus *et al,* 2004). The single nucleotide polymorphisms result in different mobilities of single-stranded fragments, thus depending on the quasispecies diversity, as well as the sensitivity of the RNA staining technique, a range of the most prevalent variants can be observed (Lee *et al.* 1997).

Unlike the indirect methods in direct sequencing, PCR products obtained are not cloned from PCR fragments, but all RNA strands with varying sequences are directly submitted en masse to sequence analysis. The presumed sequence represents the master sequence and can show degeneration at certain positions, that is, certain positions may, for example, show both an adenine and guanine residue. Degeneration can only be observed when the minor sequence is observed in 20 percent or more of the RNA strands (Maertens and Stuyver, 1997).

1.4.9 Genotyping

Several screening tests are available for the identification of HCV genotypes (Pawlotsky *et al*, 1997; Ross *et al*, 2000). However, the gold standard is sequence analysis of variants in the 5' non-coding region (5'NCR) and in the genes encoding the core protein, an envelope protein (E1) and a non-structural protein (NS-5). Available data show that the sub genomic regions of the virus such as E1 and NS-5 contain sufficient phylogenetic information for the identification of each of the 11 or 12 known types and subtypes of HCV (Simmonds *et al*, 1994).

These includes reverse hybridization line probe assay (LiPA), restriction fragment length polymorphism (RFLP) of the PCR amplicons, and nested PCR with genotype-specific parameters to the core region. LiPA technology is based on the reverse hybridization principle in that biotinylated PCR fragments are hybridized to a selection of highly specific

immobilized probes (Ansaldi *et al*, (2001). So when the biotin group in the hybridization complex is exposed by incubation with a streptavidin-alkaline phosphatase complex with the appropriate chromogen compounds it will allows the discrimination of nucleotide differences in the 5' UR. LiPA has been shown to be a reliable means of identifying all HCV types and most subtypes making very suitable for routine use (Stuyver *et al*, 1996).

However, in RFLP analysis a single PCR fragment is usually amplified from a specific region of the HCV genome with universal primers. Restriction enzyme recognition sites present in the DNA fragment usually show subtype or type-specific distribution. Thus, restriction fragments with varying lengths are created after cutting the PCR fragment with one or several restriction endonucleases. The fragments are electrophoretically separated to allow the observation of the approximate lengths of the restricted fragments and thus identify the genotype.

1.4.10 Serotyping

HCV serotyping is gradually gaining popularity as an efficient alternative to genotyping and in many developed countries it is becoming a routine test for patients with chronic hepatitis (Stuyver *et al*, 1996). The available tests are mainly based on the detection of genotype-specific antibodies directed to epitopes encoded by the NS4 region of the genome (Kobayashi *et al*, 1999). These are however, comparatively less sensitive than molecular assays and also have the disadvantage of cross-reactions between different types, which can result in mistyping. Furthermore, sub typing is not possible with the existing peptides, thus it has been found more useful for routine diagnosis. However, where sub typing is not a rule, other disadvantages are marginal and several comparative studies have found it a good alternative to molecular assays, especially in third world countries where facilities for molecular assay do not exist.

1.5 HIV and HCV co-infection

HIV and HCV have emerged as the cause of two major viral infections within the past two decades (Sulkowski *et al*, 2002), and co-infection represents a major problem in the developed countries and a growing future problem for sub-Saharan Africa countries with high prevalences of HIV and HCV.

Co-infection between HIV and HCV has been associated with higher hepatic C viral load, accelerated progression to HCV-related liver disease, and an increased risk for liver cirrhosis. The broad distribution of HCV in HIV infected persons has prompted its inclusion as an opportunistic infection in HIV-infected persons (USPHS/IDSA, 1999), however, it is yet to be accepted as an AIDS defining illness (Sulkowski *et al*, 2002). The effects of HCV co-infection on HIV disease progression are still saddled with uncertainty. There is however, a paucity of information on its distribution in most developing countries including sub-Saharan African where both infections have the highest prevalence. Some studies have provided evidence suggesting that persons infected with HIV and HCV have an accelerated decrease in CD4+ count (Kim *et al,* 2005).

1.6 Global epidemiology of HIV and HCV co-infection

Dodig and Tavil (2001) estimated that 30% to 50% of patients with HIV are co-infected with HCV. There is however, gross variation in the geographical distribution of infection globally. The prevalence therefore depends on the risk factor of the sampled population, with persons with repeated exposure to blood or its product at a greater risk (CDC 1998).

In most third world countries data on the global distribution of HIV and HCV co-infection is scanty. In the developed world most of the available data focuses on intravenous drug abusers and other high-risk groups (Coppola *et al*, 1994 and Monga *et al*, 2001). Few studies exist on the normal population at risk of these co-infections. In a study conducted in Ireland Healy *et al*, (2000) found an HIV/HCV co-infection of 4.7 percent in women of childbearing age. Similarly, Eyster *et al*, (1999) found a comparatively higher HCV prevalence among haemophiliacs infected with HIV.

Although more studies are needed to better understand the affect of HIV on HCV progression over the longer term a number of studies show that HIV can accelerate liver disease progression (Anderson *et al*, 2004; Carter, 2004), while others found no such difference (Sulkowski *et al*, 2002) It is however known that the critical damage to the immune system of an HIV infected person is what is responsible for the chronicity of HCV in HIV/HCV co-infection (Marcellin *et al*, 1994). It is also known that liver disease progression can lead to cirrhosis and liver cancer and acceleration may lead to progression in a shorter

period of time (Sulkowski *et al*, 2000). It is estimated that as many as 60-90% HIV-infected persons in the United States and Western Europe may also have hepatocellular carcinoma (HCC). It is also envisaged that the disease burden from HCV is likely to rise considerably over the next 10-20 years (Sulkowski *et al*, 2000). The projections are that there could be an increase in the number of patients with cirrhosis over the next 8 to 10 years and that this could be in the order of 500%, which could cause increasing demands on liver transplantation (Sulkowski *et al*, 2000). The cost of health care is also likely to rise significantly. The impact of this on most third world countries where health facilities are already over-stretched enhanced by dwindling poor funding cannot be over-emphasised.

1.7 HIV and CD4+ Cells.

Major variations have been reported in normal range of CD4+ lymphocyte count among different ethnic groups. A study conducted in Thailand indicated that uninfected Thai adults had significantly lower absolute CD4+ count than uninfected Caucasian adults (Stein *et al*, 1992). Furthermore, the study also revealed a significant variation in CD4+ count due to population variation.

Few documented studies have evaluated CD4+ variation in HIV patients co-infected with other viruses irrespective of the fact that HIV associated CD4+ variation has been universally accepted as a major criterion for the clinical evaluation and management of HIV infected patients and may possibly be applied as a marker for such co-infection (Stein *et al*, 1992).

CD4+ cells have essential helper/inducer functions in both cellular and humoural arm of specific immunity (Sattentau *et al*, 1992). Similarly, the CD4+ glycoprotein on the surface of human CD4+ cells and macrophages has a high affinity receptor for HIV virus and envelope glycoprotein 120, while CCR5 or CXCR4, both of the chemokine receptor family act as essential co-receptors with CD4+ to facilitate HIV entry into target cells following a successful HIV infection (Gallo *et al*, 2001). Depletion of peripheral CD4+ cells and progression to AIDS occurs as a result of HIV viral burden. Thus HIV has been shown to efficiently mediate the destruction and depletion of mature CD4+ cells in addition to the depletion of CD4+ progenitor cells in bone marrow, thymus and peripheral lymphoid organs. The ultimate end of this is the failure of compensating T-cell production and eventual collapse

of the immune system (McCune, 2001). Thus, CD4+ cells have been indisputably accepted as the single best marker of progression and survival in-patients with AIDS. The CD4+ count is also useful in monitoring therapeutic efforts in HIV infection and low counts are correlated with high plasma viraemia and low p24 antibody titres (Praphan *et al*, 1994). Furthermore, the influence of CD4+ cells by conditions unrelated to HIV infection has been documented (Stein *et al*, 1992 and Praphan *et al*, 1994). These include endemic pathogenic organisms, mostly peculiar to developing countries (Lee *et al*, 1991 and Praphan *et al*, 1994). However, the in CD4+ count variation in co-infection with other viruses needs to be established.

1.8 AIMS OF THE STUDY.

In general:

This project aims to answer three questions relating to HIV, HCV, and HIV/HCV co-infection in the Gambia.

In particular:

1. What is the prevalence of HIV, HCV and co-infection between HIV and HCV in the Gambia?

2. Does HIV co-infection with HCV complement or complicate its clinical course? Is there any significance difference in the case of mono-infection with HIV?

3. What socio-demographic and risk factors are associated with the distribution of HIV, HCV co-infection between HIV and HCV in the Gambia?

CHAPTER TWO

2.0 STUDY SITE, MATERIALS AND METHODS.

This chapter features information on the study site, materials employed and a description of the test procedures.

2.1 The Gambia.

2.1.1 Geography

The Gambia is a small coastal country with a total geographical area of 10,690 square kilometres. The diameter of the country ranges from 25 km at its eastern tip to approximately 48km at the western end. The Gambia is equidistant from the Equator and the Tropic of Cancer and lies at 15° longitude. It is bounded to the north, south and east by Senegal and by the Atlantic Ocean to the west.

The Gambian climate is characterized by a long dry season from October to early June and a short rainy season from mid-June to early October (Sudano-sahelian type). The annual rainfall ranges from 800 mm in the east to 1700 mm in the western region with a mean of 1020 mm. The Gambian economy is very dependent on rain-fed agriculture so turnover of agricultural products are usually grossly affected any year there is severe drought.

The River Gambia bisects the country into two, forming the North and South Banks. For administrative convenience The Gambia is divided into two municipal areas (Banjul and Kanifing) and five divisions, namely Central river division with headquarters at Janjanbureh, Lower river division (Mansa Konko), North Bank Division (Kerewan), Upper River Division (Basse) and Western division (Brikama). The Royal Victoria Teaching Hospital, the study site of this work, is located in Banjul, the capital and administrative centre of the country. The city is an island situated on the estuary of the River Gambia and also serves as one of the two municipal areas of the country.

2.1.2 Socio-demographic characteristic

The population of the Gambia at the end of the year 2002 was given by the World Health organization report (2004) as one million three hundred and eighty eight thousand (1, 388, 000). Close to two fifths of this is based in the Western Division with Banjul, Serekunda and Brikama the most populated, while the Central and Upper River Divisions are more sparsely populated.

The population consists of several ethnic groups. The Mandinkas (36.6%), Fulas (17.0%), Wolofs (13.2%) and Jolas (9.4%) are the predominate groups in the country. Other groups include Manjago, Serahuli, Serer and Aku. The country also has a smaller numbers of people from Mauritania, Lebanon and Europe and ECOWAS countries. The dominating group from the West African region are the Sierra Leonean, which were displaced during the war in their country.

English is the official language in the country but most people speak at least one of the local languages, while a good number, especially the uneducated, do not speak English.

The Gross Domestic Product (GDP) per capita for the year 2001 was given as $1,214(US), while the total health expenditure as a percentage (%) of GDP was 6.4%.

Similarly the total health expenditure per capita for the year 2001 was $78.00(U.S). (World Health report 2004)

Life expectancy at birth for male and females has been estimated as 55.4 and 58.9 years respectively. Similarly, healthy life expectancy at birth has been given as 48.5 and 50.5 years for male and females respectively. Male child mortality (per 1000) was estimated at 132 for the year 2001, while the female child mortality rate (per 1000) was 117. Similarly, adult male and female mortality rate (per 1000) for the same year stood at 330 and 265 respectively.

2.1.3 Social norms, traditional practices and marriage.

Gambian society like most West African communities maintain and respect several traditional practices, some which have been shown to increase the risk of transmittable blood borne infections. Early marriages, wife inheritance, female circumcision, polygamy are in most cases societal norms. Marriage is arranged and regulated by kin to support the primary goal of reproduction and accord sexual and reproductive rights (Oppong, 1992). In the words

of Caldwell, 1996 "Even after marriage, men and women remain embedded within a strong kin net work and tend to move in spheres differentiated by gender and age that intersect only in specific ways." Through marriage, men enjoy benefit of the of women's reproduction and labour while women are granted access to land (usually controlled by the men's kin), paternity for their children and the security of the men's kin group (Drapper, 1989; Caldwell, 1996). In rural Gambia after the obligations in a marriage agreement have been met to the bride's satisfaction, the wife moves her residence to her husband's compound. Early marriages are common for women among the Fula tribe with effective constrains on premarital sex (Caldwell, 1996). Generally, within the Fula tribe of the Gambia, the women marry at even 13 while the men may marry at 17. Marriages between even first cousins or members of the same kin, including a son's right to inherit the brother's wife, are seen as a protective means for the woman and to retain their protective potential within the kin (Drapper, 1989).

Within the Gambia, polygamy is common within the Muslim population. Women are usually not given the right to oppose a polygamous union for their husband. In a nationally representative survey conducted in 1990, 50 % of the women aged 15-49 and 36% of the men 18 and above were involved in a polygamous relationship (Speizer, 1995). Among the three groups represented in the study area polygamy were more common with the Mandinka and least common among the Fula. Levirate marriages are also based on substitution with broad kin categories. These marriages allow for a replacement of a dead husband through an inheritance of widows by a brother or agnatic cousin of the deceased. Female circumcisions are considered a very sacred and mandatory requirement for any female among the Fula tribe of the Gambia. The practice is declining gradually among other tribal groups. However, the extremists still consider an uncircumcised girl unfit for marriage.

2.1.4 Health system

The Ministry of Health, Social Welfare, and Women's Affairs is responsible for health care in the country. For each health division, a divisional health team coordinates health care. Village Health Workers and traditional Birth Attendants provide the lowest level of health care. These are generally illiterates but have been trained in courses of 6-8 weeks to treat minor illness and refer more severe cases to a health centre. Community health nurses

supervise them. Minor health centres are located in at bigger villages or small towns. There are manned by nurses, nurse midwifes and public health inspectors. They are the first port of referral; provide preventive services such as vaccination and antenatal care, curative services and deliveries. They generally have small number of beds for inpatients but are not encouraged to retain patients with more severe conditions. Major health centres usually have a doctor in charge, a larger number of beds for inpatients. The next level of referral is five hospitals in four different divisions and the Royal Victoria Teaching Hospital in Banjul.

Outside the government sector, health facilities are run by private practitioners mainly in urban areas and by non-governmental organisations. The Medical Research Council maintains an out-patient clinic and 40-bedded hospital caring for medical and paediatric conditions. In Sibanor, a Protestants mission society, Worldwide Evangelisation for Christ (WEC), runs a major health centre with 38 inpatient beds. Generally, the provision of health care around the country is patchy; patients may have to travel long distances to see a doctor. Clinics are often extremely busy and limited diagnostic facilities are available. Laboratory facilities for the diagnosis of HCV or any other hepatitis virus are yet to commence in any of the government-owned hospitals.

Blood for transfusion is not screened for HCV or any of the known blood borne viruses other than HIV. At the Royal Victoria Teaching hospital in Banjul screening facilities for antibodies to HCV was briefly introduced in the first quarter of 2001 and the facilities only lasted for three months (with the exhaustion of the test kit).

The officially documented annual incidence of diagnosed HIV in the Gambia is around 250 per 100,000 populations, so officially there are around 1,000 new cases per year. Around two third of new patients are males and the mean age at diagnosis is around 21 years for female and 25 for males. The sex difference in age of diagnoses may be partly due to early marriages, which is a common practice in most parts of the country (Draper, 1989; Kane *et al,* 1993). HIV cases are usually diagnosed at almost all the six government owned hospitals and at the Medical Research Council (MRC), Fajara.

2.2 The Study site.

The study site is the Royal Victoria Teaching Hospital (RVTH). The hospital, which has a 250-bed capacity, is located in Banjul, the capital city of the Gambia (Fig.6, page 58).

The hospital was approved for the training of medical students and attained a teaching hospital status in October 2002. It is the biggest hospital in the country and registered over 260,000 in and outpatients in year 2003. Her services are open to all with the bulk of patients from Banjul, Serrekunda and other nearby communities. The hospital also serves as the main referral centre for the six government-owned hospitals and health centres in the seven divisions of the country and for private clinics. The study population therefore, has a broad multi-socio-economic background.

2.3 The Subjects

The initial study population were all persons seen consecutively at the Royal Victoria Teaching Hospital, Banjul between July and December 2003 with a request for HIV test regardless of their age, sex, or clinical history until a sample population of 1500 was attained. The subject thus includes blood donors, pregnant women, patients and applicants for medical examination predominantly for employment, educational and travelling purposes among other reasons. The results of the preliminary screening tests of all study population for HIV and HCV and an evaluation of the questionnaires administered on each subject and informed consent to participate in the study were the criteria employed for the selection of subjects for the follow-up study.

2.3.1 Case Study group.

This group consisted of consented patients screened positive for either HIV or HCV or HIV and HCV following the preliminary examination of the 1500 persons.

2.3.2 Control group

These are subjects without evidence of HIV and or HCV infection age- and sex-matched with the subjects with HIV and or HCV infection. Each study subject was matched with 3 to 6 control groups depending on the number meeting up with the criteria.

2.4 Ethical approval

This study has the approval of the joint ethical committee of then then Royal Victoria Hospital, Banjul now RVTH and the Department for Health of the Gambia Government. Individual consent was also obtained from all participants. In the case of children and adolescents, the parents or guardians consents were obtained. Subjects who refused consent

were excluded from the study. Subjects were made aware that granting their consent included a decision to allow a follow-up where necessary.

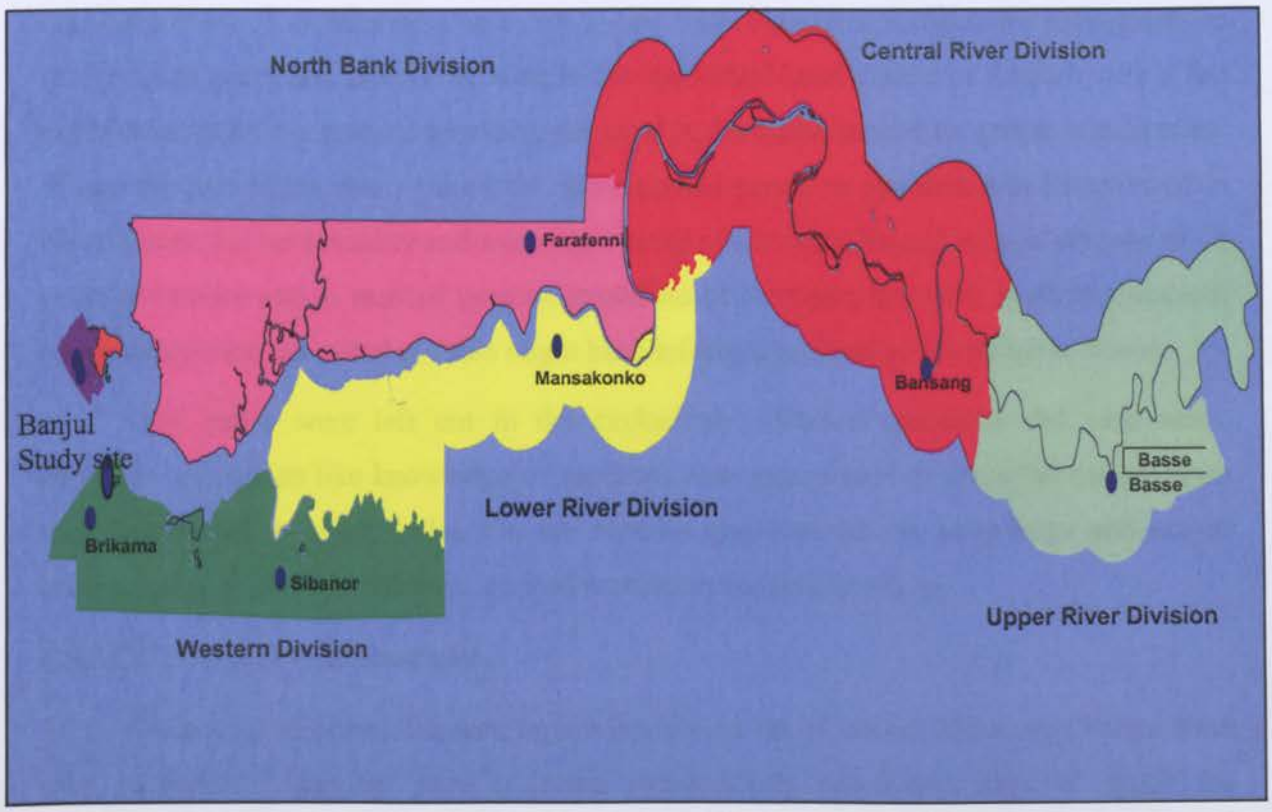

Fig. 6: Map of the Gambia showing the study site. (www.travel.state.gov/gambia.html)

2.5 METHODS

Standard laboratory methods were employed in every test carried out and in the case of test kits the manufacturer's instructions were strictly adhered to.

2.5.1 Counselling and administration of questionnaires

Each subject was informed of the study and those who accorded their acceptance were counselled. Counselling was on a one-to-one basis and was done in English language or in the local language or dialect by trained counsellors or the researcher. Post counselling acceptance to participate in the study was the consent instrument for enrolment in the study. Participants who refused consent after the counselling were excluded from the study.

Questionnaires pre-designed to collate information (appendix 1, page 213) on each subject's socio-economic/demographic/ behavioural risk factors were administered on all participants following an informed consent. Administration of the questionnaire was carried out in the form of an interview on a one to one basis making it difficult for participants to ignore some questions. Interviews were in the individual local dialect or English with a full explanation of all the medical terminologies used in the questionnaire for proper clarification. Where the participant was a minor the accompanied parent or guardian was interviewed in place. Questions on sexuality and marriage among others were limited only to persons of 18 years and above and to married persons regardless of their age, however, in-depth questions on sexuality were not asked as these might have infringed on local and or religion taboos.

The males were left out in the exclusively feminine questions and vice versa. However, a question like knowledge of condoms was opened to both sexes but questions on their use limited to adult males. On the contrast questions on the knowledge and use of contraceptive pills were limited to married women and to female adults.

2.5.2 Collection of Blood samples.

Following informed consent, approximately 10 ml of venous blood was drawn from each participant. Samples were collected consecutively (excluding days in which the researcher was not able to work) until a sample population of 1500 was attained. Samples from pregnant women were collected during their registration visit to the antenatal clinic irrespective of their trimester of pregnancy. Approximately 2 ml of each person's blood sample was dispensed into an EDTA container for CD4+ count. The remaining sample was dispensed into a non anti-coagulant container and centrifuged and the serum separated and frozen in two aliquots. One aliquot was preserved at –20°C for short-term use and the other at –70°C.

2.5.3 Screening test for HIV antibodies using ELISA test kit

Principle

The Murex HIV-1, 2, 0 test is based on the use of microwells coated with synthetic peptides representing an immunodominant region of HIV, HIV-1 (0) recombinant protein derived from the envelope proteins of HIV-1 and HIV-2 and HIV core protein. The conjugate

is a mixture of the same epitomes, all labeled with horseradish peroxidase. When samples and controls sera are incubated in the wells, antibodies to HIV bind to the antigens on the microwells if present. When test samples and any excess antibodies are washed and conjugate is added it in turn binds to any specific antibody already bound to the antigen on the well. A sample not containing any specific antibody does not cause the conjugate to bind to the well. When the unbound conjugate is removed by a washing process and solution containing tetramethlybenzidine (TMB) and hydrogen peroxide (substrate solution) is added to the wells and incubated; bound conjugate develops a purple colour which is converted to an orange colour when the reaction is stopped with sulphuric acid. The amount of conjugate, and hence colour, in the wells is directly related to the concentration of antibody to HIV in the test samples or controls, the degree of colouration is therefore read spectrophotometrically at 450nm.

Test Protocols

Preserved sera were screened every two weeks individually for HIV antibodies using enzyme linked immunosorbent assay (ELISA) kits Murex HIV–1,2,0 (Murex Biotech, UK) (Lek, 2003) following the manufacturers instruction.

Kit accompanied Microtitre test wells were selected on the basis of the number of available test samples and a work sheet prepared for all the samples and controls to be tested. This was followed by the addition of 50μl of the sample diluents to each well. For each plate 50 μl of the manufacturer's negative control sample was added into each of three wells in the first column (A1-C1) and 50 μl of the anti-HIV-1 and HIV-2 positive controls into wells D1 and E1 respectively. Similarly, 50 μl of known anti- HIV-1 and HIV-2 positive controls (Internal controls) were added into wells F1 and G1 respectively. In each circumstance this was followed by the addition of 50μl of each test sample to the wells in sequential order following the work sheet. The wells were covered with the lid and incubated in a water bath maintained at 37^0C for 30 minutes.

At the end of the incubation period each plate was washed manually using working strength wash fluid supplied by the manufacturers. This was accomplished by aspirating the first row of the wells and completely filling it with a working strength of washing fluid without allowing it to over flow; this was allowed to soak for about 30 seconds. The process

was repeated for each row of wells in turn. The whole washing process was repeated for a further four times for each row of wells in turn. At the end of the final washing exercise the wells were inverted and tapped dry on tissue paper and 50μl of conjugate added to each well using a multi-channel pipette and incubated in a water bath maintained at 37^{0}C for 30 minutes. At the end of the incubation period the plates were washed five times as previously described, drained dry and 100 μl of substrate added to each well. The wells were covered with a lid and incubated again in a water bath maintained at 37^{0}C for 30 minutes. At the end of this second incubation period 50μl of 0.5M sulphuric acid (Stop solution) was added to each well. The plates were read at 450nm within 15 minutes using an ELISA plate reader and the absorbance of each sample was recorded.

Calculation of results

The mean absorbances of the negative and positive controls were determined on each occasion the tests were carried out. Any negative control with an absorbance of 0.15 optical densities (O.D) above the mean of the three was discarded and the negative control mean calculated from the two remaining replicates.

Cut-off value

Adding 0.2 to the mean of the negative control replicates gave the cut-off value.

Negative results

Samples that gave results below the cut-off value of the kit negative control in the assay were recorded as anti-HIV negative.

Reactive results

The samples that gave an absorbance equal or above the cut-off value were considered initially reactive in the assay and were preserved for confirmatory test.

Limitations of the assays

The results of the assays were considered valid only if the mean of the absorbance of the negative control samples was less than 0.3 and the absorbance of the each of the positive controls were 0.8 above the mean of the mean absorbance of the negative controls. Assays that did not meet these criteria were repeated.

These test procedures were applied for each batch of sample screened until the sample size of 1500 was reached. A summary of these results is presented in Table 2, page 75.

2.5.4 HIV confirmatory test

All samples reactive to Murex HIV-1.2, 0 were further tested using PEPTI-LAV 1-2 (Sanofi, France) (Lek, 2003) for confirmation of the presence of antibodies to HIV and for differentiation into subtypes following the manufacturer's instructions.

The PEPTI-LAV 1-2 is a unit test using a membrane fixed on a plastic strip as a solid support. Two antigens are fixed on this membrane, HIV-1 specific peptide fixed on the membrane extremity opposite the plastic support, HIV-2 specific peptide fixed between the HIV-1 specific peptide and the control band and finally the control band located on the membrane extremity close to the plastic support.

Principle

The test is an immunoenzymatic technique based on the principle that if a sample containing antibodies to HIV-1 and or HIV-2 is incubated with a PEPTI-LAV 1-2 strip they bind to the corresponding peptide fixed on the strip and that if peroxidase–labelled anti-human IgG antibody is added after washing, it binds in turn to the specific IgG captured by the peptides on the solid phase and to the control band consisting of IgG. Finally, when the substrate is added after elimination by the washing of the fraction of conjugate left free, the presence of the enzyme fixed on the complex compounds is shown by the development of bands on the portion of the strip corresponding to the type of HIV antibody present in the sample.

Test procedures:

Exactly 30 µl of each sample screened positive using Murex HIV-1, 2, 0 was added to pre-labelled graduated test tubes containing 3ml of ready to use sample diluents supplied with the kit. One PEPTI-LAV test strip was introduced into each tube, tube capped and placed under a horizontal agitator at room temperature for 1hr. At the end of the incubation period each tube was turned upside down to remove the solution without allowing the strip to fall. Each strip was washed to remove the excess sample by the addition of the kit accompanied washing solution diluted 1/10 with distilled water. Each tube was completely filled to the

brim with the diluted washing solution, stirred and allowed to incubate for 2 minutes. At the end of the incubation each tube was emptied and the filled. The filling and empty process was repeated twice to give a total of three washings per test.

3ml of the sample diluents was added to each tube followed by the addition of 150μL of the conjugate. Each tube was capped with a colourless stopper (supplied with the test kit), homogenised and incubated at room temperature for 30 minutes. At the end of the incubation the excess conjugate of each tube was washed thrice as previously described.

The development phase of each test sample was carried out in a prepared development solution containing 3ml of reconstituted chromogen solution, plus 150μl of substrate and stirred to disperse. Each sample test strip was dipped into the development solution and tube paced on a shaker, shaken for three minutes. The development of each strip was stopped by removing from the development solution, dipped in distilled water and dried at room temperature.

Reading of results:

A negative result was marked by the absence of any band on HIV-1 and HIV-2 peptide positions of the strip. A positive result was marked by the presence of a band on the HIV-1 peptide position (HIV-1 infection) or on the HIV-2 peptide (HIV-2 infection) position or on the peptide positions of HIV-1 and HIV-2 positions (dual infection)

Samples reactive to Murex HIV 1, 2, 0 but un-reactive to PEPTI- LAV 1-2 were considered non- reactive. Those reactive to Murex HIV 1,2,0 and reactive to PEPTI LAV 1-2 either on the HIV- 1 band or HIV-2 band or on both bands were confirmed to have antibodies against HIV. The test was repeated for all the samples reactive to Murex HIV 1, 2, 0. Summaries of these results are presented in Table 3, page 76.

2.5.5 CD4+ counts

The CD4 count of every sample collected was determined using the immunomagnetic cell isolation method (Diagbouga *et al*, 2003) within four hours of collection. The test procedures recommended by the manufacturers was adhered to.

Principle:

This test is based on the use of antibody coated magnetic beads (Dynalbeads) for isolation of CD4+ lymphocytes directly from whole blood. Dynalbeads exhibits magnetic properties when placed within a magnetic field, but have no residual magnetism when removed from this field. The ability of Dynalbeads CD® and Magnetic Particle Concentrators (MPC)-Neodymium-iron-boron magnet (MPC-Q) to deplete monocytes that may also express CD4+ antigen ensures more accurate CD4+ cell counts.

Test procedures

Exactly 225 microlitres (μl) of diluent buffer was added to pre-labelled 1ml test tubes followed by 250 microlitres of each well-mixed EDTA collected blood sample. To each tube was added 25μl of Dynalbeads ® CD14 diluted 1:1 in washing/dilution buffer. Each tube was capped and moved gently by inversion. Tubes were incubated at room temperature for 10 minutes with tilting and rotation action all through. Each tube was then placed in a Dynal MPC for 2 minutes followed by the addition of 200μl of the monocytes depleted blood samples into another set of pre-labelled tubes.

Isolation of CD4+ cells

200μl of washing/dilution buffer were added to each tube containing 200μl of each sample of monocyte-depleted blood. This was followed by the addition of 25μl of Dynalbeads CD4+ to one each of the test tubes. Each tube was capped and mixed properly by inversion. Incubation was at room temperature with tilting rotation action for 10 minutes, followed by placement in a Dynal MPC for 2 minutes and discarding of supernatant. Exactly 500μl of washing/dilution buffer was added to each tube and covered with parafilm sheet. The cells were re-suspended by inverting the tubes a few times. The parafilm sheet was then safely disposed of and the magnet refitted for 2 minutes. The supernatant was discarded in each case and washing repeated once again.

Counting of cells

The cells were re-suspended in of 50 μl of lysis solution mixed, vortexed and allowed to settle for 5 minutes at room temperature. Exactly 50μl of acridine orange was added to each tube and mixed. Counting was done immediately where delay was envisaged the sample was stored refrigerated for not more than an hour. A Neubauer counting chamber was employed for each counting and the cells in an area of 1mm by 1mm with a depth of 1mm were counted. The test procedures were repeated for each of batch of samples examined until a sample size of 1500 was reached. A summary of the results obtained is presented in Table 3.

Calculation:

The number of cells counted for each sample was multiplied by 10 and expressed per microlitre of blood.

2.5.6 Screening Test for HCV Antibodies using Ortho HCV Version 3.0 Enzyme Immunoassay (EIA) Test Kit

The ORTHO HCV 3.0 EIA Test kit (Ortho Clinical Diagnostics, USA) was used to screen all the samples for antibodies to HCV (Galel *et al*, 2002 and Tobler *et al*, 2003). The Ortho HCV Version 3.0 test is a direct solid-phase enzyme immunoassay. According to the manufacturers, this test has an improved sensitivity (99%) and specificity (99.9%), compared with earlier generation tests since it includes the core and the non-structural (NS) genome regions of HCV NS3, NS4 and NS5(Galel *et al*, 2002).. The standard testing protocol, as specified by the manufacturer, was adhered to at all times.

Each collected sample was screened for antibodies to HCV on an individually basis Screening was carried out in batches on weekly basis or within 10 days of collection using the refrigerated aliquots.

Test principle

The test is based on the principle that when human serum or plasma containing anti-HCV is incubated in a microwell coated with recombinant HCV antigen and the plate is washed and peroxidase-conjugated antibody directed against human IgG is added to the well and further reincubated followed by the addition of a peroxidase-specific chromogenic substrate solution. The conjugate will react with the substrate solution specifically resulting in

the generation of an orange colour. The intensity of the orange colour generated is directly dependent on the amount of anti-HCV present in the test sample.

Test Reagents.

The following reagents prepared by the manufacturer were contained in the Ortho HCV Version 3.0 ELISA Test System (1000-test kits).

(a) Hepatitis C virus encoded antigen

This consists of recombinant c22-3, c200, and NS5, coated unto microwell plates.

(b) Conjugate

The conjugate was antibody to human IgG – anti-human IgG heavy chain (murine monoclonal) conjugated to horseradish peroxidase with bovine protein stabilizers and 0.02% thimerosal as a preservative.

(c) Specimen diluent

This is phosphate-buffered saline with bovine protein stabilizers containing 0.1% 2-chloroacetamide preservative.

(d) o-Phenylenediamine · 2HCL

This was supplied in tablets form (OPD- tablets)

(e) Substrate buffer

The substrate buffer was Citrate-phosphate buffered with 0.02% hydrogen peroxide and 0.01% thimerosal preservative.

(f) Positive control

This was inactivated human serum or plasma containing anti-HCV and non-reactive for hepatitis B surface antigen (HBsAg) and antibody to human immunodeficiency virus type 1 (HIV-1) and type 2 (HIV-2) containing 0.2% sodium azide and 0.9% EDTA

(g) Negative control

This was human serum or plasma non-reactive for HBsAg, antibody to HIV-1, antibody to HIV-2 and anti-HCV and contained 0.2% sodium azide and 0.9% EDTA as preservatives.

Test procedures

(a) Preparation of Substrate Solution

The quantity of substrate solution prepared on each occasion was dependent on the number of samples to be tested. The calculation was based on 20 microlitre of substrate solution per microwell plate. Following the operating guidelines from the manufacturer one OPD tablet provided enough substrate solution for 24 wells and two tablets for 48 wells and in similar proportion where more wells were used. The procedures enumerated here are for 48 wells, which was the average number of samples, performed on most occasions.

Procedures

(a) Two OPD tablets that have been allowed to attain room temperature were dissolved in 12μl of substrate solution contained in a plastic container. This was usually prepared prior to the second incubation and stored in a dark cupboard to protect from light until required for use. It use was however always within 30 minutes.

(b) Preparation of working wash buffer (1X) (1/2 Litre)

25μl of 20X wash buffer concentrate was added to 475mL of distilled water. This was stored refrigerated and used within two months.

Assay Procedure

(i) The required number of wells was selected on a microtitre plate

on the basis of the number of test samples available and a work sheet prepared for all the samples and controls to be tested.

(ii) Exactly 200μl of the specimen diluent was added to all wells except well A1.

(iii) 10μl of the manufacturer's negative control sample was added into each of three wells in the first column (B1-D1) and 10 μl of the positive controls into wells F1 and G1.

(iv) 10μl of known ant-HCV positive serum sample were added to wells G1 and H1 (internal control)

(v) To all the other wells were added 10 μl of each patient sample as pre-designated on the work sheet.

(v) The plate was cover sealed and incubated in water maintained at 37 °C for 1 hour.

(vi) At the end of the incubation period. The plate washed five times with the prepared working wash buffer.

(vii) The plate was inverted onto a sterile filter paper on a flat-levelled bench to completely drain the added buffer. This was followed by the addition of 200 μl of conjugate to all wells except A1.

(viii) The plate was cover sealed and incubated in a water bath maintained at 37 °C for 1 hour.

(ix) The washing process was repeated as previously described in 'vi' above.

(x) Exactly 200 μl of substrate Solution was added to all the wells including A1.

(xi) Again the plate was cover sealed and placed in a closed cupboard to provide darkness for 30 minutes.

(xii) At the end of this room incubation 50μl of 4N sulphuric acid (H_2SO_4) was added to all wells including A1.

(xiii) The reaction was read at 492 nm using a spectrophotometer and the absorbance of each well recorded.

Calculations

Negative control cut-off

The mean of all the negative controls was determined in each test. Individual negative control values accepted was greater than or equal to -0.050 absorbance and less than or equal to 0.120. Any individual negative control value that did not meet with these criteria was excluded and the mean of the negative control calculated on the basis of the other two that met up with the criteria. If two of the negative controls failed to meet with the criteria the run was repeated

The cut-off calculation was in each case was done using the following formula:

Cut-off = Mean absorbance of negative control + 0.600.

Positive control cut-off

The mean of all the positive controls was determined in each test. Individual positive control values accepted was greater than or equal to 0.800 absorbance and did not differ by no more than 0.600.

Validity criteria

The accepted absorbance of the substrate blank was less than or equal to 0.050 and greater that or equal to -0.020.

A summary of the results of the test is presented in below in table 3, page 77.

2.5.7 Serotyping of HCV

The samples positive using ORTHO HCV 3.0 EIA Test kit (Ortho Clinical Diagnostics, USA) were serotyped using Murex TM HCV Serotyping 1-6 Assay (Murex Diagnostics, Dartford, UK) (Sandres *et al*, 2001) following the manufacturers instructions.

Principle:

The Murex TM HCV Serotyping 1-6 (HC03) assay is an ELISA, which employs microtitre plates as its solid phase. The assay distinguishes type-specific antibodies to the six major HCV types. The microtitre wells are coated with synthetic HCV NS4 peptides of each type. Each strip of eight wells contains two control wells and six wells representing one each of the six major HCV types (1-6). The first control of each strip contains no soluble peptides to adsorb the serum antibodies, hence when a serum containing HCV antibodies is added the antibodies are adsorbed resulting in the generation of maximum signal ("no competition").

The second control contains HCV NS4 peptides of all types so when a serum sample containing HCV antibodies is added to it the antibodies are neutralized with the "competing solution—all", resulting in the generation of a background signal(minimum signal).The remaining six wells each contains all HCV NS4 peptides but one. Thus well 3 of each strip are specific for HCV serotype 3, Well 4 HCV serotype 4 and so on. Thus in each of the six type wells antibodies present in the serum interacts with the type-specific soluble competing peptides which are in excess of those coated on the wells and therefore block any cross-reaction. Hence type-specific antibodies present, which have not been neutralized by the

competing solution because the corresponding peptides were missing from the mix, are captured onto peptides coated on the well surface and produce a signal.

Method:

A total of 33 HCV strips were selected based on the 31 samples screened as HCV positive and an additional two strips added as internal controls. All the strips were arranged on a microtitre plate. The strips were allowed to attain room temperature while a work sheet was prepared.

Preparation of reagents:

1. Wash buffer: The wash buffer was prepared following the instructions and properly mixed.
2. Sample diluents, substrate solution, conjugate, were supplied ready for use

Serum incubation

1. A 1: 21 dilution of each sample including controls was performed in the appropriate well following the work sheet. Thus 200μl of the sample diluents was added to each well using a multi-channel pipette followed by 10μl of each appropriate sample. Samples were well mixed three to four times avoiding bubbles.
2. The plate was covered with a sealer to minimize evaporation and incubated at 37°C for 1 hour to allow the serotype-specific anti-HCV antibodies to bind to the immobilized antigens.

Wash procedure

1. A semi-automated washing device was employed for the washing process. The plate was shaken vigorously but with caution to avoid spillage. Each micro-well was filled with the working buffer, then aspirated, filled again and aspirated with total avoidance of air bubbles. This process was repeated five times to ensure the total removal of unbound material.

2. The plate was shaken several times to remove solutions from the wells and was inverted onto a sterile filter paper on a flat-levelled bench to completely drain the added buffer.

Conjugate Incubation

1. Exactly 200μl of the conjugate was added to all the wells. Peroxidase-conjugated monoclonal anti-human immunoglobulin G was added to all wells, sealed and further incubated for 30 minutes at 37°C.

2. The washing procedures as previously described was repeated at the end of the incubation period to remove excess conjugate.

Substrate Incubation

1. Exactly 200μl of a solution containing tetramethylbenzidine and hydrogen peroxide was added to all the wells using a multi-channel pipette.

2. The plate was cover sealed and placed in a closed cupboard to provide darkness for 30 minutes.

3. At the end of this room incubation 50 μl of 4N sulphuric acid (H2SO4) was added to all wells to stop the reaction. Wells containing anti-HCV were purple in colour and when the sulphuric acid was added an orange colour was produced.

Reading of plate

The microwell reader was set at a wavelength of 450nm and the optical density (OD) of each well measured against the reagent blank. The plate was read within 10 minutes of the addition of the stop solution and the absorbance reading was recorded against each sample on the work sheet.

Quality control

Cut-off value

Positive control

The cut-off of the positive control ("no competition") was calculated as the greater value of the optical density (OP) multiplied by 0.2.

Positive control ("No competition")

The positive control ("no competition") well and the six typing wells were considered positive when the ODs exceed the cut-off value.

The positive control was intended to monitor for significant reagent failure and was not to ensure precision at the assay cut-off.

Mixed reaction

A mixed reaction was entered when two typing wells still passed a new designated cut-off obtained after subtracting the OD of the negative control ("competing solution—all") from each of the other ODs and the new value for 0.2 multiplied by the OD of the positive control ("no competition").

Non-typeable samples

A sample was entered as untypeable when more than two wells passed the new designated cut-off obtained after subtracting the OD of the negative control ("competing solution-all") from each of the other ODs and the new value for 0.2 multiplied by the OD of the positive control ("no competition").

Non-reactive (NR) samples

A sample was considered non-reactive when none of the wells passed the cut-off value of the OD of the negative control multiplied by 1.5.

2.5.8 HBsAg Test

Hepatitis B surface antigen test was carried out only on patients with history of hepatocellular carcinoma using QUADRATECH CHECK 4-HBs one step generation hepatitis b surface antigen test kit (VEDALAB, France) (Pan American Health Organization, 2002) following the manufacturers instructions.

Principle:

QUADRATECH CHECK 4-HB is a qualitative immunoassay employing a combination of monoclonal-dye conjugate (colloidal gold) and polyclonal solid phase antibodies to selectively identify HBsAg of Hepatitis B viral infection with a high degree of sensitivity. When serum a sample is added directly to the sample well, it flows through an absorbent pad within the palette. The pad which is in contact with a chromatographic test strip contains a region of immobilised polyclonal anti-HBsAg antibody in the test window (B), Thus this facilitates the labelled antibody-dye conjugate to binds to HBsAg if present in the

test serum forming an antibody-antigen complex. The antibody-antigen complex moves by capillary action along the strip forming a line of immobilised complex by the zone of antibody in the test window (B), indicating the presence of HBsAg in the sample (pink line). In the absence of HBsAg the test window remains clear, while the appearance of a pink line in the control window of the test strip shows that the test has been carried out correctly.

Test procedures

1. All test samples and Check 4-HBs test palettes were allowed to reach room temperature
2. To each test palette 1 drop of physiological saline was added to its sample well A, thus moistening the paper medium and allowing smooth flow of sample without affecting test sensitivity.
3. Approximately 300μl of each test sample were dispensed into appropriately pre-labelled sample well A.
4. Each palette was allowed to remain at room temperature for 15 minutes before the reading of the test results.

Reading and interpretation of results

Negative: The appearance of a clearly distinguishable pink line in control window C with no such line in test window B.

Positive: The appearance of a clearly distinguishable pink line in test window B and in the control window C.

2.6 Result of administered questionnaires

The response of each participant who consented to the study were compiled and arranged to reflect their HIV, HCV, or HIV/HCV status.

2.7 Statistical methods

The prevalence rates of HIV, HCV and HIV/HCV co-infection were determined by simple percentages, exact binominal confidence intervals (95%) was determined for all the tests performed and significance differences in prevalence rates of HIV and HCV between males and females was determined using the Chi-square or Fisher Exact test and Odds Ratio (OR). *P*

values of <0.05 were considered significant. While multiple linear regression was employed to evaluate the trends in CD4 count and to determine the significance of associations between HIV, HCV and all enumerated variables including socio-demographic, risk factors. It was also employed to compare the trend in the test patients and control group.

CHAPTER THREE

3.0. Results.

A summary of the results of the tests conducted and responses to administered questionnaires are presented in this chapter.

3.1 HIV screening test.

The result of the screening test for HIV antibodies using Murex HIV 1, 2, 0 test kits (Murex Biotech, UK) is presented in Table 2 below. Antibodies to HIV were detected in 102 out of the 1500 (6.8%) subjects sampled.

Table 2: Summary of result of screening test for HIV antibodies using Murex HIV, 2, 0 test kits according to age of subjects.

Age range (Years)	No. screened (%) (n=1500)	No. with HIV antibodies (%) (n=102)
≤5	9 (0.6)	1 (11.1)
6-12	18 (1.2)	0
13-19	211 (14.1)	9 (4.3)
20-26	431 (28.7)	28 (6.5)
27-33	376 (25.1)	49 (13.0)
34-40	179 (11.9)	6 (3.4)
41-47	150 (10.0)	3 (2.0)
48-54	83 (5.5)	1 (1.1)
≥55	43 (2.9)	5 (11.6)

3.2 Confirmatory test for HIV antibodies.

The result of the confirmatory test for HIV antibodies and for differentiation into serotypes using PEPTI-LAV1, 2 (Sanofi, France) is presented in chapter 3.2.1, Table 3 below and in Figure 7, page 77 according to the age range and sex of the subjects sampled. The presence of HIV antibodies was confirmed in 101 out of the 102 (99.1%) samples initially screened anti-HIV positive using Murex HIV 1, 2,0 (Murex Biotech, UK). One of the samples that was reactive using Murex HIV-1, 2, 0 was confirmed to be anti-HIV antibody negative using the PEPTI-LAV1 1, 2. Cross contamination was suspected and the sample was discarded.

Table 3: Summary of the result of confirmatory test for HIV antibodies of sampled subjects according to age and sex.

Age (Yrs)	Males				Females			
	Number (%) (38.1%) (n=572)	Number with HIV antibodies (%) (n=23)			Number (%) (61.9%) (n=928)	Number with HIV antibodies (%) (n=78)		
		HIV-1 (n=14)	HIV-2 (n=8)	HIV-D (n=1)		HIV-1 (n=51)	HIV-2 (n=20)	HIV- D (n=7)
≤5	4 (0.7)	0 (0.0)	0 (0.0)	0 (0.0)	5 (0.5)	1 (20.0)	0 (0.0)	0 (0.0)
6-12	7 (1.2)	0 (0.0)	0 (0.0)	0 (0.0)	11 (1.2)	0 (0.0)	0 (0.0)	0 (0.0)
13-19	76 (13.3)	2 (2.6)	0 (0.0)	0 (0.0)	135 (14.5)	5 (3.7)	0 (0.0)	1 (0.7)
20-26	116 (20.3)	3 (2.6)	2 (1.7)	0 (0.0)	315 (33.9)	16(5.1)	6 (1.9)	2 (0.6)
27-33	167 (29.2)	3 (1.8)	4 (2.4)	0 (0.0)	209 (22.5)	26(12.4)	12 (3.8)	3 (1.4)
34-40	58 (10.1)	1 (1.7)	0 (0.0)	0 (0.0)	121 (13.0)	2 (1.7)	2 (1.7)	1 (0.8)
41-47	82 (14.3)	2 (2.4)	0 (0.0)	0 (0.0)	68 (7.3)	1 (1.5)	0 (0.0)	0 (0.0)
48-54	33 (5.7)	1 (3.0)	0 (0.0)	0 (0.0)	50 (5.4)	0 (0.0)	0 (0.0)	0 (0.0)
≥ 55	29 (5.0)	2 (6.9)	2 (6.9)	1 (3.4)	14 (1.5)	0 (0.0)	0 (0.0)	0 (0.0)

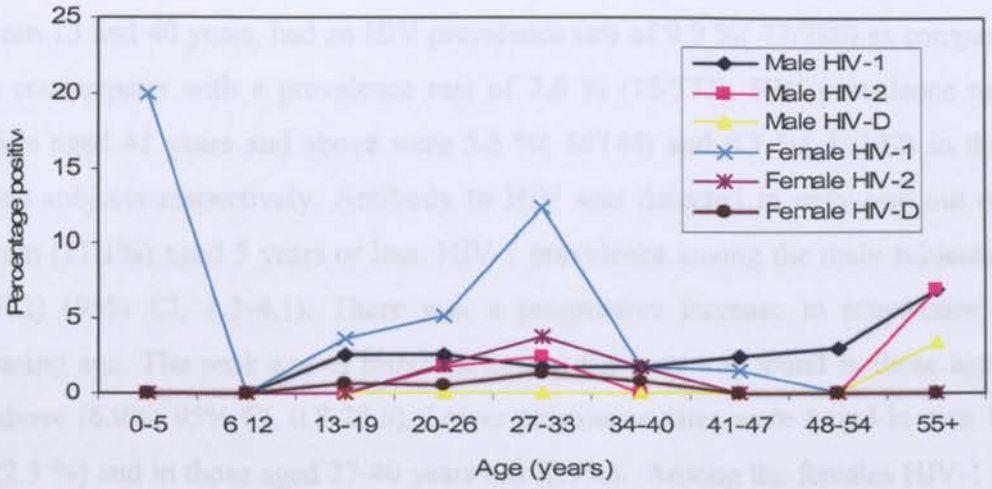

Fig. 7 Prevalence of HIV antibodies among study subjects according to age and sex.

3.2.1 Age and Sex distribution of subjects HIV antibody status

The persons examined were aged 11 months to 76 years. Their overall mean age was 30.2 years. The mean age for the men was 31.9 years and 28.7 years for the women. Women accounted for 61.9 percent (928 out of 1500) of the subjects sampled in this study and 84.1 % (780/928) of these were aged between 13 and 40 years, while, 1.7% (16/928) was 12 years or less. Only 14.2 % (132/928) of the women were aged 41 years and over (Table 3, pge 76). The men accounted for 38.1 %(572/1500) of the study population sampled but with a comparatively higher percentage aged 41 years and over (25.2% versus 14.2%). Similarly, like the women, a significant proportion of the sampled males (72.9% - 417/572) were between 13 and 40 years, while, only 1.9 percent 11/572) were below 12 years or less.

The determined overall prevalence of HIV in this study was 6.7% (101/1500) (95% CI, 5.6-8.2). The prevalence of HIV-1 was 4.3 percent (65/1500) (95% CI, 3.4-5.5) and HIV-2 was 1.9 percent (28/1500) (95% CI, 1.2-2.7), while dual infection between HIV-1 and HIV-2 (HIV-D) had a prevalence of 0.5 percent (8/1500) (95% CI, 0.2-1.0).

HIV prevalence among the women was 8.4 % (78/928) (CI, 6.7-10.4) as against a prevalence of 4.0 % (23/572) CI, 2.8-6.0) found in their male counterparts. Women aged between 13 and 40 years, had an HIV prevalence rate of 9.9 %(77/780) as compared to their male counterparts with a prevalence rate of 2.6 % (15/572). HIV prevalence rates among subjects aged 41 years and above were 5.5 %(8//144) and 0.7 %(1//132) in the men and women subjects respectively. Antibody to HIV was detected in only one out of 9 of the children (11.1%) aged 5 years or less. HIV-1 prevalence among the male subjects was 2.4% (14/572) (95% CI, 1.3-4.1). There was a progressive increase in prevalence rates with increasing age. The peak age of infection among the men was found in those aged 55 years and above (6.9%; 95% CI, 0.8-22.8). Lower prevalence rates were found in men 19 years or less (2.3 %) and in those aged 27-40 years old (2.1%). Among the females HIV-1 prevalence rates were (5.5%; 95% CI, 4.1-7.2). Unlike their male counterparts the peak age of infection among the females was in those aged 27-33 years (12.4%), (95% CI, 8.3-17.7) with lower rates in those 41 years and above (0.8%). The mean age of HIV-1 infection among the women was 26.5 years as against the 35.1 years found among the men. The women were more significantly associated ($p<0.05$) (OR 2.32; 95% CI, 1.25-4.58) with HIV-1 than their male counterparts. The HIV-2 prevalence rates for the men were 1.4 % (8/572) (CI, 0.6-2.7), with the peak age of infection in men aged 55 years and above (6.9%, 1/29) (95% CI, 0.8-22.8). No HIV-2 infection was detected in males aged 19 years or less and among the men aged 34-54 years old. Among the females HIV-2 prevalence rates was comparatively higher (2.2%, 20/928) (95% CI, 1.3-3.3). Unlike their male counterparts the peak age of infection for HIV-2 among the females was found in those aged 27-33 years (5.7%, 12/209) (95% CI, 3.0-9.8). The mean age of HIV-2 infection among the women was 28.6 and the men 35.3 years. As with HIV-1 infection the risk of infection with HIV-2 was higher in women than the males (OR 1.55, 95% CI, 0.65-4.10). The prevalence of HIV-D infection among all the subjects sampled was (8/1500). Dual infection with HIV-D was mainly found in those aged 20 years and above with the peak age of infection in those 55 years and over (2.3%, 1/43) (95% CI, 0.1-12.3). Among the males, dual infection was only found in a 67 years old man (0.2%, 1/572) while, women accounted for 7 out of the 8 cases of dual infection between HIV-1 and HIV-2 detected in this study. The infection was mainly found in those aged 13-40

with the peak age of infection among the females aged 27-33 years (1.4%, 3/209)(95% CI, 0.3-4.1). The mean age of dual infection was 24.9 years among the women.

Statistical analysis of HIV prevalence against the age and sex of all sampled subjects using the Fischer exact test showed that anti-HIV was significantly ($p<0.001$) associated with age and sex. Secondly, the females were more significantly associated ($p<0.05$) (OR 2.32; 95% CI, 1.25-4.58) with HIV-1 than their male counterparts while infection with any HIV was highly statistically significantly associated ($p<0.001$) with the females (95% CI, 1.34-3.6). Similarly, the females were more predisposed to infection with HIV-2 (OR 2.19) and dual infection (OR 4.34) than their male counterparts.

3.3 Screening test for HCV antibodies

A summary of the result of the screening test for HCV antibodies using the Ortho HCV 3.0 ELISA test kit (Ortho Clinical Diagnostics, USA) are presented in Table 4, page 80 and figure 8, page 80 according to the age and sex of sampled subjects. The determined HCV prevalence rates for this study population were 2.1% (31/1500) (95 % CI, 1.4-2.9). Antibodies to HCV were detected mainly in those aged 20 years and above. The men accounted for 71% (22/31) of the HCV infection detected with a comparatively higher prevalence rate than in the females (3.8%; 95% CI, 2.4-5.8 versus 1.0%; 95% CI, 0.4-1.8). The peak age of infection for both sex was in those aged 41-47 years: 6.0% (10/431) (95% CI, 2.8-11.1) for men and 2.9% (3/150) (95% CI, 0.4-10.2) for the women. However, the women had a comparatively lower age of infection (33.1 years versus 36.7 years). Men aged 55 years and above had comparatively lower prevalence rates (3.4% 1/ 29) than those in the age range of 34-47 years (7.8%, 11/140). No antibody to HCV was detected in women aged 48 years and above.

A highly significant association ($p=0.0001$) (OR: 4.08; 95% CI, 1.83-8.55) was found between HCV and the males. A similar association was found between HCV prevalence and the women (OR: 0.24; 95% CI, 0.12-0.54) but at significantly lower odds ratio.

Table 4: Summary of the result of anti- HCV test of sampled subjects accordingto age and sex.

Age range (Years)	Male (%) (38.1%) (n=572)	Male. Anti-HCV Positive (%) (n=22)	Female. (%) (61.9%) (n=928)	Female. Anti-HCV Positive (%) (n=9)	Total no.Anti-HCV Positive (%) (n=31)
≤5	4 (0.7)	0 (0.0)	5 (0.5)	0 (0.0)	0 (0.0)
6-12	7 (1.2)	0 (0.0)	11 (1.2)	0 (0.0)	0 (0.0)
13-19	76 (13.3)	0 (0.0)	135 (14.5)	0 (0.0)	0 (0.0)
20-26	116 (20.3)	6 (5.2)	315 (33.9)	4 (1.3)	10 (2.3)
27-33	167 (29.2)	3 (1.8)	209 (22.5)	1 (0.5)	4 (1.1)
34-40	58 (10.1)	4 (6.9)	121 (13.0)	2 (1.7)	6 (3.3)
41-47	82 (14.3)	7 (8.5)	68 (7.3)	2 (2.9)	9 (6.0)
48-54	33 (5.8)	1 (3.0)	50 (5.4)	0 (0.0)	1 (1.2)
≥ 55	29 (5.1)	1 (3.4)	14 (1.5)	0 (0.0)	1 (2.3)

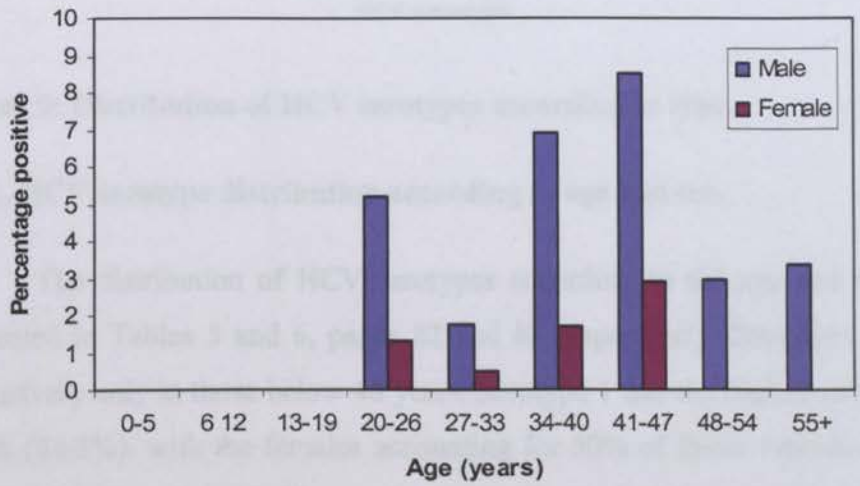

Fig. 8 Prevalence of HCV among study subjects according to age and sex.

3.4 HCV serotypes

The HCV serotyping result is presented in figure 9 (page 81) according to serotype. Twenty-six out of the 31 (83.9%) samples were successfully typed. Serotype 2 had the highest prevalence and was identified in eighteen (18) samples (58.1%). This was followed by serotype 1, which was detected in 6 samples (19.4%) while serotype 3 was found in two (2) samples (6.5 %). Serotypes 4, 5 and 6 were not identified in any of the samples. Sera from five patients (16.2%) were positive in the no competition well but did not react in any of the other wells, thus were classified as non- type specific (NT).

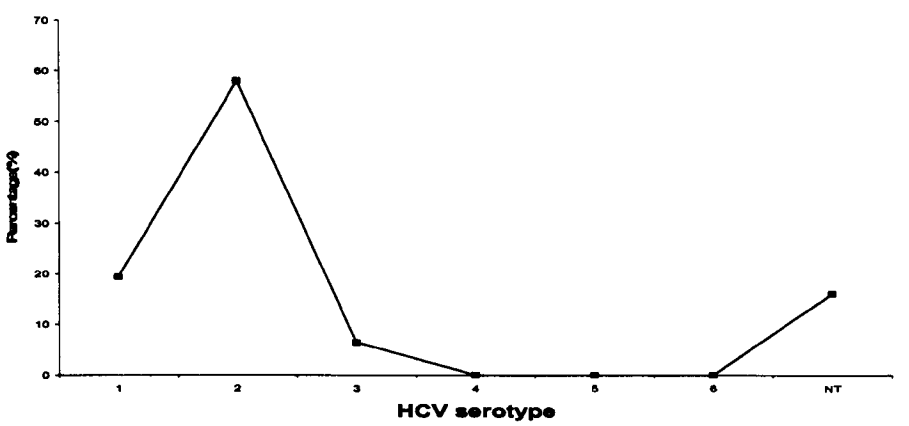

Figure 9: Distribution of HCV serotypes according to type.

3.4 1. HCV serotype distribution according to age and sex.

The distribution of HCV serotypes according to the age and sex of the subjects is presented in Tables 5 and 6, pages 82 and 83 respectively. Serotypes 1 and 3 were almost exclusively only in those below 40 years. Serotype 1 had the highest prevalence in those aged 20-26 (83.3%), with the females accounting for 50% of these. Females' aged 20-26 had the highest prevalence of HCV type 1 (75%). These differences in age and gender were not statistically significant ($p>0.05$).

Unlike serotype 1, serotype type 2 had a broader distribution pattern cutting across all age groups and gender but with its highest prevalence in those above 41 years (7/18, 38.9%). Men accounted for 72 % of the serotype 2 cases. Its lowest prevalence was found in females' aged 20-26 years. Unlike serotype 1, HCV serotype 2 was marginally associated with the age (p=0.04) (OR: 0.2; CI: 0.3-1.0) of the infected but not with their gender (p=0.3) (OR: 1.2; CI: 0.5-2.11) these differences was significantly associated with the age or sex of the infected.

Unlike serotypes 1 and 2, HCV serotype 3 showed a different pattern of epidemiology. It had the lowest prevalence and its distribution was only limited to males below the age of 40 years. These differences in age and gender were not significant (p>0.05).

Table 5: Distribution of HCV serotypes among males according to age.

Variable	Age range					
Serotype	20-26(%) (n=6)	27-33(%) (n=3)	34-40(%) (n=4)	41-47(%) (n=7)	48-54(%) (n=1)	≥55 (%) (n=1)
1 (n=3)	2 (33.3)	1 (33.3)	0	0	0	0
2 (n=13)	2 (33.3)	1 (33.3)	2 (50.0)	6 (85.7)	1 (100.0)	1(100.0)
3 (n=2)	1 (16.7)	0	1 (25.0)	0	0	0
4 (n=0)	0	0	0	0	0	0
5 (n=0)	0	0	0	0	0	0
6 (n=0)	0	0	0	0	0	0
NT (n=4)	1 (16.7)	1 (33.3)	1 (25.0)	1 (14.3)	0	0

3.4.2 HIV/HCV co-infection according to HCV serotype.

The distribution of HIV and HCV co-infection according to HCV serotype showed that HIV-1 co-infection with HCV serotype 1 had the highest prevalence of 44.4 %(4/9)

followed by HIV-1 and HCV type 2 (3/9; 33.3%). There was no co-infection with HCV serotype 3.

The four cases of HIV1 co-infection with HCV serotypel were found in two males under 24 and 26 years respectively and another in a 34 years old man. The fourth one was seen in a 27 years old housewife. While HIV-1 co-infection with HCV serotype 2 was found in three cases, two in males 41 and 48 years respectively and another in a 24 years old unmarried man. HIV-2 co-infection with HCV serotype 2 was found in 36 years old woman. One case of HIV-2 co-infected with a non-typeable HCV was detected in a 56 years old man. These differences in age and gender were not significant (P>0.05).

Table 6: Distribution of HCV serotypes among females according to age.

Variable	Age range			
Serotype	20-26(%) (n=4)	27-33 (n=1)	34—40 (n=2)	41-47 (n=2)
1 (n=3)	3(75.0)	0	0	0
2 (n= 5)	1(25.0)	1(100.0)	2(100)	1(50.0)
3 (n=0)	0	0	0	0
4 (n=0)	0	0	0	0
5 (n=0)	0	0	0	0
6 (n=0)	0	0	0	0
NT (n=1)	0	0	0	1(50.0)

3.5 Hepatitis B surface antigen in patients in HCC.

A summary of the result of the hepatitis B surface antigen carried out only on patients with history of hepatocellular carcinoma using QUADRATECH CHECK 4-HBs one step

generation hepatitis-B surface antigen test kit (VEDALAB, France) is presented below in table 7 below; In all a total of 13 (11 men and 2 women) persons aged 32 years to 76 years was screened. The mean age of men was 46 years and 43 years for the women

Hepatitis B surface antigen was detected in 5 out of the13 (38.5%) persons with a history of HCC screened. Rates were highest in patients 48 years and above (37.5%; 3/8). No patient was found with anti-HCV and anti-HBV.

Table 7: Summary of the result of HBsAg surface test according to age and sex of HCC patients.

Age range (Years)	No. tested Males (%) (n=11; 84.6%)	No. HBsAg surface test Positive Males (%) (n=5)	No. of females tested (%) (n=2)	No. of females HBsAg surface test positive (%) (n=0)	Total No. HBsAg surface test positive (%) (n=5)
27-33	1(9.1)	1(100.0)	0	0	1(20.0)
34-40	0	0	1(50.0)	0	0
41-47	2(18.2)	1(50.0)	1(50.0)	0	1(20.0)
48-54	4(36.4)	1(25.0)	0	0	1(20.0)
≥ 55	4(36.4)	2(50.0)	0	0	2(40.0)

3.6 Summary of results of HCV and HIV/HCV tests of study subjects according to age and sex

A summary of the distribution of anti-HIV antibodies and anti-HCV antibodies among the male subjects are presented in Table 8, page 85 and Figure 10, page 86 according to age. Their distributions among the female subjects are presented in Table 9, page 87 and Figure 11, page 87 according to age.

Co-infection between HIV and HCV was only detected in 9 out of the 1500 (0.6%) subjects screened with the men accounting for 77.7 percent (7/9) of the cases. Co-infection between HIV-1 and HCV was found in 7 subjects (0.5%) (95% CI, 0.2-1.0) and was detected

only in those aged 20 and above with its peak in those 34 years or above. Six cases were documented for the males and one in favour of the females. Similarly, there was a progressive increase with increasing age in HIV-1/ HCV co-infection rates in males ranging from 0.9% in 20-26 years old to 3.4% in 55 years and over.

Co-infection between HIV-2 and HCV was detected only in 2 persons (0.1 %) (95% CI, 0.0-0.5) a male and a female. The mean age of HIV/HCV co-infection among the men was 39.1 years as against 33.1 years in women.

Co-infection between HIV-1 and HCV was significantly associated ($p< 0.05$) with the age and sex of the subjects. A significantly higher association was found between those aged 27-33 years and 55 years and over. No significant association ($p>0.05$) was found between HIV and HCV co-infection and subjects aged 26 years or less. Similarly, co-infection between HIV-2 and HCV was not significantly associated ($p> 0.05$) with the age of the subjects.

Table 8: Summary of the result of anti- HCV and HIV/HCV infection in males

Age range (Years)	Male (%) (38.1%) (n=572)	Anti-HCV positive (%)(n=22)	Anti-HIV-1& anti-HCV positive (n=6)	Anti-HIV-2 & anti-HCV positive (n=1)
≤ 5	4 (0.7)	0 (0.0)	0 (0.0)	0 (0.0)
6-12	7 (1.2)	0 (0.0)	0 (0.0)	0 (0.0)
13-19	76 (13.3)	0 (0.0)	0 (0.0)	0 (0.0)
20-26	116 (20.3)	6 (5.2)	3 (2.6)	0 (0.0)
27-33	167 (29.2)	3 (1.8)	0 (0.0)	0 (0.0)
34-40	58 (10.1)	4 (6.9)	1 (1.7)	0 (0.0)
41-47	82 (14.3)	7 (8.5)	1 (1.2)	0 (0.0)
48-54	33 (5.8)	1 (3.0)	1 (3.0)	0 (0.0)
≥ 55	29 (5.1)	1 (3.4)	0 (0.0)	1 (3.4)

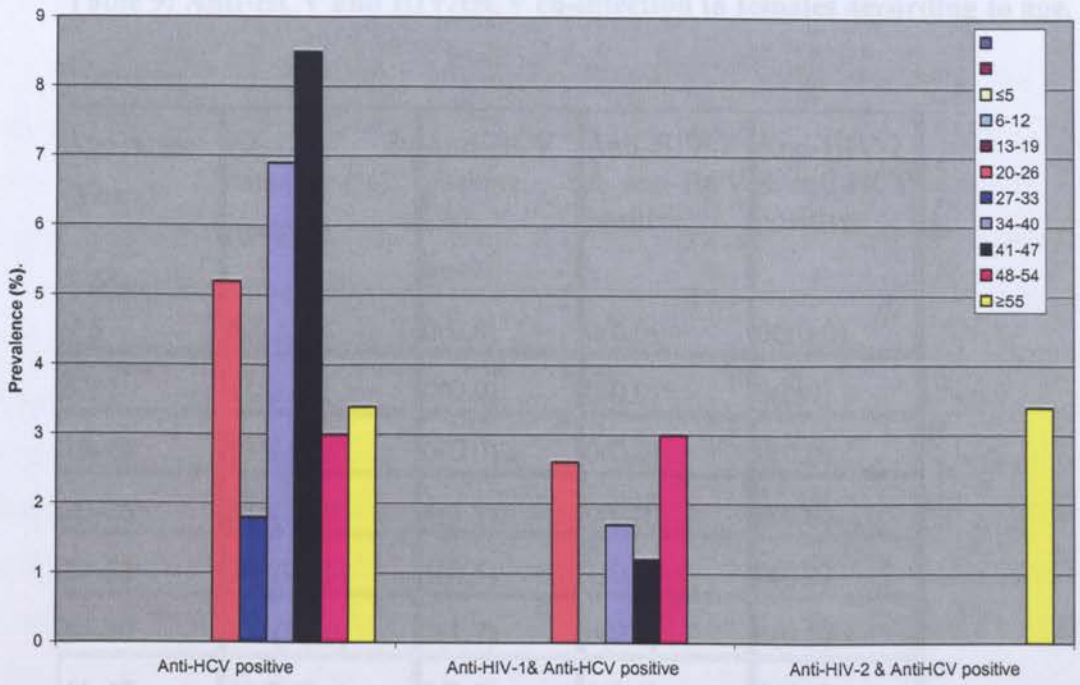

Fig. 10: Prevalence of HCV, HIV and HCV co-infection among male study subjects acording to age.

Table 9: Anti-HCV and HIV/HCV co-infection in females according to age.

Age range (Years)	No. in category (%) (n=928).	Anti-HCV positive (%) (n=9)	Anti-HIV-1 & anti-HCV positive.	Anti-HIV-2 & anti-HCV positive.
≤ 5	5(0.5)	0(0.0)	0(0.00	00(0.0)
6-12	11(1.2)	0(0.0)	0(0.0)	0(0.0)
13-19	135(14.5)	0(0.0)	0(0.0)	0(0.0)
20-26	315(33.9)	4(1.3)	0(0.0)	0(0.0)
27-33	209(22.5)	1(0.5)	1(0.5)	0(0.0)
34-40	121(13.0)	2(1.7)	0(0.0)	1(0.8)
41-47	68(7.3)	2(2.9)	0(0.0)	0(0.0)
48-54	50(5.4)	0(0.0)	0(0.0)	0(0.0)
≥ 55	14(1.5)	0(0.0)	0(0.0)	0(0.0)

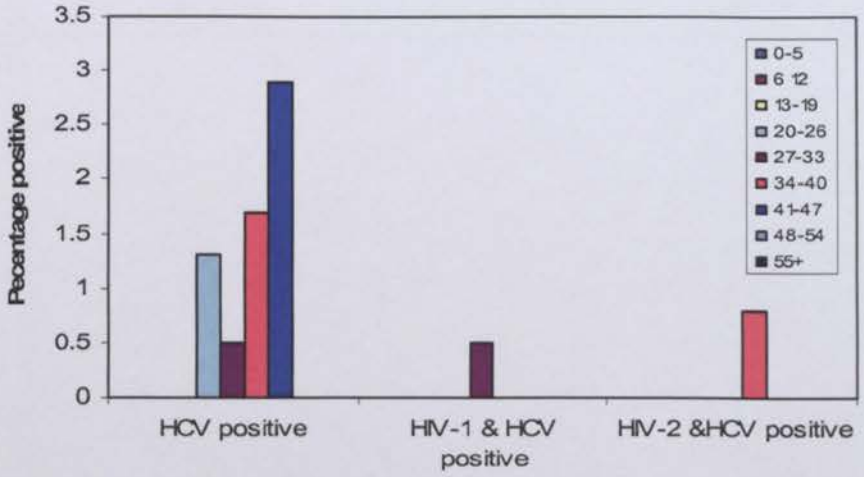

Fig.11 Prevalence of HCV, HIV and HCV co-infection among female subjects according to age

3.7 CD4+ counts in relation to the age and sex of study subjects.

The results of the CD4+ counts using the Dynal immunomagnetic cell isolation method (Diagbouga *et al*, 2003) are presented in Tables 10 and 11 (pages 89 and 90) according to age and sex respectively. Approximately half (51.0% 759/1500) of the subjects sampled irrespective of their HIV status had a CD4+ count of 500 cells/μl or above, while 77.5 % (720/928) of the females had a CD4+ count above 400 cells/μl as against 76.9%(440/572) in the men. Similarly 7.8% had counts of 300-399 cells/μl. Close to 70% (188/276) of persons aged 41 years and above had counts of 500 cells/μl or more. Counts of less than 200 cells/μl were found in approximately 6% p (89/1500) of the subjects (Table 7). Almost all the subjects (17/18) in the age range of 6-12 years had CD4+ count of 500 cells/μl and above. A comparatively lower CD4+ count was recorded among the subjects aged 20 – 26 years and 34-40 years. Counts of 500 cells/μl and above were found in more men than women (57.6% versus 46.2%). The mean count for men irrespective of their HIV status was 455 cells/μl while the women had a mean count of 447 cells/μl. Progressive decrease in CD4+ counts was observed with increasing age (Table 10). CD4+ was significantly associated ($p<0.001$) with age and sex.

Table 10: Summary of result of CD4 counts of sampled subjects according to age.

Age range (yrs)	No. (%) (n=1500	CD4+ cells/μl <100 (%) (n=1)	100-199 (%) (n=89	200-299 (%) (n=133)	300-399 (%) (n=117)	400-499 (%) (n=401)	≥ 500(%) (n=759)
≤ 5	9 (0.6)	0	2 (22.2)	1 (11.1)	0	0	6 (66.6)
6 -12	18 (1.2)	0	0	1 (5.5)	0	0	17 (94.4)
13-19	211 (14.1)	0	5 (2.4)	13 (6.2)	20 (9.5)	46 (21.8)	127 (60.1)
20-26	431 (28.7)	0	19 (4.4)	53 (12.3)	55 (12.8)	153(35.5)	151 (35.0)
27-33	376 (25.1)	0	24 (6.4)	38 (10.1)	20 (5.3)	89 (23.7)	205 (54.5)
34-40	179 (11.9)	0	6 (3.4)	16 (8.9)	12 (6.7)	80 (44.7)	65 (36.3)
41-47	150 (10.0)	1(0.6)	10 (6.7)	5 (3.3)	6 (4.0)	27 (18.0)	101 (67.3)
48-54	83 (5.5)	0	10 (12.0)	2 (2.4)	2 (2.4)	1 (1.2)	68 (81.9)
≥ 55	43 (2.9)	0	13 (30.0)	4 (9.3)	2 (4.7)	5 (11.6)	19 (44.1)

Table 11: Summary of result of CD4+ count of sampled subjects according to sex

CD4+ cells/μl	Male (%) (n=572)	Female (%) (n=928)
$\leq$ 100 (n=1)	0	1 (0.1)
100-199 (n=89)	37 (6.5)	52 (5.6)
200-299 (n=133)	51 (8.9)	82 (8.8)
300-399 (n=117)	44 (7.7)	73 (7.9)
400-499 (n=401)	110 (17.7)	291 (31.4)
$\geq$ 500 (n=759)	330 (57.6)	429 (46.2)

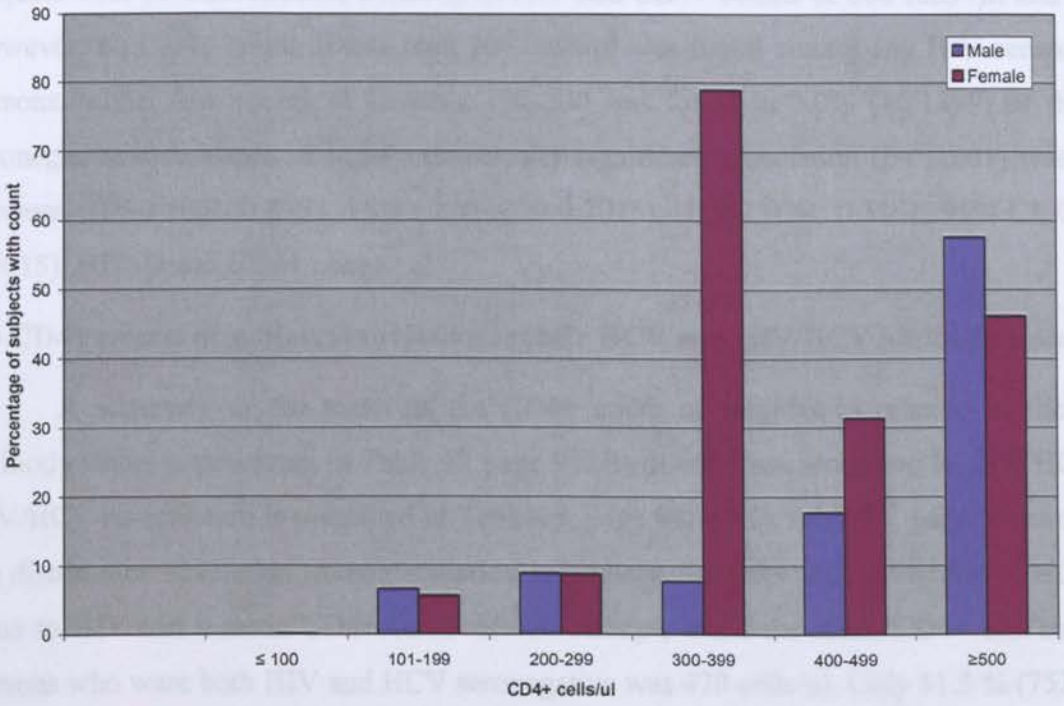

Fig. 12: CD4 count of study subjects according to sex.

3.8 CD4+ count of study subjects in relation to their HIV antibody status.

A summary of the results of the CD4+ count of all the subjects sampled according to their HIV status is presented in table 12 below. Approximately 93.3% (1399/1500) of the persons evaluated in this study were HIV seronegative. The rest were seropositive.

The mean CD4+ count of the HIV seronegative persons was 467 cells/μl, while the seropositive persons had a count of 310 cells/μl. Persons who were HIV-1 seropositive had a mean CD4+ count of 298 cells/μl, (95% CI, and 0.0249-0.269) while the HIV-2 seropositive had a mean count of 320 cells/μl. A mean count of 248 cells/μl was found for HIV-D seropositive persons.

Lower CD4+ counts of less than 200 cells/μl was found in 42% and 62% of persons with HIV-1 and HIV-D respectively. Only 7.7% (5/65) of the HIV-1 seropositive persons had CD4+ counts of 500 cells/μl and above as compared to 25% (7/28) recorded in those with HIV-2 antibody. Similarly, none of the subjects with dual infection with HIV-1 and HIV-2 (HIV-D) had a CD4+ count of 500 cells/μl and above. Only about half (53.4 %) of the

subjects with no demonstrable antibody to HIV had CD4+ counts of 500 cells /μl and above. However, no CD4+ count of less than 100 cells/μl was found among any HIV seronegative persons, while, low counts of between 100-200 was found in 3.0% (46/1399) of the HIV seronegative individuals. A highly statistically significant association ($p< 0.001$) was found between HIV-1 (OR: 0.0753, 95% CI, 0.0329-0.2015), HIV-2 (OR: 0.3191, 95% CI, 0.0329-0.2015), HIV-D and CD4+ count.

3.9 CD4+ counts of subjects in relation to their HCV and HIV/HCV antibody status.

A summary of the result of the CD4+ count of subjects in relation to their HIV antibody status is presented in Table 12 page 93. Its distribution according to their HCV and HIV/HCV co-infection is presented in Table 13, page 94, while Table 14, page 95 summaries the distribution in relation to their statistical inferences. Subjects with antibodies to HCV, but none to HIV had a mean CD4+ count of 460 cells/μl, while the mean CD4+ cell count for persons who were both HIV and HCV seronegative was 470 cells/μl. Only 51.3 % (753/1469) of the HCV seronegative persons had a CD4+ count of 500 cells/μl and above. Over 80% (25/31) of the HCV seropositive subjects had CD4+ counts of 400 cells/μl and above, while 41.9%(13/31) of these same group had counts of CD4+ count of 500 cells /μl and above.

HIV-1/HCV seropositive persons had a mean CD4+ count of 306 cells/μl, HIV-2 /HCV persons had a mean count of 365 cells/μl. None of the subjects with HIV-1 and HCV or HIV-2 and HCV co-infections had a CD4+ count of 500 or above. Comparatively, lower CD4+ counts were associated with HIV-1 and HCV co-infection, than with HCV alone or HIV-2 and HCV co-infection. CD4+ count was significantly associated ($p<0.05$) with HIV/HCV co-infection but not with HCV.

Table 12: Summary of the result of CD4+ counts of sampled subjects according to their HIV antibody status.

CD4+ count (cells/μl)	Anti-HIV negative (%) (n=1399)	Anti-HIV-1 positive (%) (n=65)	Anti-HIV-2 positive (%) (n=28)	Anti-HIV-D positive (%) (n=8)
≤100 (n=1)	0	1 (1.5)	0	0
100-199 (n=89)	42 (3.0)	30 (46.1)	12 (42.9)	5 (62.5)
200-299 (n=133)	121 (8.6)	7 (10.8)	5 (17.9)	0
300-399 (n=117)	96 (6.9)	17 (26.1)	2 (7.1)	2 (25.0)
400-499 (n=401)	393 (28.1)	5 (7.7)	2 (7.1)	1 (12.5)
≥500 (n=759)	747 (53.4)	5 (7.7)	7 (25)	0

Table 13: Summary of results of CD4+ counts of subjects according to their HIV and HCV status.

CD4+ count cells/	HCV sero-negative (%)n=1469	HCV sero-positive (%)n=31	HIV-1 & HCV sero-positive (%) n=7	HIV-2 & HCV sero-positive (%) n=2
≤100 (n=1)	1(0.06)	0	0	0
101-199 (n=89)	84(5.7)	3(9.7)	3(42.9)	0
200-299 (n=133)	125(8.5)	1(3.2)	1(14.3)	0
300-399 (n=117)	111(7.6)	2(6.5)	1(14.3)	1(50.0)
400-499 (n=401)	391(26.5)	12(38.7)	2(28.6)	1(50.0)
≥500 (n=759)	753(51.2)	13(41.9)	0	0

Table 14: CD4+ counts of HIV and HIV/HCV co-infected persons at diagnosis of their HIV/HCV status.

Variable	CD4+ count	No. in category		Statistical inference		
		Male	Female	CI:	OR	*P*-value
HIV-1						
	<200	3	22	0.38-2.37	0.92	0.31
	200-499	2	24			
	≥ 500	1	6			
HIV-2						
	<200	4	6	0.65-4.0	2.44	0.22
	200-499	2	5			
	≥ 500	1	6			
HIV-D						
	<200	1	5	0.88-2.24	-	0.75
	200-499	0	2			
	≥ 500	0	0			
HIV/HCV	coinfection					
	<200	3	0	0.84-1.72	-	0.67
	200-499	5	1			
	≥ 500	0	0			

3.9.1 CD4+ counts of Study subjects in relation their clinical history or clinic seen.

A summary of the CD4+ counts of all the study subjects in relation to their clinical history or clinic seen are presented in Table 15, page 96.

3.9.2 CD4+ count among pregnant women

Close to half (46.2%; 693/1500) of the subjects of this study were pregnant women seen at the antenatal clinic of the Royal Victoria Teaching Hospital, Banjul (Table 15). About

eighty percent (79.8%) of these were in their first trimester of pregnancy and the remaining in their second (16.2%) or third trimester (4.2%).

The mean CD4+ count of the antenatal women was 461 cells/μl, counts of less than 200 cells/μl were found in 5.9% (41/693) of this group, while counts of 500 cells/μl and above was found in only 38.2% (265/693)(95% CI, 0.56-0.69) of them. Borderline counts of 400-499 cells/μl were found in 35.6 %(247/693). CD4+ count of 100 or less was not associated with any of the 693 pregnant women sampled irrespective their HIV status. The non-pregnant HIV/HCV seronegative women had a mean count of 498-cells/μl. There was a significant statistical association between CD4+ count ($p<0.001$) and pregnant women.

Table 15: CD4+ counts of sampled subjects according to clinical history or clinic seen

Clinic seen or History (%)	CD4+ counts/ μl					
	<100 (%) (n-3)	100- 199 (%) (n=89)	200–299 (%) (n=133)	300–399 (%) (n=117)	400-499 (%) (n=401)	≥500 (%) (n=759)
Antenatal 693(46.2)	0	41(5.9)	78(11.2)	62(8.9)	247(35.6)	265(38.2)
Blood donors 460(30.7)	0	0	2(0.4)	12(2.6)	100(21.7)	346(75.2)
Family planning 28(1.9)	0	0	3(10.7)	9(32.4)	16(57.1)	0
Hepatocellular carcinoma (LP) 13 (0.8)	1(7.7)	0	4(30.8)	1(7.6)	1(7.6)	6(46.1)
Other class of patients 277(18.4)	0	48(17.3)	44(15.9)	33(11.9)	37(13.3)	115(41.5)
Others (Med Examination) 29 (1.9)	0	0	2(6.9)	0	0	27(93.1)

3.9.3 CD4+ counts among blood donors.

Summaries of the CD4+ count of all blood donors are presented in Table 15, page 96. Blood donors accounted for 30.7 percent (460/1500) of the persons sampled in this study. Over 75.2 % (346/460) of the blood donors had a CD4+ count of 500 cells/μl and above, while those in the borderline (400-499 cells/μl) class were 21.7%. All but two of all the blood donors (458/460) sampled in this study were males. The mean count for these donors exclusive of gender was 484 cells/μl. The mean count for HIV/HCV seronegative donors was 496 cells/μl. High CD4+ count and blood donors were highly statistically associated ($p< 0.001$).

3.9.4 CD4+ counts among family planning attendees

Summaries of the CD4+ count of all family planning clinic attendees sampled are presented in Table 15, page 96.

Pre-family planning check-up at the family-planning clinic attached to the RVTH often includes HIV test among others. Fewer women patronize the clinic as many strict Muslims consider family planning incompactible with their religious beliefs. In this study 50 % (14/28) of the women sampled from the family planning clinic were non-Gambians. Seven of these were students while the remaining had an average of four children. The mean CD4+ counts found among these family planning clinic attendees was of 465 cells/μl. None of the women had a count below 200 cells/μl or above 500 cells/μl. There was no significant association ($p<0.05$) between CD4+ count and the family planning attendees.

3.9.5 CD4+ count among patients with a history of Hepatocellular carcinoma.

Hepatitis B prevalence in the Gambia has been shown to be quite high (Kirk *et al*, 2000; Mbaye *et al,* 2000) and it has been documented as the major cause of HCC in the region. However, the contributory role of HCV in HCC in the Gambia is yet to be fully defined. Routinely, facilities for the diagnosis of HBV or HCV do not exist in any of the government owned hospitals. In this study all the 13 subjects with a history of hepatocellular carcinoma had a mean CD4+ count of 403 cells/μl (Table 15, page 96). Among this group was

an AIDS patient in WHO clinical stage 3 (Stein *et al*, 1992). There was no significant association ($p<0.05$) between CD4+levels and the patients with a history of hepatocellular carcinoma.

3.9. 6 CD4+ counts among patients other than those with history of HCC

Patients other than those with history of hepatocellular carcinoma made up 18.4% (277/1500) of the persons sampled in this study. Close to 30 percent (80/277) of these were in-patients while the remaining were outpatients. Most of these (67%, 185/277) visited the hospital following symptoms of acute malaria. The in-patients sampled included 22 AIDS patients in WHO clinical stage 3 or 4 (Stein *et al*, 1992). The mean CD4+ counts of all patients in this group were 382 cells /μl. Similarly, the mean CD4+ counts for the 22 confirmed cases of AIDS were 116 cells/μl. 40.8% of this group of patients (113/277) had CD4+ count of 500 cells /μl or above. None of the defined AIDS patients had a count of 500 cells /μl or above. There was a statistically significant association ($p<0.001$) between CD4+ count and this class of patients. A summary of the CD4+ count of this class of patients is presented in Table 15.

3.9.7 CD4+ counts among persons for medical examination.

Summaries of the CD4+ count of the persons sampled on the ground of medical examination for fitness mainly for employment or educational purposes are presented in Table 15, page 96. Medical examination for fitness is a necessary pre-requisite of many prospective employers and some educational institutions for admission purposes. The persons were all in the age range of 17-48 years. In this study 93% of the persons evaluated under the platform of medical examination (72% for employment purposes and 28% for admission purposes) had CD4+ count of 500-cells/μl or above. Only 7% (2/29) of these persons had a CD4+ count of less than 300 cells/μl. No significant statistical association was found between CD4+ counts and the persons for medical examination.

3.9.8 Summaries of anti-HIV, anti-HCV and anti-HIV/HCV tests results in relation to study subjects clinical history or clinic seen.

Summaries of anti-HIV, anti-HCV and anti-HIV/HCV tests results in relation tostudy subject's clinical history or clinic seen are presented in Table 16, page 98. The pregnant women screened had an HIV and HCV prevalences of 4% (95% CI, 0.29-0.68) and 0.9 %(

6/693) (CI, 0.11-0.67) respectively. Co-infection between HIV and HCV was detected in 2 out of the 693 (95% CI, 018-1.16) of the pregnant women screened. A statistically significant ($p<0.001$) relationship was found between HIV, HCV and pregnant women, however no such relationship ($p>0.05$) was found between HIV/HCV co-infection among the pregnant women.

Blood donors have an HIV and HCV prevalence of 2.4 percent (11/460) (95% CI, 0.14-0.51) and 1.1 percent (5/460) 95% CI, 0.16-1.12 respectively. Patients other than blood donors, antenatal cases and those with history of hepatocellular carcinoma had an HIV prevalence of 19.1 % (53/277). This group also had an HCV prevalence of 6.5% (18/277) (95% CI, 3.03-12.3) and an HIV/HCV co-infection rate of 1.4 percent (4/277) (95% CI, 1.49-20.4). The patients with a history of hepatocellular carcinoma had an HIV prevalence rate of 30.1 %(4/13), an HCV rate of 7.7 % (1/13) and an HIV/HCV co-infection rate of 7.7 %(1/13). HIV/HCV co-infection was significantly associated ($p<0.05$.) with patients with other ailments other than hepatocellular carcinoma.

Table 16: Summary of the results of HIV and HCV antibodies tests in relation to subjects' clinical history or clinic seen.

Clinical history or clinic attended	Number in category (%)(n=1500)	HIV seropositive (%) (n=101)	HCV seropositive (%) (n=31)	HIV&HCV seropositive (%) (n=9)
Ante natal	693 (46.2)	28 (4.0)	6 (0.9)	2 (0.2)
Blood donors	460 (30.7)	11 (2.4)	5 (1.1)	2 (0.4)
Family planning	28 (1.9)	4 (14.3)	0 (0.0)	0 (0.0)
Hepatocellular carcinoma patients	13 (0.9)	4 (30.1)	1 (7.7)	1 (7.7)
*Patients other than HCC cases	277 (18.4)	53 (19.1)	18 (6.5)	4 (1.4)
Others	29 (1.9)	1 (3.4)	1 (3.4)	0 (0.0)

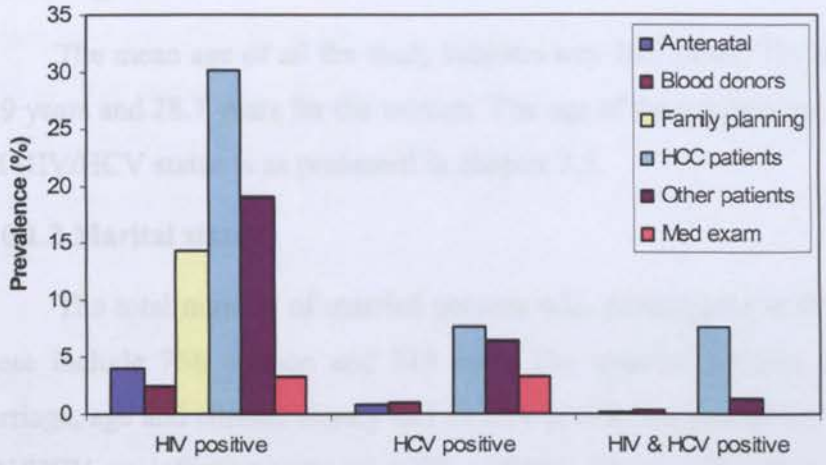

Fig 13: Prevalence of HIV, HCV, HIV co-infection among Study Subjects in relation to Subject's Clinical history or Clinic seen

3.10 Summary of responses to questionnaire

The counselling technique and interview format applied in administrating the questionnaires made participation and responses to the questionnaires very high. Pre-counselling refusal was about 6%, while post counselling refusal was only 2%. The pre-counselling refusal reasons mainly bordered on the issue of lack time, while most post counselling refusal was mainly based on the reason of non –availability or acceptability for follow-up. Similarly, responses to the questions in the questionnaire by all participants were close to 100%. All the 1500 participants who had their samples collected were interviewed directly or indirectly (in case of minors). Full interview covering all sections of the questionnaire was administered on a total of 1367(91.1%) persons. This was made of 523 males (38.2%) and 844 females ((61.7%). A summary of participants responds to these questions in relation to their HIV, HCV, and HIV/HCV co-infections are presented in Tables 17-22.

3.10.1 Socio-demographic characteristics of study subjects in relation to their HIV and HCV antibody status.

A summary of the results of the subjects HIV and HCV results in relation to their age and other demographic characteristics are presented in Table 17, page 103.

3.10.1.1 Age

The mean age of all the study subjects was 30.2 years. The mean age for the men was 31.9 years and 28.7 years for the women. The age of the subjects as related to their HIV, HCV and HIV/HCV status is as presented in chapter 3.5.

3.10.1.2 Marital status

The total number of married persons who participated in the study was 972 (64.8%). These include 758 women and 214 men. The married persons exclusive of sex, type of marriage, age and clinical history had an HIV prevalence rate of 6.1% percent (59/972) and an HIV/HCV co-infection rate of 0.7% (7/972). Those who were single had an HIV and HIV/HCV rates of 7.0% (33/476) and 0.2 %(1/476) respectively. HIV antibody rate was 18.8 percent (3/16) among the divorce/separated. Widows had an overall HIV and HIV/HCV prevalence of 16.7 percent (6/36) and 2.8% respectively (1/36) respectively. HIV prevalence was marginally associated ($p<0.05$) with marriage in this study.

3.10.1.3 Duration of marriage

Couples of three years or less of marriage accounted for 42.6 %%(414/972) of all the married persons, while ten years and above accounted for 18.1 %(176/972). Those that have been married for 4-9 years made the remaining 39.3%. HIV prevalence in couples of three years or less in marriage was 6.0% (25/414) (OR: 0.9589; 95% CI, 0.6019-1.613) HIV-1 was 4.1% (17/414) (OR: 1.095; 95% CI, 0.583-2.041), HIV-2 was 1.7% (7/414) (OR: 1.354; 95% CI, 0.4764-3.813). Similarly, HIV/HCV co-infection rate was 0.5% (2/414) (CI, 0. 3821; 95% CI, 0.0804-1.8443).

Persons who have been married for 10 or above years had the lowest prevalence of 4.5% (8/176). HIV and HIV/HCV rates decreased significantly with increasing duration of marriage. There was no significant association ($p>0.05$) between HIV, HCV or HIV/HCV co-infection and duration of marriage.

3.10.2 Tribe

A summary of the distribution of HIV, HCV and HIV/HCV test results on the basis of tribe is presented in Table 17, page 103. In this study 32.3% of the persons sampled belong to the Mandinka tribe, while the 19.1% and 18.5% were Jolas and Fulas respectively. The

Wolofs made up 13.6% while the Serahulis, Manjago. Aku, Bambara and Serer made up the remaining population of Gambians sampled. Non-Gambians predominantly Senegalese and persons from other West African countries accounted for 7.3 percent (110/1500). Antibodies to HIV, HCV and HIV/HCV were generally distributed among most of the persons irrespective of the tribe. However, significantly high HIV prevalence was found among persons of the Wolof (16.2%), Fula (8.3%), Jola (5.6%) and Mandinka (3.9%) tribes.

The lowest rate of 3.7% was found among the Manjago, none was found among persons of Aku and Bambara tribe origin. Persons of Senegalese origin had an HIV seropositive rate of 4.6 %(4/69). Similarly, HIV/HCV co-infections was found mainly among the Wolofs (2.5%, 5/204) (OR: 7.4246; CI, 1.9679-26.83) Mandinkas (0.4%, 2/493) (OR: O.5179; CI, 0.1084-2.4925), Jolas (2.5%) (5/204), Serahulis (1.7%) (1/58) and Serer tribe (2.7%) (1/36). No co-infections were found among the subjects from Manjago, Aku and Bambara. Similarly, no HIV and HCV co-infection was observed among the 110 non-Gambians other than Senegalese tested in this study. HIV-1 prevalence was significantly associated ($p<0.001$) with the Mandinkas (OR: 0.402 95% CI, 0.21-0.788) but not with all the other tribes. Similarly, HIV-2 was associated ($p<0.05$) with the Senegalese (OR: 23.7; CI, 10.33-26.66) persons but not with all other groups sampled. HIV/ HCV co-infection was marginally associated with persons of the Wolof tribe origin but no such association was found with other tribal groups.

Table 17: Summary of result of socio-demographic factors in relation to subjects' HIV and HCV antibody status.

Variable	No. in category (%)	No. with HIV-1 ab* (%) (n=65)	No. with HIV-2ab (%) (n=28)	No. with HIV D ab(n=8)	No. with HIV-1 &HCV ab (n=7)	No. with HIV-2 &HCV ab (%)(n=2)
Marital status						
Married(Male & Female)	972(64.8)	39(3.6)	14(1.2)	6(0.6)	6(0.9)	1(0.1)
Single	476(31.7)	20(4.5)	12(2.4)	1(0.1)	1(0.1)	0(0.0)
Divorce/Separated	16(1.1)	2(12.5)	1(6.3)	0(0.0)	0(0.0)	0(0.0)
Widowed	36(2.4)	4(11.1)	1(2.8)	1(2.8)	0(0.0)	1(2.8)
Duration of marriage (years)(n=972)						
≤3	414(42.6)	17(4.1)	7(1.7)	1(0.2)	2(0.5)	0(0.0)
4-6	198(20.4)	9(4.5)	3(1.5)	2(1.0)	1(0.5)	1(0.5)
7-9	184(18.9)	7(3.8)	2(1.1)	2(1.1)	2(1.1)	0(0.0)
≥10	176(18.1)	5(2.8)	2(1.3)	1(0.6)	1(0.6)	1(0.6)
N/A*2	528(35.2)	26(4.7)	14(2.6)	2(0.4)	1(0.2)	0(0.0)
Tribe						
Mandinka	493(32.9)	11(4.5)	7(1.4)	1(0.2)	2(0.4)	0(0.0)
Wolof	204(13.6)	24(11.8)	6(2.9)	3(1.5)	4(2.0)	1(0.5)
Jola	286(19.1)	9(3.1)	5(1.7)	2(1.7)	0(0.0)	0(0.0)
Fula	277(18.5)	14(5.1)	7(2.5)	2(0.7)	0(0.0)	0(0.0)
Serahuli	58(3.9)	1(1.7)	2(3.4)	0(0.0)	0(0.0)	1(1.7)
Manjago	27(1.8)	1(3.7)	0(0.0)	0(0.0)	0(0.0)	0(0.0)
Aku	5(0.3)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)
Bambara	4(0.3)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)
Serer	36(2.4)	2(5.6)	0(0.0)	0(0.0)	1(2.7)	0(0.0)
Senegalese	69(4.6)	3(4.3)	1(1.4)	0(0.0)	0(0.0)	0(0.0)
Others *NG)	41(2.7)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)

Variable	No. in category (%)	No. with HIV-1 ab* (%) (n=65)	No. with HIV-2ab (%) (n=28)	No. with HIV D ab(n=8)	No. with HIV-1 &HCV ab (n=7)	No. with HIV-2 &HCV ab (%)(n=2)
Religion						
Muslim	1206(80.4)	57(4.7)	25(2.1)	8(0.7)	7(0.6)	1(0.1)
Christian	221(14.7)	6(2.7)	2(0.9)	0(0.0)	0(0.0)	1(0.4)
[b]Others	66(4.4)	1(1.5)	0(0.0)	0(0.0)	0(0.0)	0(0.0)
None	7(0.5)	1(14.3)	1(14.3)	0(0.0)	0(0.0)	0(0.0)
Number of people in household						
≤ 5	109(7.3)	2(1.8)	0(0.0)	0(0.0)	0(0.0)	0(0.0)
5-8	260(17.3)	6(2.3)	2(0.8)	1(0.4)	2(0.8)	0(0.0)
9-12	488(27.1)	16(3.3)	8(1.6)	2(0.4)	4(0.8)	1(0.2)
≥13	643(42.9)	41(6.4)	18(2.7)	5(0.8)	10(1.6)	1(0.2)

[a]NG- Non Gambians,

* [b] Persons belonging to other faith other than Christianity or Mohammedanism (Islam)

3.10.3 Religion

It is estimated that close to 90 percent of the Gambia populations are Muslims (Table 13, page 94). In this study, 80.4 percent of the subjects sampled were Muslims. This group accounted for 89% (8/9) of all the co-infection between HIV and HCV observed, while the Christians accounted for the remaining 11% (1/9). HIV-1 was marginally associated (p=0.04) with the Muslims. However, no significant relationship was found between HIV/HCV co-infection and the religion of the persons sampled in this study.

3.10.4 Number of people in household

Subjects belonging to a large household of thirteen or more formed 42.9 percent (643/1500) of the sampled population, while subjects belonging to household of five or less accounted for 7.3 percent (105/1500). HIV rates were high in those with 9 or above (OR: 0.3103, 95% CI, 0.1772-0.604), people in their household as against those with 8 or less (8.0% versus 3.0%) (95% CI, 0.1772-0.604). Co-infection was also comparatively higher in subjects belonging to a large household. The HIV/HCV rates were 0.8% (2/260) for those

belonging to a household with 5-8 persons, and 0.4% (2/488) for 9-12 persons household and 0.5% (3/643) for household with 13 or more persons. There was a significant association ($p<0.05$) between HIV prevalence and number of people in ahousehold, but no association ($p>0.05$) was found with HIV/HCV co-infection and number of people in household.

3.11 Education, Income level, Duration in present village or town of residence and HIV/HCV antibody status of sampled subjects.

A summary of the influence of education, income level, and duration in present village or town of residence on the HIV, HCV antibody of the sampled subjects is presented in Table 18, page 109.

3.11.1 Education

Illiterates accounted for 34% (510/1500) of the subject's sampled, while primary school leavers accounted for 39.6% (594/1500) and others with secondary education and above accounted for the remaining number (26.4%). HIV prevalence was highest among the illiterates (9.4%; 48/510) (95% CI, 1.207-2.559) followed by primary school leavers. Subjects with secondary education had an HIV prevalence of 4.5% (22/488) and those with one form of post secondary education or the other had the lowest HIV prevalence of 4.0%(4/100).

Similarly the illiterates had the highest HCV prevalence of 5.8 %(18/510) (95% CI, 1.32-5.44, OR: 2.75), followed by the most educated 2.0% (2/100). Co-infection with HIV and HCV had a prevalence rate of 0.8 %(4/510) (95% CI, 0.412-5.76, OR: 1.56) among the illiterates and 0.5% (95% CI, 0.173-2.38, OR: 0.64) among the educated. HIV prevalence was significantly associated ($p<0.001$) with both the illiterates and the educated, while infection with HCV was marginally associated ($p=0.003$) with the illiterates and the educated. Co-infection between HIV and HCV was not significantly associated ($p<0.05$) with both the illiterates and the literates.

3.11.2 Employment Status

Job applicants which full-time housewives accounted for almost a third of the people sampled in this study while government and extra-governmental workers accounted for 16.9% (254/1500). Self employed, predominately tradesmen, petty traders accounted for 12.7% (191/1500), Students and infants made up 33.5% (502/1500) and private company workers made up the remaining 3.2% (48/1500).

HIV rates were highest among self-employed persons (10.5%) and private company workers (10.4%) as against its rate in applicants /full-time housewives (6.5%) and government/ extra-governmental workers (5.9%). Infection with HIV-2 was also detected among all class of workers averaging a prevalence rate of 2.0%. Infection with HIV-1 was twice (8.3%) as prevalent among self-employed subjects than among government workers (3.5%), private companies (4.2%) un-employed persons and housewives (3.6%) and was the lowest among students/ infants (1.0%). Private company workers and government/ extra-governmental workers had rates of 2.0 percent (1/48) and 1.2 percent (3/254) respectively. HIV and HCV co-infection had an average prevalence of 1.0 %(2/191) among the self-employed, government and extra-governmental workers, and the unemployed/housewives. No co-infection was detected among the private company workers, students and infants.

A comparative analysis showed that the working class had a higher HIV prevalence than the un-unemployed 8.1 %(40/493) (95% CI, 0.95-1.54) versus 6.1% (61/1003) (95% CI, 0.51-1.11). Conversely, a higher HCV prevalence was found among the un-employed than among the working class [2.3% (23/1003) versus 1.6 %(8/493)]. However, the same rate (0.6%) of HIV and HCV co-infection was found in both groups of subjects. HIV was marginally associated ($p < 0.05$) with the employment status of the subjects; however, there was no significant association between HCV and HIV/HCV co-infection and the subject's employment status.

3.11.3. Income

Less than half of the people sampled in this study were employed, however, most claimed to receive one form of monthly support or the other from relations often from relations' abroad. However, more than three-quarters (76.5%) of the employed or self employed persons with a guaranted income earned less than two thousand Dalasis (D2000 per month), while 15.7% (77/493) were within the middle income bracket of D2000-3999 per month and 2.6% (39/1500) earned D4000 or more per month. The HIV prevalence according to the income of the subjects showed progressive increase with increasing income. The highest income earners (*D4000 per month) in this study had the highest prevalence of 10.3% (4/39) as against the 6.4 percent (20/312) found among the less income earners (* D2000 per month). A similar trend was observed in dual infection with HIV-1 and H1V-2. The lowest

income earners had an HIV 1 prevalence of 4.0% (12//312) as against a rate of 7.7% (3/39) seen among the high-income earners.

Infection with HIV-D had an almost equitable distribution (0.4%) among all income groups except for those in the income group of D3000- D3999 or above per month. This group had an HIV-2 prevalence of 1.9% (1/54), while no HIV-D infection was detected in the highest income group. In a similar manner infection with HCV was almost independent of the income group except for those in the income bracket of D4000 per month and above. Anti-HCV had a prevalence of 2.2% (7/312) among the low-income group (*D2000 per month) and 0.9 %(1/93) among those earning D3000 and above per month. HIV and HCV co-infection was equally independent of income of the subjects' sampled. Co-infection was found in almost all income groups with an average prevalence rate of 0.6% (3/493). The lowest income earners (D1000 per month) had rate of 1.0 % (2/204) as compared to 2.6% (1/39) found among those earning D3000 and above. Co-infection between HIV-2 and HCV was only found in one person within the low earners (D1000 per month).

3.11.4 Duration in present town or city of residence.

Over 70% (1054/1500) of the subjects sampled have spent 16 years or more in their present town or village of residence and only 10% 149 (1500) have stayed for 5 years or less. Similarly, approximately 20 percent (198/1500) have stayed in their present town or village of residence for between 6-15 years. HIV prevalence was highest (10.7%) among those who have stayed for 5 years or less and lowest among these who have stayed for 16 years or more (5.4%), those who have stayed for 6-10 years had a prevalence rate of 8.4 percent (11/131) and 6.0 percent (10/167) for those who have stayed for 11-15 years in their present town or village. The rates decreased with the increasing number of years of residing in one station.

Infection with HCV was found with similar prevalence in those with less than 5 years of residency in present town or village (2.7%) and in those with 16 years or more of residency in their present town or village (2.3%). Similarly those with 6-10 years of duration in present town or village and those with 11-15 years had prevalence rates of 0.8 percent (1/131) and 1.2 percent (2/167) respectively. Co-infection between HIV and HCV was highest in those with low number of years of residency in their present location (2.0% for < 5 years versus 0.1% for

6-15 years). In a similar vein co-infection between HIV-2 and HCV was only found in those with less than 5 years in their present location.

Table 18: Summary of Education, type of employment and income of samples subjects in relation to their HIV and HCV antibody status.

Variable	No. involved (%) (n=1500	Anti-HIV-1 positive (%) (n=65)	Anti-HIV-2 positive (%) (n=28	Anti-HIV-D positive (%) (n=8)	Anti-HCV positive (%) (n=31)	Anti-HIV-1 &HCV Positive (%) (n =7)	Anti-HIV-2 HCV Positive (%) (n=2
Education: Highest level attained							
Illiterate	510(34)	28(5.4)	16(3.1)	4(0.8)	18(5.8)	3(0.6)	1(0.2)
[a]1°	594(39.)	18(3.0)	6(1.0)	3(0.5)	6(1.0)	2(0.3)	1(0.2)
[b]2°	288(19.)	16(3.2)	5(1.0)	1(0.2)	5(1.0)	1(0.2)	0
[c]3°	108(7.2)	3(3.0)	1(1.0)	0(0.0)	2(2.0)	1(1.0)	0
Employment							
Self-employed	191(12.)	16 (8.3)	3(1.6)	1(0.5)	4(2.1)	2(1.0)	1(0.5)
Govt/ Govt Agencies	254(16.9)	9(3.5)	5(2.0)	1(0.4)	3(1.2)	3(1.2)	0(0.0)
Private Compans	48(3.2)	2(4.2)	4(2.1)	2(4.2)	1(2.0)	0(0.0)	0(0.0)
Applicants/ Full-time wives	505(33.7)	21(4.2)	10(2.0)	2(0.4)	18(3.6)	1(0.2)	1(0.2)
Students/infants	502(33.5)	17(3..3)	6(1.2)	2(0.4)	5(1.0)	1(0.4)	0(0.2)
Income: Dalasis([1]D) per month							
1000	204(63.3)	9(4.4)	4(1.9)	1(0.4)	6(2.3)	1	1(0.2)
1000-1999	108(18.4)	3(2.9)	2(1.8)	1(0.4)	1(1.4)	0	0(0.0)
2000-2999	88(10.6)	5(5.7)	2(1.9)	1(0.6)	1(1.9)	1	0(0.0)
3000-3999	54(5.1)	11(2.6)	1(1.3)	1(1.2)	1(1.2)	0(0.0)	0(0.0)
≥4000	39(2.6)	3(37.7)	1(2.6)	0(0.0)	0(0.0)	0	0(0.0)

[1] D31.25=$1.00 (US) (As per December, 13 2005)

[a]1° - Primary Education

[b]2° - Secondary Education

[c]3° - Post Secondary Education

3.12 Summary of behavioural risk factors in relation to subjects HIV and HCV antibody status

A summary of the subjects' behavioural risk factors in relation to their HIV and HCV antibody status is presented in Table 19, page113.

3.12.1 Marital status and number of wives

Married women accounted for 77.8% (757/972) of the married subjects in this study. Similarly, 37.5% (215/572) of the men were married. The men involved in monogamous marriage were 68 (37.4%), polygamous marriage with two, three or four wives was 55(26.1%), 51(22.3%) and 41(13.9%) respectively. In a similar vein, 206 (27.2%) of the married women were involved in monogamous marriage, while 551(72.3) of them were involved in polygamous relationship.

HIV prevalence rate among the married persons exclusive of gender was 6.1 %(59/972; 95% CI, 0.56-1.2). HIV-1, HIV-2 and HIV-D rates among this class of subjects were 4.0% (39/972; 95% CI, 0.53-1.42), 1.4 %(14/972; 0.26-1.13) and 0.6 %(6/972; 95% CI, 0.33-8.04) respectively. Similarly, the married persons had an HIV-1 and HCV co-infection rate of 0.6 %(6/972; 95% CI, 0.39-27.3) and 0.1 %(1/972; 95% CI, 0.034—0.66) for HIV-2 and HCV co-infection.

The HIV prevalence for those involved in polygamous relationship independent of gender was 6.4% (45/698) as compared to 5.1% (14/274) for those in monogamous relationship. Similarly, HIV-1 and HIV-2 rates for those in polygamous marriage was 4.3 %(30/698) and 1.4% (10/698), HCV 2.4% (17/698), while HIV and HCV co-infection rate for those in polygamous relationship was 0.6% (4/698). The rates for those in monogamous relationship were 3.3 % (9/274) for HIV-1, 1.4 % (4/274) for HIV-2, 1.1% (3/274) for HCV. Co-infection between HIV and HCV for this group was 1.1% (3/274). The women in monogamous relationship had an HIV prevalence of 5.8 % (12/206 as compared to the rate of 2.9 % (2/68) found among their male counter parts. Unmarried persons had a comparatively higher HIV prevalence rate than the married (7.9 % versus 6.1%). Among the un-married, HIV-1 and HIV-2 prevalences were 4.9% (26/528) and 2.6% (14/528) respectively. Similarly, HIV-D and HCV had a prevalence of 0.4 (2/528) and 2.1 % (11/528) respectively. Co-infection rates between HIV-1 and HCV, and HIV-2 and HCV was each 0.4% (1/528).

The married men independent of the number of wives had an overall HIV prevalence of 5.6 % (12/215). HIV-1 rates for the married men was 3.3 %(7/215), HIV-2 (1.4 % (3/215) and HIV-D 0.9 %(2/215). Similarly the married men had an HCV prevalence of 5.1 % (11/215). The overall HIV/HCV co-infection rate for this group was 1.9 % (4/215). Co-infection between HIV-1 and HCV was 1.4% (3/215) and HIV-2 and HCV 0.5 % (1/215). There was a marginally significant relationship ($p<0.05$) between HIV, HIV-2 and the married. No significant relationship ($p>0.05$) was found between HIV-1, HIV-D and HIV/HCV co-infection with the married persons.

3.12.2 Female circumcision

In this study about a third (36.7%) (341/928) of the females sampled were circumcised (Table 19.). HIV prevalence among the circumcised females was 11.7 % (40/341) (OR: 1.9; CI, 1.18-2.76). A break down of the rates among the circumcised was 6.7% (24/341) (95% CI, 0.92-2.72) for HIV-1, 3.5% (12/341) (95% CI, 1.06-6.25) for HIV-2 and 1.2% (4/341) for HIV-D. The prevalence of HCV among the circumcised females was 0.9 %(3/341) 95% CI, 0.21-3.41). Similarly the HIV and HCV co-infection rates among the circumcised women were 0.6 %(2/341). The uncircumcised female subjects had an HIV prevalence of 6.5% (38/587) (OR: 0.52; CI, 0.36-0.84). HIV-1 rates for this group were 4.6 % (27/587) (CI, 0.38-1.11), while HIV-2 was 1.4% (8/587) (CI, 0.15-0.93), HIV-D was 0.5% (3/587) and HCV 1.0% (6/587) (CI, 0.29-4.61). A comparison of the circumcised versus uncircumcised women showed that HIV prevalence was significantly associated ($p=0.002$) with both circumcised and uncircumcised women such association was found ($p>0.05$) was not found between female circumcision and HCV or HIV/ HCV co-infection.

3.12.3 History of jaundice or hepatitis since birth

Only 67 of the subjects (4.5%) sampled gave a history of jaundice or hepatitis since birth, while 93.4 % (1401/1500) were certain that that they have never suffered from both conditions since birth and 2.1% (32/1500) could not recall suffering from either conditions (Table19, page 113). HIV and HCV rates among those with history of jaundice or hepatitis was 7.4 %(5/67) (95% CI, 0.43-2.2.45) and 3.0% (2/67) (95% CI, 0.09-5.14) respectively. The prevalence of HIV/HCV co-infection was 3.0% (2/67) (95% CI, 1.29-28.8). The HIV and HCV rates in those with no history of jaundice or hepatitis since birth were 6.9 % (96/1401)

and 2.1 % (29/1401) respectively. No significant statistical relationship ($p>0.05$) was found between jaundice or hepatitis and HIV or HCV prevalence.

3.12.4 History of skin tattoo

Only 1.3 percent (19/1500) of the sampled subjects admitted having their skin tattooed in the past (Table 20, page 118) Antibodies to HIV-2 only were detected in 1 out of these 19 persons (5.3%). No statistical significance relationship was found between skin tattoos and HIV prevalence.

3.12.5 History of blood oath

HIV rates among the subjects with a history of blood oath were 4.3 % (1/23). No HCV antibodies were detected among this class of subjects (Table 19, page 113). No statistical significant ($p >0.05$) relationship was found between blood oath and HIV prevalence.

3.12.6 Alcohol consumption

Only 1.1% (16/1500) of the subjects (all males) admitted alcohol consumption as shown in Table 19, page 113. Approximately 0.4 % (6/1500) took it occasionally, while 0.7% (10/1500) were regular consumers. No HIV or HCV antibodies were detected among this class of subjects.

Table 19: Summary of personal/behavioural risk factors of sampled patients in relation to their HIV and HCV antibody status.

Variable	No. involved (%)	No Anti-HIV 1 Positive (%) (n=65)	No Anti-HIV 2 Positive (%) (n=28	No. HIV D Positive (%) (n=8)	No. HCV positive (%) (n=31)	No. HIV 1 & HCV Positive (%) (n =7)	HIV-2 & HCV Positive (%) (n=2)
Type of marriage (No. involved=972)							
Monogamous	274(18.2)	9(3.3)	4(1.4)	1(0.4)	3(1.1)	2(0.7)	1(0.3)
Polygamous	698(46.5)	30(4.3)	10(1.4)	5(0.7)	17(2.4)	4(0.6)	0(0.0)
Unmarried.	528(35.2)	26(4.9)	14(2.6)	2(0.4)	11(2.1)	1(0.4)	1(0.4)
No of wives							
1	68(37.4)	1(2.3)	1(2.3)	0(0.0)	3(4.4)	1(1.5)	0(0.0)
2	55(26.1)	2(6.7)	1(3.3)	1(3.3)	2(3.6)	1(1.8)	1(1.8)
3	51(22.3)	2(4.5)	0(0.0)	0(0.0)	3(5.9)	0(0.0)	0(0.0)
>4	41(13.9)	2(12.5)	1(6.3)	1(6.3)	3(7.3)	1(2.4)	0(0.0)
Female Circumcision (Female subjects only, n=928)							
Yes	341(36.7)	24(7.0)	12(3.5)	4(1.2)	3(0.9)	1(0.3)	1(0.3)
No	587(63.3)	27(4.6)	8(1.4)	3(0.5)	6(1.0)	0	0
History of Jaundice or Hepatitis since birth (All subjects, n=1500)							
Yes	67(4.5)	2(3.0)	2(3.0)	1(1.5)	1(1.5)	1(1.5)	1(1.5)
No	1401(0.9)	62(4.4)	24(1.7)	7(0.50)	29(2.1)	5(1.00	1(1.1)
Do not know	32(2.1)	1(3.1)	2(6.2)	0(0.0)	1(3.1)	1(3.1)	0(0.0)
History of skin tattoo (All subjects n=1500)							
Yes	19(1.3)	0(0.0)	1(5.3)	0(0.0)	0	0(0.0)	0(0.0)
No	1479(98.6)	65(4.4)	27(1.8)	8(0.5)	31(2.1)	7(0.4)	2(0.1)
Cannot recall	2(0.13)	0(0.0)	0(0.0)	0(0.0)	0	0(0.0)	0(0.0)
History of blood oath (All subjects n=1500)							
Yes	23(1.5)	1(4.3)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)
No	1477(98.5)	64(4.3)	28(1.9)	8(0.5)	31(2.1)	7(0.5)	2(0.1)

3.13. Summary of subjects' knowledge of HIV/AIDS, attitude and personal behaviour in relation to their HIV and HCV antibody status.

A summary of study subjects' responses to questions on knowledge of HIV/AIDS, attitude and personal behaviour in relation to their HIV and HCV antibody status are presented in Table 20, page 118 and 21, page 121.

3.13.1 Knowledge of HIV/AIDS transmission and condom awareness

Over 80% (1121/1367) of all the persons interviewed knew at least of sex as a mode of transmission of HIV, 16.8% also had heard of the virus but do not know how it is transmitted, while 1.2% (16/1367) had never heard of the virus at all (Table 20, page 118). The prevalence of HIV was highest in those who have never heard of the virus (12.5%; OR: 2.3002; 95% CI, 0.5745-7.9539) and lowest in those who did not know how the virus is transmitted (5.7%; OR: 0.9435; CI, 0.5321-1.6844). Conversely HCV rates were marginally higher in those who knew how HIV/AIDS is transmitted than those who did not (2.2% versus 2.1%) while, HIV/HCV co-infection had a comparatively higher prevalence among those who do not know how HIV/AIDS is transmitted than those who knew (0.9%, OR: 1.416 versus 0.6%, OR: 0.766). HIV and HIV/HCV co-infection prevalence rates were not statistically associated ($p>0.05$) with the men and women's knowledge of HIV. However, there was a significant relationship ($P=0.0198$; OR: 0.1611; 95% CI, 0.0226-1.1203) between those who have never heard of HIV/AIDS and HCV prevalence but not with HIV or HIV/HCV co-infection.

3.13.2 Knowledge and use of the condom

Almost all (95.7%) the men interviewed admitted having heard of condoms, while 2.3 % (12/523) used them regularly, and 10.3% used them occasionally. Conversely, 4.3 %(22/523) had never heard of the condom, while 87.3 % (457/523) who know about the condom had never used it. No anti-HIV was detected in all the 12 men who claimed regular use of the condom, while the occasional users had a lowest prevalence (3.7%; OR: 0.95; 95% CI, 0.23-3.97) as compared to the non-users (4.1%; OR: 1.39). Similarly, HCV rates were marginally higher among the men who have never heard of condoms than those who have heard (4.5%, OR: 1.08 versus 4.2%, OR: 0.92).

Condom awareness among the women interviewed was significantly lower than their male counterparts, 78 % (662/844) of them admitted having heard of the male condom, while 21.6% (182/844) have never heard of the condom. HIV-1, HIV-2 and HIV-D prevalence rates among the women who admitted knowledge of condoms was 5.3 % (35/662), 2.0 % (13/662) and 0.9 %(6/662) respectively. The women who have never heard of condoms had a comparatively higher HIV-1 prevalence rate (6.6%; 12/182), a lower HIV-2 (1.6%; 3/182) and HIV- D rates (0.5%; 1/182). However, unlike their male counterparts, HCV prevalence rates among the women were independent of the level of their condom awareness (1.1%). However the Odds ratio was higher among the women with no knowledge of condoms than those with knowledge of condoms (1.03 versus 0.96).

3.13.3 Sex during the menses

In this study all the 844 women interviewed responded to the question on sex during menses. More than eighty percent (80.6%) of them felt sex during menses was incmpactible with their religious beliefs. Only 1.3 % (11/844) of the women admitted having had sex during their menses (Table 20). No antibody to HIV was detected among the subjects who reported sex during menses as against prevalence rates of 5.6 % (47/833) for HIV-1, 1.9 % (16/833) for HIV-2 and 0.8 % (7/833) for HIV-D found in subjects who reported no sex during menses. Conversely, HCV rates were comparatively higher among those who had sex during their menses than those who did not [9.1% (1/11), 95% CI, 1.2898 versus 1.0% (8/833), 95% CI, 0.0144-0.7953]. Similarly, the Odds ratio for HCV infection was significantly higher among women who admitted sex during menses than those who did not (10.3 versus 0.09). Furthermore, no HIV/HCV co-infection was found among the women who reported sex during menses while a rate of 0.4% was found among those never had sex during their menses. A test of significance using the Fischer exact test showed no statistical significance relationship ($p>0.05$) between sex during menses and HIV, HCV and HIV/HCV rates among the women.

3.13.4 History of non-menstrual bleeding following sexual intercourse and painful sexual intercourse.

Twenty six out of the 844 women (3.1%) interviewed reported a history of non-menstrual bleeding following sexual intercourse, while 5.9% reported having experienced painful intercourse on regular basis within the past five years. HIV rates were higher among the women with history of non-menstrual bleeding following sexual intercourse than other who did not (11.5%, 95% CI, 0.4742-4.1848 versus 8.2%, 95% CI, 0.239 -2.1087). Similarly the odds ratio for HIV infection among the women with a history of non-menstrual bleeding following sexual intercourse was more than double that than other who did not report such a history (1.46 versus 0.68). Similarly higher HCV prevalence was recorded among subjects with history of non-menstrual bleeding following sexual intercourse and or painful sexual intercourse (Table 20, page 118). However, there was no statistical significance association ($p>0.05$) between subjects' histories of non-menstrual bleeding following sexual intercourse or painful sexual intercourse and HIV or HCV or HIV/HCV co-infection.

3.13.5 History of STIs/STDs amongst the women

Questions bordering on the history of sexually transmitted infection or disease for the past five years were answered by 90.5% (765/844) of the women interviewed; 8.1% (68/844) reported non-ulcerative venereal disease and 1.3% (11/844) ulcerative venereal disease. Women, with a history of ulcerative VD had an HIV prevalence rate of 36.3% (4/11), OR: 6.64, CI, 2.03-10.37 while non-ulcerative VD history had a rate of 14.7% (10/68), OR: 2.06, 95% CI, 1.02-3.54 (Table 20, page 118). The lowest rate of 7.3 % (56/844) (OR: 0.37) was found among those with no history of VD. Similarly women who reported a history of ulcerative VD had an HCV rate of 18.2% (2/11) OR: 26.22, CI, 5.05-92.67 and an HIV/HCV co-infection rate of 9.1% (1/11) OR: 83.2, CI, 5.05-1135.0. A comparatively lower rate was found among those who reported a history of non-ulcerative VD and those with no history of VD (Table 20). A test of significance using the Fischer exact test showed a significant relationship ($p<0.05$) between HIV and all the women irrespective of their history of VD.

3.13.6 History of STIs amongst the men.

All the 523 men interviewed responded to the questions on history of STIs/STDs. A history of urethra discharge was given by 8 of them (1.5%, 8/523), while 0.4 % (2/523 reported having received treatment for genital ulcers in the past and the remaining 513 claimed never to have had any form of sexually transmitted disease within the past five years. HIV was significantly associated ($p=0.033$) (OR: 8.95; 95% CI: 1.93-25.0) with persons with a history of urethral discharge and among those who claimed never to have had STIs ($p=0.005$) (OR: 0.035; 95% CI: 0.041-0.33) (Table 17, page 103). However, no association ($p>0.05$) was found between males with or without a history of STIs and HCV or HIV/HCV co-infection (Table 20, page 118).

3.14 Summary of selected socio-demographic variables, risk factors and CD4+ count of the study subjects according to their HIV status.

A summary of the selected socio-demographic variables and risk factors, CD4+ count of the study subject according to their HIV positivity is presented in Table 20, page 118. HIV infection was significantly associated ($p<0.01$) with the age, CD4+ count, education, employment, marital status, female circumcision and history of STD but not ($p>0.05$) with tribe, number of wives, duration of marriage, HIV awareness, use of condoms, blood transfusions during menses, injection drug use, alcoholism, blood oath, or wife inheritance.

Table 20: Description of socio-demographic, risk factors and CD4+ count of HIV infected persons in relation to their Confidence intervals, Odds ratio and *P*-trends.

Variable	Number of Subjects	Number HCV +ve	CI:	OR	*P*-value
Sex					
Male	572	23	0.03-0.75	0.46	0.0003
Female	928	78			
Age (years)					
≤ 19	238	9	0.49-1.06	0.7	0.02
20-26	431	28			
27-33	376	49			
34-40	179	6			
41-47	150	3			
48-54	83	1			
≥55	43	5			
CD4+ count					
<200	90	49	22-19.7	29.3	0.00001
200-499	651	410			
≥ 500	759	12			
Tribe					
Aku	5	0	0.7-1.5	1.02	0.08
Bambara	4	0			
Fula	277	23			
Jola	286	16			
Mandika	493	19			
Manjago	27	1			
Serer	36	2			
Serahuli	58	3			
Wolof	204	33			
Senegalese	69	4			
Others	41	0			

Variable	Number of Subjects	Number HCV +ve	CI:	OR	*P*-value
Education					
Illiterates	510	48			
Educated	979	53	1.03-2.12	1.52	0.009
**Not Applicable	11	0			
Employment					
Employed	493	8			
Unemployed	505	18	1.04-2.21	1.56	0.009
*** Students/ FT wives	502	25			
Marital Status/Type of Marriage					
Monogamous	274	14			
Polygamous	698	45	0.4-1.24	0.71	0.06
N/applicable	528	42			
No. of wives (n=215)					
1	68	2			0.15
2 or more	147	10	0.09-1.92	0.42	
Duration of Marriage (Years) (n = 972)					
≤3	414	28			
4-9	382	25	0.71-1.86	1.15	0.09
≥10	176	8			
Female Circumcision (n=928)					
Yes	341	40			
No	587	38	1.19-2.77	1.92	0.002
History of STDs within the past ten years (n =1288)					
Yes	89	14			
Yes	1199	85	1.3-3.74	2.45	0.003
HIV/AIDS awareness (n=1321)					
Yes	1121	66			
No	230	13	0.54-1.72	0.97	0.12

Variable	Number of Subjects	Number HCV +ve	CI:	OR	*P*-value
Condom use (n = 523)					
Yes always	12	0	0.22-3.82	0.91	0.29
Yes Occ	54	2			
Never	457	19			

(*Non-Gambians and non-Senegalese)

**Not Applicable – These were teenagers/children

*** Occ - Occasionally

3.15 Summary of selected socio-demographic variables, risk factors and CD4+ of the study subjects according to their HCV status.

A summary of the selected socio-demographic variables and risk factors, CD4+ count of the study subject according to their HCV positivity is presented in Table 21, page 121. HCV infection was significantly associated ($p<0.01$) with the sex and the age of the subjects, tribe, education, and history of STDs but not ($p>0.05$) with CD4+ count, employment, marital status, female circumcision, number of wives, duration of marriage, wife inheritance, HIV awareness, use of condoms, blood transfusion's during menses, injection drug use, alcoholism and blood oath.

Table 21: Selected socio-demographic variables, risk factors, CD4+ count of HCV infected subjects in relation to their Confidence intervals, Odds ratio and *P*- trends.

Variable	Number of Subjects	No. HCV positive	CI:	OR	*P*-value
Sex					
Male	572	22	1.84-8.50	4.1	0.0001
Female	928	9			
Age (years)					
≤ 19	238	0	0.28-1.24	0.6	0.5
20-26	431	10			
27-33	376	4			
34-40	179	6			
41-47	150	9			
48-54	83	1			
≥55	43	1			
CD4+ count					
<200	90	3	0.52-5.42	1.70	0.17
200-499	651	15			
≥ 500	759	13			
Tribe					
Aku	5	0	0.7-1.5	1.02	0.08
Bambara	4	0			
Fula	277	10			
Jola	286	6			
Mandika	493	8			
Manjago	27	0			
Serer	36	1			
Serahuli	58	1			
Wolof	204	5			
Senegalese	69	0			
Others	41	0			

Variable	Number of Subjects	No. HCV +ve	CI:	OR	*P*-value
Education					
Illiterates	510	18	1.32-5.44	1.52	0.003
Educated	979	13			
**N/A	11	0			
Employment					
Employed	493	8	1.32-5.44	2.75	0.003
Unemployed	505	18			
*** Students/ FT wives	502	25			
Marital Status/Type of Marriage					
Monogamous	274	3	0.15-1.57	0.47	0.10
Polygamous	698	17			
N/applicable	528	11			
No. of wives (n=215)					
1	68	3	0.24-3.57	0.92	0.27
2 or more	147	7			
Duration of Marriage (Years) (n = 972)					
≤3	414	9	0.46-2.64	1.10	0.17
4-9	382	5			
≥10	176	6			
Female Circumcision (n=928)					
Yes	341	3	0.22-3.42	0.86	0.27
No	587	6			
History of STDs within the past ten years (n =1288)					
Yes	89	6	4.65-42.97	15.1	0.00004
Yes	1199	25			
HIV/AIDS awareness (n=1321)					
Yes	1121	25	0.38-2.21	0.91	0.18
No	230	6			
Condom use (n = 523)					
Yes always	12	2	0.21-369	0.88	0.29
Yes Occ	54	0			
Never	457	9			

(*Non-Gambians and non-Senegalese)
**Not Applicable – These were teenagers/children
*** Occ - Occasionally

3.16 Summary of selected socio-demographic variables, risk factors and CD4+ count of the study subjects according to their HIV and HCV co-infection status

A summary of the selected socio-demographic variables and risk factors, CD4+ count of the study subject according to their HIV and HCV co-infection status is presented in Table 22, page 124. Co-infection with HIV and HCV was significantly associated ($p<0.05$) with the sex of the subjects, CD4+ count, tribe, education, employment and history of STD but not ($p>0.05$) with marital status, income, female circumcision, number of wives, duration of marriage, wife inheritance, HIV awareness, use of condoms, blood transfusions during menses, injection drug use, alcoholism and blood oath.

Table 22: Description of HIV and HCV co-infected person in relation to their socio-demographic, risk factors and CD4+ count with the statistical inferences.

Variable	Number of subjects	No. HCV positive	CI:	OR	*P*-value
Sex					
Male	572	7			
Female	928	2	1.18-27.2	5.74	0.02
Age (years)					
≤ 19	238	0			
20-26	431	3			
27-33	376	1			
34-40	179	2	0.14-2.1	0.53	0.17
41-47	150	1			
48-54	83	1			
≥55	43	1			
CD4+ count					
<200	90	3			
200-499	651	6	2.09-30.8	8.1	0.01
≥ 500	759	0			
Tribe					
Aku	5	0			
Bambara	4	0			
Fula	277	0			
Jola	286	0			
Mandika	493	2			
Manjago	27	0	0.02-0.56	0.12	0.003
Serer	36	1			
Serahuli	58	1			
Wolof	204	5			
Senegalese	69	0			
Others	41	0			

Variable	Number of subjects	No. HCV positive	CI:	OR	*P*-value
Education					
Illiterates	510	4			
Educated	979	5			
**N/A	11	0	0.41-5.7	1.52	00.2
Employment					
Employed	493	6			
Unemployed	505	2	1.0-1.62	4.12	0.03
***Students/ FT wives	502	1			
Marital Status/Type of Marriage					
Monogamous	274	3			
Polygamous	698	4	0.5-8.9	2.3	0.15
N/applicable	528	2			
No. of wives (n=215)					
1	68	1			
2 or more	147	3	0.08-6.8	0.72	0.41
Duration of Marriage (Years) (n = 972)					
≤3	414	9			
4-9	382	2	0.11-2.77	0.54	0.24
≥10	176	3			
Female Circumcision (n=928)					
Yes	341	2			
No	587	0	-	-	0.13
History of STDs within the past ten years (n=1288)					
Yes	89	3			
No	1199	6	1.7-26.5	6.94	0.02

HIV/AIDS awareness (n=1351)					
Yes	1121	7			
No	230	2	0.08-2.0	0.42	0.18
Condom use (n = 523)					
Yes always	12	0			
Yes Occ	54	1	0.14-9.44	1.16	0.04
Never	457	6			

(*Non-Gambians and non-Senegalese)

**Not Applicable – These were teenagers/children

*** Occ - Occasionally

3.17 Identification / Description of study/control group.

Based on the test results all the subjects with antibodies to HIV and or HCV who consented to the follow-up study were enrolled for further evaluation. These subjects were categorised into three groups.

Category 1 (HIV seropositive subjects)

This group was made up of all the anti-HIV seropositive persons who consented to the follow-up. Eighty-seven persons were listed for participation in this category out of the 101 found HIV positive. Nine were co-infected with HCV and were classified under a different category, four were lost to death and one declined a follow-up after post-test counselling. The 87 persons were as follows: 72 females including 49 HIV-1 infected with an age range of 3 years to 47 years (mean age 26.5 years), 17 HIV-2 infected patients with an age range of 20-26 years (mean age 28.6 years) and 6 dually infected aged 16-36 years (mean age 24.9 years).

The men totalled fifteen (15) and included seven (7) HIV-1 patients with an age range of 19-58 years (mean age 35.1), seven (7) HIV-2 infected, ranging from 20-58 years in age (mean age 35.3 years) and a 58 years old dually infected man. The overall mean age of the men and women as per the time of enrolment was 36.1 years and 26.9 years respectively.

Category 2 (HCV seropositive persons)

The persons enrolled in this category were persons who tested anti-HCV positive and but HIV negative. In all a total of 15 men in the age range of 22 to 52 years and 7 women in the age range of 22 to 44 years were enrolled. The mean age of the men and women as per the time of enrolment was 36.7 years and 33.1 years respectively.

Category 3 (HIV and HCV co-infected patients- Case study)

The third group were patients with HIV and HCV co-infection. In all nine persons consisting of seven men in the age range of 26–58 years (Mean age: 39.1 years) and two women aged 32 and 33 years (Mean age- 31.5 years) were listed for participation in this class. Six of the men had HIV-1/HCV co-infection and one HIV-2/HCV co-infection. Two women one in her second trimester of pregnancy as per time of enrolment had HIV-1 and HCV co-infection and the other a full-time housewife had HIV-2/HCV co-infection. Thus the total number of persons with either HIV or HCV or HIV/HCV co-infection listed for participation in this study totalled 118. This thus formed the case study group.

3.18 Matched Control group

A total of 306 persons were enrolled initially as a control group. However this number was reduced to 288 following the need to withdraw participants with CD4+ counts of 200 cells/μl or less and subsequently increased to 250 cells/μl. Thus, this group was made up of 222 women confirmed as HIV and HCV seronegative and selected on basis of age to match the 74 HIV positive women, 66 men confirmed as HIV and HCV negative selected on basis of age to match the 22 HIV positive men, Secondly it was not possible to match all subjects with control groups on the basis of submitted risk factors. All case study subjects were matched with two or three HIV and HCV seronegative control subjects.

CHAPTER FOUR

4.0 Follow-up Studies

This chapter features all the follow-up work carried out on all the persons enrolled for follow-up study. It includes the case and case control subjects.

4.1 Collection of follow-up blood samples

The last month of every six-month following the preliminary screening test was devoted for sample collection. The first two weeks of the sample collection months was employed for the collection of blood samples from the control subjects, while the last two weeks was for collection from all study patients. Thus, the months of June and December 2003 and June 2004 were exclusively for sample collection. All class of participants were given a specific appointment with an option of two weeks of grace within the stipulated month to keep the appointment. Participants who failed to keep the appointment within one month of the appointed date were excluded from further participation. Thus the sample collection often spilled over to the better part of the following month. The blood collection procedures were as previously described in section 2.5. In all, a total of three follow-up samples were collected from each participant over a period of 18 months.

4.2 Follow-up HIV test

All the HIV seronegative control groups and HCV infected patients employed, as case study groups were re-tested every six months for HIV antibodies to rule out HIV seroconversion (Fiebig *et al,* 2003). In all a total of three follow-up HIV tests were carried out on each participant over a period of 18 months and a total of 307 persons were involved. This was made up of 19 persons (15 men and 4 females) all HCV positive and 288 people enrolled as negative control groups (66 men and 222 females). A participant who seroconverted or who missed any of the three appointments was withdrawn from further participation. The test materials and procedures are as previously described in chapters 2.5.3 and 2.5.4. These results are presented in chapter five.

4.3 Follow-up HCV test

A repeat of the HCV tests were carried out on all the HIV seropositive persons (case study group) and on all the HIV/HCV seronegative persons enrolled as negative control groups to rule out HCV seroconversion every six months. A total of three follow-up HIV test were carried out on each participant over a period of 18 months. In all a total of 376 persons were initially involved. This was made up of 14 males and 66 females all HIV positive and 288 persons enrolled as negative control groups (66 men and 222 females). HCV seroconverts were to have been withdrawn from further participation, while participants who missed any of the three appointments were withdrawn from further participation.

The test materials and procedures were as previously described in section 2.5.6. These results are presented in chapter five.

4. 4 Follow-up CD4+ count

The CD4+ count of every participant involved in the follow-up test was repeated every six months and the result recorded as a means of monitoring the trend of the count. In all a total of 387 (288 negative control, 19 HCV and 80 HIV positive) persons were initially enrolled for participation. Participants with counts of 250 cells/μl or less at any stage were referred to the Medical Research Council Laboratories clinic for the commencement of antiretroviral treatment. Such participants were therefore excluded from further participations in the study. Initially, a count of 200 cells/μl or less at diagnosis of status (CD4+dx) was employed for referral, this was however, upgraded to 250 cells/μl after the preliminary test to accommodate an envisaged decline in count within the 6 months of in-waiting before the next test. The procedures employed were as previously described section in 2.5.5. These results are presented in chapter five

CHAPTER FIVE

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5.0 FOLLOW-UP TEST RESULTS

5.1 Follow-up HIV test Result

A total of 291 persons out of the 307 (94.8%) initially listed for HIV follow-up tests fully participated in the three rounds of the follow-up tests. This was made up of 72 males (12 HCV infected and 60 seronegative control group) 219 females (5 HCV infected and 214 negative control group).

One case of HIV seroconversion was recorded against one of the 66 men (1.5%) initially enrolled as HIV negative control subject during the second round of the follow-up study (12 months later). The man a 32 years illiterate old driver of Wolof tribe was married to two wives and was excluded from further participation. He gave no previous history of blood transfusion or blood oath or any form of skin scarification. However, he admitted receiving injections from some chemists but with new syringe and needles. Similarly he also admitted involvement in extra-marital sex without the use of a condom and gave a history of urethral discharge, which was treated in a chemist store seven months before the diagnosis of his HIV status. The man had been married for over twenty years to the first wife, while his second wife was only a year old as his wife. He admitted not taking his second the wife to the hospital for HIV screening prior to their marriage, while the wife claimed ignorance of her HIV status. Screening of the wife revealed her as HIV sero-positive. It was not possible to screen the wife's two months old baby as per the time of the diagnosis of her serostatus. The man admitted knowing how HIV is spread and was aware that a condom can prevent its spread but claimed never to have used them.

Among the females three cases of HIV seroconversion was recorded among the 222 (1.44%) of them enrolled as HIV and HCV seronegative control subjects over the 18 months follow-up period. Two of the HIV seroconverts were detected during the second round of the follow-up period (12 months after enrolment) and the other during the third and final round of the follow-up (18 months later). Two out of the three women who seroconverted were of Jola tribe and the third was a Mandika. They were aged 24, 26 and 29 years as per the time of

their diagnoses. All were married, two polygamously. Two out of the three (66.7%) of the female seroconverts gave a history of persistent vaginal discharge for which treatment was on-going as per the time of the diagnosis of their HIV status. Only one out of the three (33.3%) admitted to have been circumcised.

One out of the three women completed primary school the remaining two were illiterate.The three women were all full time housewives with an average of two children. Similarly two out of the three women admitted having heard of HIV/AIDS and how it can be transmitted and prevented. One admitted having heard of HIV/AIDS but was not aware of how it be prevented. Analysis of the socio-demographic and selected risk factors of the HIV positive seroconverts and that of the non-seroconverts showed a statistically significant association ($p=0.0006$) (OR: 167.4; CI: 15.1-120.1) between a history of STDs and HIV seroconversion but not with blood transfusion ($p=0.9$) or female circumcision ($p=0.1$). Similarly, HIV seroconversion was not associated ($p > 0.05$) with the age, gender, marital status, religion, education, condom use, HIV/AIDS awareness, injection with contaminated needles, and or number of wives of the seroconverts. A summary of the submitted socio-demographic and risk factors of seroconverts and non-seroconverts are presented in Table 23, page .

Table 23: Selected demographic and submitted Risk factors associated with HIV seroconversions and non-seroconversions HIV/HCV seronegative control groups.

Variable	HIV sero-conversions (n=4)	Non-HIV Seroconversions (n=284)	Cl:	OR	*P*-value
Sex					
Male	1	66	0.2-4.6	1.12	0.4
Female	3	218			
Age (years)					
≤ 19	0	0	0.3-6.0	2.5	0.3
20-26	2	3			
27-33	1	1			
34-40	0	2			
41-47	1	1			
48-54	0	1			
≥ 55	0	1			
Religion					
Muslims	4	274		0.9	
Xtians	0	9			
Others	0	1			
Tribe					
Aku	0	1	0.2-9.5	1.4	0.4
Bambara	0	0			
Fula	0	40			
Jola	2	79			
Mandika	1	113			
Manjago	0	2			
Serer	0	2			
Serahuli	0	0			
Wolof	1	47			

Variable	HIV sero-conversions(n=4)	Non-HIV Seroconversions (n=284)			
Education (n=288)					
Hliterates	3	110	0.49-4.41	4.75	0.1
Educated	1	174			
Marital Status (n=288)					
Single	0	7	-	-	0.9
Married	4	277			
Type of Marriage (years) (n=281)					
Monogamous	1	91	0.14-4.2	0.7	0.4
Polygamous	3	186			
Duration Marriage (Years) (n=281)					
<6	3	119	1.0-3.2	4.2	0.2
6-9	0	113			
≥10	1	52			
Female circumcision (n=222)					
Yes	1	138	-	-	0.1
No	2	0			
Histroy of STDs within the past ten years (n=288)					
Yes	3	5	15.1-120.1	167.4	0.00006
No	1	279			
HIV/AIDS awareness (n=288)					
Yes	3	258	0.5-1.5	0.3	0.3
No	1	26			
Condom use (n=66)					
Yes always	-	7	0.14-9.4	1.16	0.40

* Yes Occ	-	5			
Never	1	53			
History of blood transfusion within the past ten years (n=288)					
Yes	0	2	-	-	
No	4	282			

* Occ- ocassionally

5.2 Follow-up HCV test result

A total of 293 out of the 376 (78%) persons listed for HCV follow-up tests participated fully in all the three rounds of the follow-up tests. Only 19 out of the 88 (21.6%) HIV infected persons participated in all the three rounds of the tests, while 274 out of the 288 (95.1%) negative control persons participated in all the three rounds of the follow-up test. No HCV seroconversion was recorded among any of these participants.

5.3 CD4+ Trends in HIV–1 infected persons

The mean age of the participants at the commencement of the programme was 39.4 and 26.5 years for males and females respectively. The mean CD4+ lymphocyte count of males and females as per the diagnosis of their HIV status was 375 cells and 291 cells per microlitre (cells/μl) respectively. A comparatively larger proportion of women presented with a lower CD4+ count than the men as per the time of the diagnosis of their status (44.9%; 22/49 versus 42.9%; 3/7). Only 4 out of the 7 men (57.1%) and 25/49 (51.0%) women participated in the first round of the follow–up test six months later. The mean CD4+ lymphocyte count for the 4 male participants was 464 cells/μl and 339 cells/μl for the 25 females.

During the second round of the follow-up test 12 months later only three males (42.9%) and 18 (36.3%) females participated. The mean CD4+ count of these was 328 cells/μl and 303 cells/μl for male and females respectively. There was a 29.3% and 10.6% decline in the mean CD4+ count of the male and female participants over the 6-months interval. Similarly, the last round of the follow-up test had only 2 male and 10 female participants and their mean CD4+ count was 316 cells/μl and 285 cells/μl for males and

females respectively. The cumulative decline in CD4+ of the participants who fully completed the programme was 43.2% and 44.4% for male versus female respectively. A highly significant association ($p<0.0001$) was found between CD4+dx and the declining CD4+ trends when CD4+dx was regressed against the first, second and third CD4+ counts, however, no such association was found between CD4+ counts and age or gender due in large part to the small number of participants who completed the programme. The CD4+ trends in those who participated fully are shown in Table 24, page 135 while its trends in all the enrolled participants is contained in Appendix 9, page 236.

Table 24: CD4+ trends in HIV-1 infected male and female subjects

Serial Number	Age Years	Sex	CD4+dx cells/μl	1st count cells/μl	2nd count cells/μl	3rd count cells/μl
1	20	F	586	522	476	401
2	16	F	384	345	271	201
3	13	F	389	345	256	188
4	20	F	365	323	265	201
5	22	F	393	345	292	224
6	28	F	483	435	368	295
7	30	F	588	542	482	421
8	22	F	579	523	468	408
9	35	F	475	433	378	303
10	33	F	376	322	264	204
11	47	M	458	405	372	307
12	32	M	472	436	370	324
Mean	26.5		462	415	355	290

5.4 Trends in CD4+ cell count of HIV-2 infected male patients

Seven HIV-2 infected males aged 29-55 years with a mean age of 32.3 years were initially listed for follow-up. Their mean CD4+dx count at enrolment (CD4+HIV) was 287 cells /μl. The lowest count was 172 cells/μl and the highest 504 cells/μl. Four out of the seven persons had counts of 250 cells /μl or less were referred for antiretroviral therapy and were not given further appointments. The three participants during the next round of the count had a mean count of 345 cells /μl and were aged 25, 30 and 55 years. A fifty-five years (55) old man among this three had a count of 237 cells/μl cells/μL and was referred for antiretroviral therapy and was not given any further appointment. . The second and third round of the counts had only one participant with a count of 328 cells/μl and 269 cells/μl respectively. One participant aged 25 years did not show up after the first count. Thus only one participant aged 30 years completed the follow-up exercise. No HCV sero-conversion was recorded among any of these classes of participants. The 30 years old man showed a persistent decline in CD4+ count ranging from 12.1% after 6 months to 16.2 % after 12 months and 23.1% after 18months. The cumulative decline in his CD4+ count after 18 months was 51.3%. The trend in his CD4+ count is shown in Table 25, page 137 while its trends in all the enrolled participants are contained in Appendix 12.

5.5 Trends in CD4+ cell count of HIV-2 infected female patients

A total of 17 females aged 20 to 40 years with a mean age of 28.5 infected with HIV-2 were initially enrolled for follow-up study. The mean CD4+ count of the females upon enrolment was 354 cells /μl. The count ranged from 169 to 564 cells /μl. Seven of them had CD4+ counts of 250 or less and were referred for retroviral therapy without further appointments. Ten of the females kept the next appointment six months later and recorded a mean CD4+ count of 403 cells /μl with a range of 196-508 cells/μl. Two of these ten had counts below 250 cells /μl and were referred for retroviral therapy without further appointments. Seven participants showed up during the second round of the count another six months later. Their mean CD4+ count was 400 cells /μl with a range of 213 to 468 cells /μl One of the participants further recorded a count below 250 cells /μl was also referred for antiretroviral therapy with no further appointment. Only 5 out of the 17 (29.4%) females participated fully in all the rounds of the counts. The mean CD4+ count of these at enrolment

for the programme was 556 cells/μl cells. Six months later there was a 10.6 % decline in their mean CD4+ count; this was increased to 13.9% and 17.1 %, 12 months and 18 months latter respectively. Thus they was a 36.1% decline in the mean CD4+ count of these five females after 18 months. The lowest count recorded by any them after the 18 months of monitoring was 340 cells /μl, while the highest was 410 cells /μl. No HCV sero-conversion was recorded among any of the females through out the 18 months of follow-up. A highly significant association ($p<0.0001$) was found between CD4+dx and the declining CD4+ trends when CD4+dx was regressed against, the first, second and third CD4+ counts, however, no such association was found between CD4+ counts and age due in large part to the small number of participants who completed the programme. The CD4+ trends in the five females and one male who completed all rounds of the count are shown Table 25, page 137 its trends in all the listed participants is contained in the Appendix 13, page 244.

Table 25: CD4+ trends in HIV-2 infected male and female study patients

Serial no.	Age Years	Sex	CDdx cells/μl	1st count cells/μl	2nd count cells/μl	3rd count cells/μl
1	31	F	564	496	407	341
2	28	F	551	485	417	342
3	20	F	547	492	423	340
4	40	F	564	508	427	341
5	34	F	554	504	468	410
6	30	M	504	443	371	285
Mean	30.5		547	488	418	343
% Decline				10.8	14.3	17.9

5.6 Trends in CD4+ cell count of HIV-D infected male and female patients

A total of seven HIV-D infected females and one HIV-D infected man aged 67 years were initially enrolled for follow-up counts. The mean age of these participants was 30.1 years. The females were aged 14 years to 34 years with a mean of 25 years. The lowest count recorded among the females upon enrolment was 168cells/μl and the highest was 474 cells/μl with a mean of 250cells/μl. All participants in this class including the only man had counts of below 250 and were referred for retroviral therapy without further appointments. One out of the two remaining participants aged 20 years had over a 46% decline in her CD4+ count within six months bringing her count from 280 cells/ to 151 cells/μl and was referred for retroviral therapy with further appointments. Only one out of the eight (12.5 %) participants participated fully in all the rounds of the count and had a final count of 258 cells from an initial count of 474 cells upon detection of HIV/HCV status, thus showing 54.8% decline in count within 18 months. The CD4+ trend in the person who participated fully is shown in Table 26, page 138 and its trends in all those initially enrolled are contained in the Appendix 14, page 245

Table 26: CD4+ trends in HIV-D study patients (females)

S/N	Age	Sex	CD4+dx	1st count cells/μl	2nd count cells/μl	3rd count cells/μl
1	27	F	474	412	328	258
% Decline				13.1	20.4	21.3

5.7 Trends in CD4+ cell count of HCV seropositive male and female subjects

A total of 15 males aged 22 years to 52 years were enrolled as HCV positive persons for follow-up. The mean age of the men as per the time of enrolment was 35.7 years. The mean CD4+ count of the men upon enrolment was 486 cells /μl. The lowest count of 404 cells /μl was recorded in a 46 years old man, while the highest count of 555 cells /μl was seen in a 24 year old man. A total of 14 of the men kept the second and third appointments six months and 12 months later respectively. Their mean CD4+ count after six months was 481cells/μL showing a mean decline of 1%. Similarly, their mean CD4+ count after 12 months was

491cells/μL depicting a 2% increase in the mean count. While the lowest count recorded among this group was 455 cells/μl cells/μl μL and the highest was 590cells/μl μL. Two other men did not keep the last appointment; the remaining 12 participants had a mean CD4+ count of 493 cells/μl with a range of 430-560 cells/μl. No HIV sero- conversion was recorded among any of these participants through out the duration of the study. The trends in CD4+ count of the 12 participants who fully participated in the counts are shown in table 27 below.

Similarly, seven females aged 22 to 45 years were enrolled for follow-up studies as HCV-seropositive. Five participated fully in all the counts, while one dropped after the preliminary count and another did not show-up for the last appointment.

The mean age and mean CD4+ count of the females as per the time of enrolment were 31.2 years and 524-cells/ μl respectively. The lowest count recorded among this group as per enrolment was 410 cells/μL seen in a 38 years old mother of seven and the highest was 606 cells/μl. seen in a female aged 45 years involved in a polygamous relationship. Six females kept the appointment six months later while only 5 responded 12 months later. The mean age and mean CD4+ count as per the time of enrolment of the five females who fully participated in all the counts were 31.2 years and 524 cells /μl.

A decline of 4.3% in the mean CD4+ count was recorded among this group six months after enrolment in the programme. This was followed by a 2.2 % increase in count after 12 months bringing the mean count of the five participants who submitted blood samples to 506cells/μl. The five participants who participated fully in the programme had a mean count of 499 after 18 months with the lowest as 450 and the highest as 556cells/μl. There was a gross decrease of 3.5% in the mean CD4+ count of the five participants over the 18 months of monitoring. This decrease was not uniform over the period. Multiple regression analysis of these trends showed no significant association (p=0.27) between CD4+ at diagnosis (CD4+dx) and the subsequent counts. Similarly, there was no association (p=0.17) between the age of the participants and their CD4+ counts. The CD4+ trends in all the listed participants are contained in the appendix while its trends in those who participated fully are shown in Table 27, page 140.

Table 27: Trend in CD4+ cell count of HCV seropositive male and female patients.

Serial number	Age (years)	Sex	CD4dx cells/μl	1st count cells/μl	2nd count cells/μl	3rd count cells/μl
1	45	F	606	554	580	556
2	26	F	542	490	478	460
3	38	F	410	380	424	450
4	20	F	521	506	488	514
5	22	F	506	545	562	516
6	26	M	501	467	512	524
7	46	M	404	521	498	472
8	33	M	452	456	455	432
9	22	M	534	612	590	560
10	45	M	476	433	480	466
11	32	M	500	478	512	478
12	32	M	412	453	466	490
13	24	M	555	488	472	455
14	25	M	456	478	472	502
15	40	M	552	434	455	430
16	52	M	539	470	512	494
17	22	M	550	512	476	489
Mean	32.3		501	487	496	488

5.8 Trends in CD4+ cell count of HIV-1/HCV co-infected male and female patients

A total of seven persons made up of six men aged 23 to 48 years (mean 31.9 years) and a woman aged 27 years co-infected with HIV-1/HCV were initially listed for the follow-up CD4+ count. The mean age of all the men was 31.9 years, while their mean CD4+ cells count upon enrolment was 350 cells. The mean CD4+ count of all the participants upon enrolment was 360cells/μl. Three of the participants all males aged 26, 41 and 48 years had CD4+ counts of 187, 168 and 190 cells/μl respectively and were excluded from further participation. During the next round of the counts the female member had a 17.1% decline in

her CD4+ count (280 to 232 cells/μl), while three of the remaining men had a mean count of 350cells/μl during this round of the count and two of them kept the appointment for the second and third rounds of the count and had a mean count of 272 cells/μl and 171 cells/μl respectively. One did not show up after the first round of the count. No HCV seroconversion was recorded among this class of participants. There was a cumulative decline of 64.4 % in CD4+ within 18 months. The trends in CD4+ count of these two who participated fully in all rounds of the follow-up is shown in Table 28 below and its trends in all the participants are contained in appendix.

Table 28: CD4+ trends in HIV/HCV co-infected patients who participated fully in the follow-up study

Serial number	Age (years)	Sex	CD4+dx count (cells/μl)	1st count (cells/μl)	2nd count (cells/μl)	3rd count (cells/μl)
1	34	M	474	370	251	160
2	23	M	485	407	293	182
Mean	28.5		480	389	272	171
% Decline				19.0	30.1	36.8

5.9 Trends in CD4+ cell count of HIV-2/HCV co-infected male and female patients

Only two participants a man and a woman aged 55 years and 36 years respectively were HIV-2/HCV co-infected. The man had a CD4+ count of 305 cells /μl upon enrolment, while the woman had a count of 425cells/μl. The man had a 16.4% decline in CD4+ count after six months while the woman had 18.1 % decline. The man was referred for retroviral therapy without further appointments, while the woman did not keep the subsequent appointments. Hence no HIV-2/HCV infected patient completed the follow-up exercise.

5.10 Trends in CD4+ cell count of HIV and HCV seronegative females-control subjects.

Of the 222 confirmed HIV and HCV seronegative females enrolled as control subjects for the 72 HIV positive female patients, 214 (96.4%) successfully participated in the three successive CD4+ counts. Two persons did not keep the appointment for the first round of the follow-up count. The mean CD4+ count for the 220 participants was 503cells/μL. The second round of the follow-up counts had 216 participants two of these persons were found to have seroconverted to HIV positive, while another two did not attend and were dropped from further participation. The third and last round of the follow-up CD4+ count had 215 participants with one found to have equally seroconverted while another did not attend. All sero-converters were infected with HIV-1 and had a mean CD4+ of 403 cells /μl at the time of detection of their sero-status. No HCV sero-version was recorded among any of the participants throughout the follow-up period.

The mean CD4+ count of the all the 222 female control subjects upon enrolment for the programme was 496 cells cells/μl. Their mean CD4+ counts during the first second and third count were 503, 499 and 491 cells /μl respectively. Multiple regression analysis of these trends showed a highly significant ($p<0.0001$) association between CD4+ at diagnosis (CD4+dx) and the subsequent counts. A similar association was found between age and the counts but at a lower level of significance ($P=0.02$).

5.11 Trends in CD4+ cell count of HIV and HCV seronegative male control subjects

A total of sixty-six males aged 18-66 confirmed as HIV and HCV seronegative were enrolled as a control group for the HIV seropositive males. Their mean age and mean CD4+ count as per the time of enrolment were 35.8 years and 489 cells /μl respectively. The lowest count recorded as per the time of enrolment was 239 cells seen in a 56 years old pensioner, while the highest count of 633 was seen in an 18 years old student. Repeated tests did not reveal any evidence of HIV infection in the pensioner. The first follow-up count conducted six months later had 65 out of the 66 participants keeping the appointment. Their mean CD4+ count was 492 cells/μl and the lowest count recorded was 270 cells /μl seen in a 63 years old Fisherman while the highest count was 601 cells/μl seen in two men aged 18 and 29 years old. The second follow-up count had 60 participants keeping the appointments. Their mean CD4+ count was 509 cells /μl. One of the participants a 32 years old driver developed antibodies to HIV and was with drawn from the programme. The third follow-up count had 59

of the participants keeping the appointments, their mean CD4+ count was 513 cells /μl. There was a 4.9 % increase in the mean CD4+ count of these participants after 18 months of enrolment in the programme. The CD4+ trends of all who participated fully are contained in Table 29 below and values in all enrolled participants is contained in the appendix 21.

Table 29: Trends in CD4+ count of HIV and HCV seronegative control male subjects.

Serial number	Age (years)	CDdx (cells/μl)	1st count (cells/μl)	2nd count (cells/μl)	3rd count (cells/μl)
1	18	540	511	520	536
2	18	633	601	578	590
3	19	522	506	496	511
4	19	516	534	503	526
5	22	418	434	450	446
6	22	579	524	543	555
7	22	444	460	488	502
8	23	402	424	434	455
9	24	455	468	442	402
10	25	457	472	505	490
11	25	556	548	532	560
12	25	430	411	452	438
13	26	423	451	448	457
14	26	422	455	434	460
15	26	552	534	525	543
16	26	438	450	448	452
17	26	599	580	572	586
18	26	486	490	510	498
19	27	523	545	560	555

Serial number	Age (years)	CDdx (cells/μl)	1st count (cells/μl)	2nd count (cells/μl)	3rd count (cells/μl)
20	27	567	580	566	576
21	27	467	456	470	466
22	28	415	422	434	453
23	28	504	534	524	511
24	28	517	490	509	526
25	28	506	551	533	521
26	29	589	601	578	598
27	29	512	523	502	496
28	30	509	498	511	520
29	29	566	546	560	533
30	29	445	422	452	458
31	29	414	456	460	446
32	30	508	515	524	515
33	30	513	556	563	537
34	30	486	517	511	498
35	30	556	560	548	560
36	31	511	542	534	524
37	31	534	546	523	528
38	31	402	456	472	487
39	32	522	503	528	542
40	33	523	546	558	542
41	42	520	512	477	486
42	44	489	474	441	424
43	44	516	552	526	542
44	44	509	531	522	536

Serial number	Age (years)	CDdx (cells/μl)	1st count (cells/μl)	2nd count (cells/μl)	3rd count (cells/μl)
45	45	556	567	540	538
46	45	556	523	544	537
47	46	520	534	527	538
48	47	409	398	426	452
49	47	535	540	548	560
50	55	532	502	549	534
51	55	515	535	521	542
52	55	545	560	533	557
53	56	409	411	428	453
54	56	505	525	540	548
55	57	509	543	526	528
56	58	506	488	511	534
57	58	448	478	490	488
58	59	521	552	536	544
59	60	524	511	528	506

CHAPTER SIX

6.0 DISCUSSION

Association between HIV and various other agents including other viruses has been globally recognized (El-Serag *et al*, 2003, Tedaldi *et al*, 2003b, Udo *et al*, 2003). Despite growing global concerns of the clinical consequences of the complementary actions of these associations, and the emerging information of Africa as an endemic area or origin for most of these infections (Tanaka *et al*, 1998; Menendez *et al*; 1999, UNAIDS/WHO; 2002), several issues bordering on the natural history and distribution of these infections remains unclear. To complicate the scenario, most European-based studies are predominately on groups (Bodsworth *et al*; 1996, Fabriz *et al*; 1997, Darby *et al*, 1997, Beld *et al*, 1998, Eyster *et al*, 1999) uncommon in the West African region making it difficult to extrapolate their findings to this region.

In the Gambia, it is only recently that laboratory-based data has begun to emerge on the distribution of HIV in the country (UNAIDS/WHO, 2002 and Schim van der Loeff *et al*, 2003) despite the fact that the infection is over two decades old in this country. Similarly, little or no laboratory- based data exist on the distribution of either HCV or its coinfection with HIV in Gambia. This present study on HIV and HCV coinfection in the Gambia therefore aims to contribute generally to our understanding of the natural history of HIV coinfections with HCV. The eighteen months follow-up study of cases and age-matched control group facilitated the evaluation of the trends in CD4+ count of both the infected and the uninfected and for the identification of the risk factors associated with the incidence of the infections in the region.

6.1 HIV in The Gambia.

The United Nations AIDS programme report (2002) documented an HIV national prevalence of 1.8% for the country, while the national sentinel surveillance found a prevalence of 1.2% for the country. In this study, the overall prevalence of HIV was 6.7 % (101/1500), HIV-1 was 4.3 percent; while that of HIV-2 was 1.9 percent and dual infection between HIV-1 and HIV-2 (HIV-D) had a prevalence of 0.5 percent. More than 90 %

(93/101) of the HIV-seropositive persons were asymptomatic, in WHO clinical stage 1 (Stein *et al,* 1992). The 6.7% HIV prevalence rate found in this study is three fold higher than the documented national average (UNAIDS/WHO, 2002), however, it is comparable to the prevalence reported for adults in Cote d'Ivoire (Da Camara-gomes, 1997) five years ago and is within the highest range for the West African sub-region.

The major disparity between the UNAIDS/WHO, (2002) estimated HIV prevalence for the country and the findings in this report may be due to the fact that this study included patients and children. When these groups were excluded the determined HIV prevalence was 3.6 percent (43/1189). Although this latter rate is twice the UNAIDS/WHO estimate for the country (UNAIDS/WHO, 2002), it may be in line with the changing trend in HIV prevalence within the country since the year 2000 (from laboratory records-unpublished data). Similarly, the rate of 4 percent found among pregnant females was slightly higher than the determined average for the healthy subjects. The higher prevalence found in this group may be due to the comparatively higher prevalence found among females involved in polygamous relationship. Married females are generally considered a low risk group and their HIV prevalence rates accepted as a reflection of the prevailing rate in the community (UNAIDS/WHO, 2002). However other studies have shown that females in polygamous relationship may be classified as a high-risk group (Mboto and Epoke, 2000). Similarly the lower prevalence rate of 2.4 percent found among blood donors may not be considered the prevailing rate within the healthy populace at large. This is because in this study only two out of the 460 donors were females hence extrapolating the HIV prevalence rate for blood donors for the general population at large may therefore be statistically biased. Based on these confounding variables, the acceptable HIV prevalence for the country may be taken as its prevalence among the females involved in monogamous relationship 5.8% (12/206) (OR: 0.61; CI: 0.35-1.16) and its rate among blood donors (2.4%; 11/458) (OR: 0.24;CI: 0.14-0.47) excluding the two females donors. Based on these the determined HIV prevalence for the low risk group in this study is 3.5 % (23/664) (OR: 0.61; CI: 0.24-0.58)

The finding of a comparatively lower mean age of infection for the females as compared to the men (24.9 years versus 36.2 years) may be suggestive of early marriage of the females. There is no minimum age for marriage in the Gambia. Among the Fula tribe, marriage of females at 13 years is not uncommon. A report by Kane *et al,* published in 1993

showed that in Banjul, the capital City of the Gambia 58% of the females were married by the age of 20, a much greater percentage is envisaged for the rural communities. Senderowitz and Paxman (1985) had earlier defined the mean marriage age for females in sub-Saharan Africa to be between 16 and 18 years. Their report showed that Senegal a country that shares an extensive border, language and cultural similarities with the Gambia had the lowest marriage age of 16.4 years. The finding of a highly significant association (p= 0.0003) between HIV prevalence and females in this study and the men in this study may therefore in line with this report. This finding also supports WHO/UNAIDS (2002) report of twice as many young females than as men infected in sub-Sahara Africa.

Studies have also shown that young females tend to marry men several years older who might be more sexually exposed and that their risk of infection increases if a husband is three or more years older than they are (Lagarde *et al*, 1996). WHO/UNAIDS (2002) report had earlier shown that ignorance about sexual and reproductive health and HIV/AIDS is widespread in countries with generalized AIDS epidemics in Africa with up to 80% of females aged 15-24 shown to lack sufficient knowledge about HIV/AIDS. This combined with the fact that the females are more biologically vulnerable to sexually transmitted infections (Latif, 1989) due to the receptacle nature of their genitalia which permits exposure to a larger volume of semen for a longer period of time (Bamberger *et al*, 1999) may help to explain the high difference in HIV prevalence between females and males observed in this study. Furthermore, the estimated risk of transmission of HIV by receptive intercourse is higher in a male to female sexual intercourse than the reverse (Bamberger *et al,* 1999). In addition, unlike the males, the female vaginal mucosa is usually subjected to trauma during heterosexual contact which may enhance HIV transmission (Miller and Lu, 2003).

Generally, disparities in HIV distribution have been reported in various studies globally with several reasons advanced for them (Allen *et al,* 1991 and Auvert *et al*, 2001). However, the finding of a comparatively higher HIV prevalence among persons of the Wolof (16.2%), Fula (8.3%), Jola (5.6%) and Mandinka (3.9%) tribes cannot be explained with clear-cut reasons. One possible explanation may be in terms of the number of persons of these tribes sampled. Another may be in terms of mobility associated with these tribal groups. However, with a poor history of previous blood transfusion, absence of intravenous drug use and sex as the major risk factor and a common vice in the society; individual behavioural risk

factor may be a major contributor to the HIV distribution pattern observed in this study. In the Gambia the Mandinkas are the most predominant tribe, followed by the Wolofs, the Jolas, and the Fulas. The main occupation in the country is farming; the Mandinkas and Serahulis are the leading business persons in the country. Most working class groups are predominantly the Wolofs and recently the Jolas while, the Fulas are the mainly cattle rearers, and the Serer's both men and females are predominately fishers. The most educated elites of the Gambia society are predominately the Akus. Farming is mainly the occupation of the uneducated tribal groups except the Fula groups residing outside the main towns. Polygamy is associated with all the tribal groups except the Aku's that are mainly Christians. In a similarly vein also, the observation of comparatively high HIV rates in persons belonging to a large household as compared to those in less populated houses cannot be justifiably explained. The average Gambian family found in one household often extends to include grand father/grandmothers where these are still living and most often their sons and daughter in-laws, children and grandchildren all under the umbrella of a father or grandfather. Where the original family head may have two or more wives the population within one household may be quite large. This is further compounded by a custom that permits the marriage of first cousins. There is therefore need for a longitudinal study in this regard.

An un-common HIV epidemiology found in this study is the significantly high HIV prevalence found in men aged 55 years and above as against the rates in men aged 13-40 years (17.2% versus 3.1 %). This pattern of HIV epidemiology differs from what has been reported in the developed countries (Bodley-Tickell *et al,* 2004; Stanekova *et al,* 2004). Generally, higher HIV prevalence is globally associated with the youths (UNAIDS/WHO 2002 and Rehan 2003). Two possible explanations that can be advanced for this finding is the few number of men aged 55 years who were sampled. Another reason is probably the comparatively higher number of wives associated with the men aged 55 years and above. In this study only 29 (5.1%) of the male participants were aged 55 years and above as against 417 aged 13-40 years (72.9%). However, the five men in the age range of 55 years and above with HIV antibodies had an average of three wives. Generally, high HIV prevalence in a polygamous relationship has been attributed to the marriage of females by sexually advanced men into a polygamous relationship where HIV infection already exists or by the marriage into a polygamous relationship of an already infected woman (Allen *et al,* 1991). This

assertion is however weakened by the finding of a non-significant statistical relationship between number of wives and increased HIV prevalence ($p>0.05$). However, a study conducted in Pakistan reported the finding of more STIs in older, married men living with their families than the young and unmarried living alone (Rehan 2003). The finding in this study therefore may support Rehan's suggestion of the existence of a different pattern of STIs in developing countries than the type seen in the developed world and underscores the need for more STIs related studies on males and the inclusion of males in the annual sentinel surveillance studies. The current focus in most developing countries is females predominately the pregnant who obviously are more available for sampling.

Patients other than those with history of hepatocellular carcinoma accounted for 18.5% (277 /1500) of the subjects in this study. The finding of an HIV prevalence of 19.1% (53/277) among this group confirms the report of Da Costa (1994) of close at hand AIDS epidemic. Similarly, the finding of a comparatively higher prevalence of HIV-1 in favour of HIV-2 (4.3% versus 1.9%) (28/1500) also confirms earlier reports by Da Costa, in 1994 and Schim van der Loeff *et al*, (2003) of the gradual replacement of HIV-2 by HIV-1 within the region. HIV-2 originated from the West African sub-region and is known to be endemic in the region (Lemey *et al*, 2003). Its comparatively longer incubation period before the development of immunodeficiency coupled with its prolonged period of low levels of virus in the blood of infected persons (Kanki *et al, 1994*, Berry *et al*, 1998) has probably delayed the AIDS disease burden in most parts of the West African sub-region (Kanki *et al*, 1994). Its gradual replacement by HIV-1 may emerge as a major health burden to countries already saddled with a fragile health system.

The finding of comparatively higher prevalences of HIV-1 and HIV-2 in this study contradicts the 2001 national sentinel surveillance report of an overall prevalence of HIV-1 as 1.0% and 0.8% for HIV-2 (Schim van der Loeff *et al*, 2003). Though there was a two years gap between the surveillance and this study. The rates found in this study may be said to strongly differ taken into consideration that the virus has been described as stable in the region (Schim van der Loeff *et al,* 2003). The question is however "Can there be a sudden four-fold up-surge in the virus in the region within two years?" Whatever the answer may be, this calls for further studies, which may be outside the scope of this work. However, the

possible explanations that can be advanced for this disparity may be in terms of differences in sites sampled and probably the comparatively fewer number of people sampled in this study.

In this study the number of females sampled was almost twice the population of the men (61.9% versus 38.1%), while the population of children sampled was comparatively insignificant. This obviously was due to the project design, which allows for the first consecutive 1500 persons referred to the serology laboratory for HIV test during the study period. With a higher female than male population seen in most hospitals in the region, this outcome was therefore not un-expected. The aim of this was to allow a broader coverage of the females- the pregnant and patients for possible extrapolation of the results to related groups. Another reason is that since blood donors are almost exclusively males in the Gambia, pregnant females must be incorporated as group for a balanced study of the low risk society. Thus, a study carried out on pregnant females and blood donors would allow for a balanced comparism of the low risk group of men and females.

Dual infection between HIV-1 and HIV-2 has been reported in several West African regions where HIV-2 predominantly co-exists with HIV-1. However, despite this common presence actual data on the rate of this dual infection are often limited. In this study 88 % (7/8) of the dual infection with HIV (HIV-D) was found among the females. Several reasons may be advanced for this. One is the comparatively greater number of females sampled and probably also the higher prevalence already associated with this group. However, even though there was a comparatively higher prevalence rate of HIV-D among the females subjects, this association was not significant ($p>0.05$).

6.2 HCV in The Gambia

Not many published data exist on the distribution of hepatitis C in the Gambia. Similarly provision for routine diagnosis is yet to be provided in government owned hospitals in the country. Hence screening of donated blood for the virus or for HBV is yet to commence. This has made it difficult to get baseline information on the distribution of the virus within the community. However, a recent report seems to have reduced this burden (Kirk *et al*, (2004). The Gambian hepatitis intervention project and the current research project at hand at the hepatitis unit of the Medical Research Council laboratories, Fajara mainly focus on Hepatitis B (Montesano, 2002), which has been shown to have a

significantly higher distribution in the country than HCV. This study may therefore be the first to provide an extensive baseline data on HCV among blood donors, patients, pregnant females and persons for medical examination for fitness in the Gambia.

In this study the determined overall HCV prevalence was 2.1% (95 % CI, 1.4-2.9). Its rate of 1.0 % (12/1210) among patients and an apparently healthy population respectively may be considered significant because of the clinical and public health implication of the infection. However, its rate among apparently healthy population is below the estimated worldwide prevalence of 3% (WHO weekly Epidemiological records, 2001) and below that of WHO. Madhava *et al*, (2003) estimated an HCV prevalence of 2.4% for the region. It is also below the prevalence rate of 3.0% found by Kirk *et al*, (2004) among a total of 382 apparently normal populations of Gambians recruited as a control group. The comparatively higher prevalence found by *Kirk et al* (2004) may be due to the comparatively smaller population employed in their work and also to differences in the site of population sampled.

Some studies have reported comparatively higher HCV prevalences within the West African region. Earlier studies by Coursaget *et al*, (1990) have reported an anti-HCV prevalence of 4.2% in an apparently healthy adult population in Senegal, Burundi, Tunisia and Madagascar with lower rates reported by Ka *et al* (1996) six years later. This disparity may not be unconnected with the test kit employed (Aach *et al*, 1991) or it may be influenced by the increasing cases of HIV (Terrault, 2002). Disparities in HCV tests due to the generation of HCV test kits employed have been globally accepted (Aach *et al*, 1991). Similarly, an increased prevalence of HCV influenced by HIV coinfection has been established (Terrault, 2002). The contributory roles of these coinfections on HCV prevalences are yet to be evaluated in most of communities of the developing world where HIV and HCV are becoming an increasing concern.

In this study the peak age of HCV infection found for both sexes was in those aged 41-47 years, with the men accounting for 71 % (22/31) of the infection and they had a comparatively higher prevalence rate than the females (3.8%; 95% CI, 2.4-5.8 versus 1.0% 95% CI, 0.4-1.8).

Several studies have provided conclusive evidence for a progressive increase in the prevalence of HCV infection with increasing age (Guadagnino *et al,* 1997), while, high HCV

distribution in those below 49 years (young adults) in the developed countries has generally been accepted as suggestive of recent infection (Wasley and Alter, 2000). However, it is known that regional variability in age-specific HCV prevalence is influenced by prevailing contributory risk factors and that males are at a greater risk for cirrhosis as well as liver cancer when infected with HCV (Poynard *et al,* 1997). However, because of the long incubation period of HCV it's more frequently diagnosed among middle aged and older persons (Poynard *et al*, 1997) most especially in communities where intravenous drug use is not the principal route of its transmission.

The relationship between HCV and age has been demonstrated in several other studies (Amin *et al,* 2004; Leone and Rizzetto 2005, Farley *et al*, 2005) with varying reasons. However, in this study the detection of HCV antibodies in those in their twenties, its absence in females aged 48 years and above coupled with its higher prevalence in persons aged 34-47 years may be suggestive of sex as a possible route of transmission of the virus. This is because intravenous substance abuse, the principal route of transmission of the virus in most developed countries, (Feldman *et al*, 2004, Pybus *et al,* 2005) is a rare event in the Gambia. Irrespective of the fact that HCV is less efficiently transmitted sexually, like HIV, with the existence of a sizeable reservoir of HIV and sex as common behaviour, attributing sex as the predominant mode of transmission of the virus in this study may not be an under statement. This finding may therefore be in line with Fletcher (2003) who reported of increasing sexual transmission of HCV particularly among HIV-positive men. Terrault, (2002) had earlier observed that the "Risk of HCV transmission by sexual contact differs by the type of sexual relationship. Persons in long-term monogamous partnerships are at lower risk of HCV acquisition (0% to 0.6% per year) than persons with multiple partners or those at risk for sexually transmitted diseases (0.4% to 1.8% per year). This difference may reflect differences in sexual risk behaviours or differences in rates of exposure to nonsexual sources of HCV, such as injection drug use or shared razors and toothbrushes." Terrault (2002) further reported a range of 1.6% to 25.5% for HCV seroprevalence for those at risk for sexually transmitted diseases. This assertion is supported by the finding of a comparatively higher significant relationship between the females and presence of HCV antibodies ($p<0.001$) (OR 0.24 95% CI, 0.10-0).

Although very little is known about the natural history of HCV infection in children it has been suggested that asymptomatic infection and complications resulting into liver failure may occur as in adults (Bortolotti *et al*, 1997). In this study anti-HCV was detected mainly in persons aged 20 years and above. However, in a study reported in China, Chen and Xia (1999), found an HCV prevalence rate of 0.35% among 4,055 healthy children of 14 years or less in Beijing. The risk factor reported for this group was blood transfusion.

The HCV prevalence rate of 0.9 percent found among pregnant females in this study is comparable to its overall prevalence of 1.0% found among all the females. This finding further substantiates the possible role of sex in the transmission of HCV in the region and also the contributory role of HIV in enhancing its transmission. However, it is comparatively lower than Njouom *et al,* (2003) finding of a prevalence rate of 1.9% among pregnant females in Yaoundé, Cameroon using a 3rd generation ELISA test kit; Menendez *et al*, (1999) report of 5% among pregnant females in Tanzania and Ahmed *et al,* (1998) a finding of 16.5% among a similar target group in Malawi. However, none of these enumerated studies have evaluated the contributory role of HIV in enhancing HCV transmission in their region. Filippini *et al,* in 2001 had earlier demonstrated that in subjects with only sexual behaviour or gender as a risk factor for the parenterally transmission of HCV, HIV may enhance its sexual transmission. This assertion provides support for the finding of comparatively higher prevalence of HIV/HCV coinfection among females than men in this study.

Another striking finding here is the comparatively lower prevalence rates found in males aged 55 years and above than in those in the age range of 34-47 years (3.4 % versus 7.8%). In most developed countries HCV is associated with injection drug use, blood transfusion (before the 1990) (Alter, 1997), poverty, high risk sexual behaviour, and low level of education, thus the most afflicted age are those in their twenties to forties. In most developing countries these factors with the exception of intravenous drug use still contributes to high HCV prevalence thus this comparatively higher HCV prevalence among this age group is unclear and makes the need for further studies.

Furthermore, the finding in this study indicates that history of blood transfusion, income, level of education even though endemic in the Gambia were all not significantly associated ($p>0.05$) with HCV transmissions.

The finding in the study of a comparatively higher HBV prevalence than HCV in patients with a history of hepatocellular carcinoma (38.3%, 5/13 versus 7.7%, 1/13) corroborates the finding of Coursaget in a study conducted in Senegal (Coursaget *et al*, 1992) and further supports Mbaye *et al,* (2000) reports of the minor role HCV plays in the onset of hepatitis disease in hospitalised patients in Senegal. It may also be in line with Ka *et al,* (1996) earlier report of low prevalence of anti-HCV-antibodies in Senegal, a country with a history of high incidence of hepatocellular carcinoma (HCC) which shares an extensive border, native language and culture with the Gambia.

Generally, Hepatitis B virus has been accepted as the major aetiological agent of hepatocellular carcinoma in Africa (Laskus *et al,* 1998). Despite the comparatively lower HCV prevalence of 0.4% found among blood donors this data highlights the potential public health hazard of HCV infection in the Gambia, where anti-HCV testing is generally not performed in HIV-infected populations or among blood donors.

6.3 HCV Serotypes

Full-length genomic sequence analysis followed by phylogenetic analysis is the reference method for HCV genotype determination. Available data however, shows that the sub genomic regions of the virus such as E1 and NS-5 contain sufficient phylogenetic information for the identification of each of the 11 or 12 known types and subtypes of HCV (Simmonds *et al*, 1994), making the need full -length genomic sequence analysis for routine diagnosis less necessary.

However, facilities for such work are often outside the reach of most third world countries. The lapses of the molecular biology- based methods is it non-adaptation for clinical studies or large-scale routine use. Furthermore it is time-consuming and is often the sequence determination of more limited regions of the genome that is use in most cases. These inadequacies saw the emergence of serotyping assays in 1996 based on the detection of genotype-specific antibodies by immunoenzymatic methods. Furthermore; serotyping assays have the advantages of being useful in determining the HCV genotype in HCV RNA-negative patients and in identifying infections with multiple genotypes.

Studies on the distribution of HCV genotypes conducted in China, the United States of America, and some European countries have shown genotypes 1 or 3 as the most prevalent (Lu *et al*, 2005, Nakano *et al*, 2005; Balogun *et al*, 2003). The finding in this study of HCV type 2 with the highest prevalence and its comparative occurrence in those aged 41 years and as compared to serotypes 2 and 3 that were almost exclusively limited to those 40 or less years may be supportive of the assertion of West Africa as the origin of HCV serotype 2 (Candotti *et al*, 2003). This assertion is further reinforced by the comparatively higher cases of HIV-1 coinfection with HCV serotype 1 and 3 instead of serotype 2 that had a higher prevalence. It also corroborates the report of independent small scale surveys conducted in some West African countries including Burkina-Faso (Mellor *et al.*, 1995), Gambia (Ruggieri *et al.*, 1996), Ghana (Wansbrough-Jones *et al.*, 1998) Benin and Guinea (Jeannel *et al.*, 1998) that have shown the predominance of type 2 in the region. While the finding in this study of more than eighty percent of the HCV infected persons as apparently healthy highlights the potential public health hazard the infection poses in the country.

HCV serotypes are particularly important in the epidemiology of , and pathogenesis of HCV related diseases. There have been shown to significantly relate to the source of the infection (Pawlotsky *et al*, 1995; Haushofer *et al*, 2001). Some studies have shown grave disparities in the severity of liver disease (Feray *et al*, 1995; Puoti *et al;* 2001; Hnatyszyn, 2005) and response to therapy due to differences in the type of HCV infection (Soriano *et al*, 2005; Daniel, 2005). Similarly, some studies have shown differences in serological reactivity due to HCV genotypes (Dhaliwal *et al.* 1996). Furthermore the regional distribution of HCV genotypes shall influence the configuration of diagnostic assays and vaccine design. In addition HCV genotype detection shall be an important tool for tracing sources of infection since the route of transmission of HCV infection remains unknown in a significant number of infected individuals (Zcuzem *et al*, 1994). This disparity has made it necessary for the advocation of routine genotypic determination of HCV isolates as a necessary requisite for the effective management of HCV patients (De Cock and Vranckx, 2003). The implication of these is that vaccines against multiples HCV genotypes is overdue making it is for an understanding of the geographical distribution of HCV genotypes necessary.

The finding of five cases of un-typeable serotypes could be due to infection with variants of HCV genotypes, which were not covered by the competing peptides used in the

assays, or due to the non-specificity of the NS4 peptides used to coat the plates (Songsivilai *et al,* 1998). This finding reveals some of the limitations associated with HCV serotyping as compared to molecular assays.

Relatively few data exist on the genotypic characteristics of HCV in most developing countries of the world including the Gambia and where such data is available it often lacks correlation with the severity of the liver disease, pathology and even the possible sources of the infection. These data therefore highlight the potential public health hazard of HCV infection in the Gambia, where anti-HCV testing is generally not performed in HIV-infected populations or even among blood donors or any other class of patients. It also shows the need for a more elaborate longitudinal study to fully clearly characterise the HCV genotypes in the Gambia using molecular assays Further studies are necessary to evaluate the clinical course of these apparently healthy carriers.

6.4 Risk factors for HCV transmission.

Reports of high HCV prevalence without any evidence on the route of transmission (Coursaget *et al*, 1990, Ola *et al*, Candotti *et al*, Ndjomou *et al*, 2002) have continued to emerge from several parts of the West and Central Africa. The finding in this study of 29 out of the all 31(93.5%) HCV positive persons with no reported apparent risk factors add only to this dilemma. While two persons gave a history of blood transfusion of more than a decade ago each, the rest gave no apparent risk factor. A decade old report by Strasser *et al*, (1995) indicated that 27 per cent of 342 consecutive anti-HCV positive patients seen in a liver clinic in a major Australian metropolitan general hospital had no definite percutaneous risk factor, while Wiese *et al.*, (2000) and Seeff *et al.*, (2000) in two very independent studies have shown that long-term asymptomatic carriage of HCV may occur among persistently infected individuals. Similarly, Kenny-Walsh, (1999) reported the observation of a benign course of HCV infection 22 years after the receipt of anti-D immunoglobulin.

The finding in this study is therefore in agreement of the existence of yet to be identified salient risk factors associated with the transmission of HCV most especially in the developing countries and advocates for the need further studies in this area, while the long asymptomatic stage of HCV infection and its slow disease progression may pose a major

problem to most third world countries where routine testing is often not done until full fledge disease sets in. Thus if all infected with HCV were to progress to disease stage, this would have been a big burden to countries already saddled with fragile health services. Thus it is vital that the future outcome of HCV infection and its natural history be understood to allow for proper HCV management, treatment and health planning. Furthermore due to differences in interruption strategies required for each pattern of HCV transmission, it is necessary that the prevailing risk factors for HCV transmission in each community be identified.

6.5 HIV and HCV coinfection in the Gambia.

In sub-Saharan Africa including the West Africa region, studies of HIV and HCV coinfection have become importantly necessary because of the emerging evidence of a high HCV prevalence (Halim and Ajayi, 2000, Hassan *et al*, 2001, Blanton *et al*, 2002) along with a concurrent HIV epidemic (Madhava *et al*, 2002, UNAIDS, 2003). Available data indicates that sub-Saharan Africa has the highest HCV prevalence (5.3%) in the world (Madhava *et al*, 2002). This is further enhanced by information revealing Africa as an endemic area and or origin for some HIV (UNAIDS/WHO, 2002) and HCV infections (Menendez *et al,* 1999, Candotti *et al*, 2003, Njouom *et al*, 2003). Furthermore, most studies conducted mainly in industrialized countries have shown that coinfection between HIV and HCV is significantly higher among haemophiliacs (Lessens *et al*, 1999, Laurent *et al*,2001; Sulkowski and Thomas 2005) homosexuals (Bodsworth *et al*, 1996) and injecting drug users(Beld *et al*, 1998; Bodsworth *et al*, 1996; Eyster *et al*, 1999) groups uncommon in the West African region making it difficult to extrapolate their findings to this region. Above all, in most of the countries in the West African region despite the increasing reports of high HCV prevalence (Attia, 1998; Halim and Ajayi, 2000; Hassan *et al*, 2001, Blanton *et al*, 2002) amidst a current HIV epidemic (Madhava *et al*, 2002), the respective contributions of parenteral and non-parenteral routes of transmission are not known or are the potential roles of sexual transmission (Laurent *et al*, 2001) known in a region where sexually transmitted infections (STI) are frequent (Atrah *et al*, 1994; Morison *et al.* 2001) thus advancing the need for this study.

In the Gambia, it is only recently that laboratory-based data had begun to emerge on the prevalence of HIV in the country (UNAIDS/WHO, 2002; Schim van der Loeff MF, *et al*,

2003) despite the fact that the infection is over two decades old. Similarly, baseline data on HCV prevalence or its risk factors are lacking in the country. This study is therefore is the first documented work on HIV/HCV coinfection in the Gambia. Its finding of a prevalence of 0.6% among all the subjects sampled and 8.6% in HIV positive persons is low when compared to rates of up 10 % reported in the United States among HIV infected persons (Brau *et al*, 2003) and 2.5 - 4.9% of the general population in Brazil (Brandao and Fuchs, 2003). It is however, similar to the report of a prevalence arte of 8.2% found by Agwale *et al*, 2004 in what is probably the first study of HIV/HCV coinfection study reported in the region. Furthermore it is far below Yerly *et al* (2001) report of an HIV/HCV coinfection prevalence of 47 percent among children in Libya. The source of these epidemics was later attributed to nosocomial transmission. However, of particular importance is its detection among blood donors (0.4%; 2/460) and pregnant females (0.3%; 2/693). This is a major public health concern especially in a system where blood for transfusion is not screened for HCV. Also important is that all the four HIV/HCV seropositive individuals found among blood donors and pregnant women reported no previous history of blood transfusion, surgery or sharing of injection needles thus suggestive of sex as the possible route of transmission.

6.6 HCV serotypes associated with HIV coinfection

Previous studies conducted in developed countries have reported HIV-1 coinfection with HCV genotypes 1 and 3 as the most predominant HCV genotype associated HIV coinfection (Berger *et al*, 1996), while some have suggested significant association between HCV genotype, most especially genotype 1 and progression of HIV disease in coinfected persons (Sabin *et al*, 1997, Goedert *et al*, 2001; Delladetsima *et al*, 2002, Yoo *et al*, 2005). In some studies the association was independent of age at seroconversion (Sabin *et al*, 1997), while some found this association to be unrelated to HIV and age (Goedert *et al*, 2001).

This study is probably the first to report the distribution of HCV genotypes in HIV infected persons in the Gambia. Most of the reported studies have found significant association between HCV genotype with HCV viral load but not with HIV coinfection (Berger *et al*, 1996). In this study HCV viral load was not measured, however, persons coinfected with HCV serotype 1 were more associated with lower CD4+ count at diagnosis and also had a higher decline in CD4+ count than persons coinfected with other serotypes.

However, these differences did not reach statistical significance because of the few number of persons involved; that notwithstanding, the comparatively higher level of HCV serotype 1 coinfection with HIV-1 which is associated with more severe ESLD (Yoo *et al*, 2005) has serious health implications most especially in terms of treatment and makes need for the provision of facilities, not only for the detection of HCV infected individuals, but also for the identification of its serotypes and treatment.

6. 7 CD4+ counts in HIV, HCV, HIV and HCV coinfected persons

The finding in this study of a significant variation in CD4+ counts between non-HIV infected and HIV- infected persons (467 cells/μl versus 310 cells/μl) confirms the role of CD4+ an important evaluator of HIV/AIDS disease. The CD4+ count of 467 cells /μl observed in apparently healthy individuals in this study is in line with the reported range of 294-1597 in absolute count (Nag *et al*, 2002). Furthermore the finding of a significantly lower count in those infected with HIV-D than those infected with HIV-1 or HIV-2 provides evidence for the variation in the severity of these infections (Popper *et al*, 1999) Furthermore the observation of a greater association of significantly lower CD4+ count between coinfection with HIV-1 and HCV than HIV-2 and HCV coinfection (306 cells/μl versus 365 cells/μl) all adds credence to the variation in the severity of the infections This finding is in line with the global concept of the outcome of HIV infection and the observed variation in CD4+ count found here confirms the accepted grading of the severity of the infection (Stein *et al,* 1992). The CD4+ count correlation with the HIV serotypes is also similar to the findings of Schim van der Loeff *et al,* 2002).

The finding in this study of a significantly higher mean CD4+ count for HIV/HCV-seronegative blood donors than for other HIV/HCV-seronegative persons may be suggestive of the high criteria of fitness demanded from all blood donors. Similarly the finding of a significantly higher CD4+ count among non-pregnant HIV and HIV/HCV-seronegative females than their pregnant counterparts is in line with the finding of Kapiga *et al* (2000) in a study conducted in Tanzania.

However, an unusual finding is the low level of CD4+ count found among persons with no demonstrable antibodies to HIV. In this study only 51.1% of the HIV and HCV seronegative persons had a CD4+ count of 500 cells /μl and above, while 1% (15/1500) of

apparently healthy persons had CD4+ counts of between 168-199 cells/μl. Following WHO guidelines on the clinical staging of HIV/AIDS CD4+ counts of below 200 cells/μl may be suggestive of HIV infection (Stein *et al,* 1992). The reasons for these are obviously unclear and calls for further studies to identify the underlying causes. Similarly the finding of only 51.1% of the HIV and HCV seronegative persons having CD4+ count of 500 cells /μl is particularly low when compared to the findings in some European countries (Stein *et al,* 1992). However, a study conducted in the United States reported CD4+ counts less than 200 cells/μl in 17% of HIV-negative patients admitted into intensive care (Aldrich *et al*, 2000). Similarly, a study conducted in Thailand indicated that uninfected Thai adults had significantly lower absolute CD4+ count than uninfected Caucasian adults (Stein *et al,* 1992), while Ramalingam *et al* (2001) advocated for a new CD4+ count staging following comparative difference in CD4+ counts he found between normal and HIV-infected south Indian adults. Also in India Nag *et al* (2002) found similar disparities in the CD4+ count of healthy HIV seronegative Indians.

Another further explanation for the disparity in CD4+ count found in this study is that not all HIV infected individuals or all HIV/HCV coinfected individuals that develop lower CD4+ counts or viraemia (Puoiti *et al*, 2001). Similarly some studies have shown that most antiretroviral drugs influence CD4+ count and viral load (Moore and Chaisson, 1999 Ledergerber *et al*, 1999, Resino *et al*, 2004). In these studies all sampled persons including the patients in various WHO clinical staging of HIV infections were not on any documented antiretroviral treatment. Furthermore, some studies have shown that females generally have higher CD4+ counts on the average than men (Aldrich *et al*, 2000). However, in this study blood donors who were predominately males had a slightly higher mean CD4+ count than the females. This could be in line with the several other factors other HIV infection that influences CD4+ count (Maini *et al,* 1997). More so, CD4+ count in females fluctuates with the menstrual cycle, while the use of oral contraceptives has been associated with its low count. Again, CD4+count decreases following rest, while higher counts have been seen in HIV-positive smokers, as compared with non-smokers (Maini *et al,* 1997). These factors have not been evaluated in this study. While, a longitudinal study may be required for a proper understanding of this disparity in CD4+ count between males and females, there is an

apparent need for each population to have its own clinical staging of CD4+ count most especially countries outside the Caucasian race.

6.8 Hepatitis B virus in the Gambia

Hepatitis B surface antigen (HBsAg), which is a primary marker of current infection hence infectivity can mainly detect infection with standard viruses and some variants with a significant proportion of individuals who are infected with some variants undetected. PCR thus remains the gold standard for the diagnosis of HBV. The HBsAg test however offers the advantage of simplicity and cheapness without expensive equipment making it suitable for most third world countries,

In Gambia HBV has been documented to have a very high prevalence as compared to HCV (Lakus *et al,* 1997). Its investigation is outside the scope of this. It was however, employed as a means of comparing its role with HCV work in HCC patients. Its comparatively higher significance in association with HCC patients than HCV found corroborates a previous report suggesting a minor role of HCV in HCC patients in the region (Kirk *et al,* 2000; Mbaye *et al,* 2000). In the Shashi district of China, Wu (1998) using a second-generation HCV test kit found cross infection between HBV and HCV as the major cause of active cirrhosis of the liver. He also found an anti-HCV antibody prevalence of 20% in patients with active cirrhosis of the liver.

The finding of no HCC patient with HBV and HCV coinfection in this study however, could be due to the few number of HCC patients involved in this study. Further studies are therefore required in this direction involving a cohort of HBV, HCV and HCC patients.

6.9 Trends in CD4+ count

Measurement of trends in CD4+ count is particularly useful in the determination of when to commence antiretroviral therapy and for monitoring of patients' response is to such therapy. In this study none of its participants was on any known antiretroviral therapy. The finding of a highly statistically significant ($p=0.0001$) progressive decrease in CD4+ count in all HIV-infected and HIV/HCV coinfected persons in this study but not in the HIV seronegative control persons only confirms the effect of infections with HIV on CD4+, while the more significant cumulative decline in CD4+ counts that was found in HIV-D than HIV-

1 or HIV-2 infected persons further adds credence to the severity associated with the different types of HIV infection (Popper *et al*, 1999). However, the finding of a higher level of cumulative decline in HIV-2 infected persons than HIV-1 infected persons (43% versus 41.6%) though not significant may be explained in terms of the fewer number of HIV-2 infected persons who participated fully in all the follow-up test as against HIV-1infected person. Furthermore, the finding of a hundred fold increase in the cumulative decline in CD4+ count of persons coinfected with HIV and HCV than those infected with HIV alone may be suggestive of the greater impart of HIV and HCV confection on CD4+ than HIV alone. The finding in this study of a persistent decline in the CD4+ count of apparently healthy (HIV and HCV seronegative) women over the 18 months period is unclear. Although the rate of decline was quite low it was statistically significant and was not seen among the males. A possible explanation for this is that in addition to the numerous factors which may influence CD4+ count (Abuye *et al*, 2005) most especially among women, the duration of the follow-up period was probably too short for a coherent conclusion to be made. Furthermore, more than 90% of the women employed as control group in this study were either pregnant women or lactating mothers. The contributing role of reproductive hormones such as chorionic gonadotropin (CG), estradiol and progesterone, and their physiological combinations which are expressed during pregnancy and postpartum period on CD4+ count have not been evaluated in this study. Little or no data exist on the influence of reproductive hormones on CD4+ count in apparently healthy women. The few available studies have focused on HIV-infected women with varying findings (van Benthem *et al*, 2002; Shirshev *et al*, 2003). There is therefore need for a more elaborate longitidutinal study employing a cohort of pregnant and non-pregnant women to validate these findings.

In this study, the rate of decline in CD4+ varied proportionally according to the type of HIV infection. Although this variation was not significantly associated with the type of HIV, the finding of persons with HIV and HCV coinfection with the highest level of decline within the 18 months period as compared to persons infected with HIV alone and HIV-D infected person with a higher decline than HIV1 or HIV-2 infected persons may be suggestive of the degree of severity associated with these infections (Tedaldi *et al*, 2003 and Carter, 2004).

Some studies have advanced reasons for the accelerated HCV progression in HIV coinfected persons. A study reported by Kim *et al,* (2005) showed HIV/HCV coinfected persons with CD4+ counts of less than 500 cells/μl had less immune response to HCV compared to persons with counts greater than 500 cells/μl, while individuals with count less than 200 cells/μl had worse response. In this study no individual coinfected with HIV and HCV had a CD4+ count above 500 cells/μl at diagnosis (CD4+dx). CD4+ decline was progressively higher in HIV-1/HCV infected individual than in HIV-2/HCV infected person. Similarly the rate of decline was higher in coinfected individuals than in persons infected with HIV or HCV alone. However these differences did not reach statistical significance. The accelerated decline in CD4+ count observed in these individuals as compared to those infected with HIV or HCV alone may therefore be in line with these reports. This assertion is further supported by studies that have provided evidence of less stimulation of CD8 HCV cells that target HCV (Manfras *et al*, 2004) thus contributing to quicker HCV progression in HIV infected individuals. This finding underscores the need for early diagnosis of people at risk of HIV/HCV coinfection and supports reports suggesting the commencement of therapy at a comparatively higher CD4+ count than in coinfected persons.

6.10 Socio-demographic and risk factors for HIV, HCV and HIV/HCV coinfection

Several sociodemographic and risk factors have been associated with the transmission of HIV and HCV. These often vary from one community to another and are significantly determined by the individual personal life style most especially as regards drugs abuse and sexuality. In the Gambia religion and local taboos made it difficult to ask in-depth questions about sexuality especially with females. In this study, this precaution has been taken into consideration in designing the questionnaires; however, these limitations did not serve as a major impediment to this work.

6.10.1 Education, Employment Status, Income and HIV, HCV, HIV/HCV infection/coinfections.

The finding in this study of the highest HIV prevalence among the illiterates (9.4%; 48/510) (95% CI, 1.207-2.559) and a progressive decrease with increasing level of education may be suggestive of poor level of awareness. Awareness and knowledge of HIV/AIDS have been shown to be limited in the Gambia (Wilkins *et al,* 1989). A survey reported over a

decade ago revealed that only 17% of the females in rural areas in the Gambia were aware of the existence of AIDS, with secondary educated urban males showing better understanding of the disease (Wilkins *et al,* 1989). Unlike the work reported by Wilkins *et al*, 1989, the persons sampled in this study were predominantly urban based; and the ability of over 80% (1121/1367) of them to confirm the transmission modes of HIV shows a significant improvement in HIV awareness in the Gambia. Similarly, the high level of condom awareness found in this study among the male and females not it use and its non-significant association with HIV prevalence shows the minor role of this in HIV transmission in the region. Similarly the finding of the highest HCV prevalence 5.8 %(18/510) (95% CI, 1.32-5.44, OR: 2.75), among the illiterate class, 99% having no history of blood transfusion or needle sharing or surgery, could be suggestive of sex as a route of transmission, while its highly significant statistical relationship ($p>0.01$) with HIV prevalence in both illiterates and the educated shows that HCV transmission in the Gambia was independent of the educational status of the participants.

Income status and the ability to pay for sex have been shown to important factor for the transmission of STIs (Sopheab *et al*, 2003). In this study, close to 70% of the persons involved were either student, applicants or full-time housewives, while only 20.1% earn a monthly wage and 12.7% were self-employed tradesmen or petty traders. The finding of a progressive increase in HIV prevalence with increasing income and a marginally significant statistical association ($p<0.05$) between HIV prevalence and the employed thus corroborates Sopheab *et al,* (2003) observation. This finding could serve as a frame work for designing an intervention programme and also creates the need to include men in all the sentinel surveillance studies.

6.10.2 Duration in present station, number of people in household and HIV, HCV, HIV/HCV infection/coinfections.

The finding of a significant relationship between increasing prevalence in both HIV and HIV/HCV coinfections and duration in one station confirms the contributory role of migration and spatial mobility in the spread of HIV among other STIs. Some studies have shown that mobility enhances HIV transmission (Boerma *et al,* 2003). In the Gambia, migration from the rural area to urban towns is a common practice for most people seeking employment or business prospects among a host of reasons.

The finding of comparatively higher rates of high HIV and HCV prevalence among persons belonging to large households cannot be sufficiently explained within the scope of this work. However, with the possible living together of all members of the extended family in one house coupled with polygamy and a culture that permits marriage to uncles and first cousins, this disparity may be due marriage into a polygamous home of an already infected person or the interrelated marriages with an already infected member within the same compound. This data could serve as a frame work for designing an intervention programme.

6.10.3 Tribe, Religion and HIV, HCV, HIV/HCV infection/coinfections.

In this study, even though HIV was found in among almost all tribal groups its prevalence was only significantly associated with the Mandinkas and the Jolas. The reasons for disparity are not clear. Similarly, the finding of a marginal association between persons of the Jola tribe origin with HIV/HCV coinfection but with other tribes cannot be clearly explained. However, while this will obviously involve another study a general explanation that may be given is that urban migration is particularly more common among the Mandinkas and the Jolas. However, the finding of a marginally significant association between HIV and persons belonging to the Moslem religion may be due the significant association found between polygamy and HIV prevalence, with more Muslims predominantly involved in polygamy, this outcome may not be un-expected. Another possible reason for the disparity could be due to the small population of members of other religions involved in this study as compared to the Muslims. However, this finding also offers an important frame work for the designing of an intervention programme.

6.11 Behavioural risk factors

6.11.1 Marital status, type of marriage, duration of marriage and HIV, HCV, HIV/HCV infection/coinfections.

High prevalence of most sexually transmitted diseases is most often associated marital status, while, HIV prevalence among other STIs is generally considered lower among married persons (Rehan, 2003). Furthermore, married females are generally considered a low risk group for the transmission of these infections (UNAIDS, 2000). The finding in this study of single persons independent of their gender having a higher HIV prevalence than married

females [(7.0 percent (53/758) versus 5.4 percent (37/690)] may be considered but is however in line with the global epidemiology of HIV. On the other hand the finding of males 48 years and above with a significantly higher prevalence than those below 25 years provides an HIV epidemiological pattern entirely different from what has been reported in most developed countries (UNAIDS, 2003) but is in line with Rehan (2003) finding in Pakistan. While, the finding of decreasing HIV rates with increasing duration of marriage, and a marginal association (p=0.04) between unmarried persons and HIV prevalence are all in line with the most reported pattern of HIV epidemiology. The observed epidemiological pattern seen in among the males 48 years and over reinforces the need for the inclusion of men in sentinel surveillance studies in the country. There is therefore a need to develop an intervention programme for the epidemiological pattern of HIV found in this study.

6.11.2 Number of wives, female circumcision, history of jaundice or hepatitis since birth.

The finding in this study of a significant progressive increase in HIV prevalence with increasing number of wives and a higher HCV, HIV and HCV coinfection prevalence in those involved in polygamous marriages than those involved in monogamous marriages, all adds to provide additional evidence in favour of the sexual transmission of HCV. Similarly, female circumcision or vaginal mutilation has been postulated to contribute significantly in facilitating the transmission of HIV infection through numerous mechanisms (Brady, 1999). In a study reported by Mboto and Epoke (2000), a significant level of association was found between HIV and female circumcision in a rural community of the Cross-river state of Nigeria. In this present study HIV and HCV prevalence were independent of the female's circumcision status even though a comparatively higher number of them admitted to having been circumcised. Similarly, even though a significant number of persons reported a history of jaundice or hepatitis in this study no association was found between these and HCV. HCV prevalence in this group was almost the same with persons with no previous history of jaundice or hepatitis. Thus this finding confirms the minor role of female circumcision, history of jaundice or hepatitis since birth as risk factors in the transmission of HIV or HCV in the region.

6.11.3 History of STIs, skin tattoo, history of blood oath, alcohol consumption.

In this study, antibody to HIV was found among males and females and independent of their history of STIs or venereal disease. Ten percent (5/50) of the females who reported a history of painful intercourse after sex and 11.5% (3/26) who documented non- menstrual bleeding after intercourse all within the past five years demonstrated antibodies to HIV.

These rates are comparatively higher when compared to the 8.2 % (65/794) and 8.1 % (67/818) found among females with no history of painful intercourse or non-menstrual bleeding after intercourse respectively: thus suggestive of the contributory role of other undiagnosed STIs in the enhancement of HIV transmission in this region. This assertion was not supported by the finding of a non-statistical relation ($p>0.05$) between history of painful intercourse after sex or non- menstrual bleeding after intercourse and HIV positivity. Generally, several other STIs positively favour HIV and even HCV transmissions. In men common STIs symptoms include urethral discharge and ulcerative genital ulcers. In females, genital vaginal discharge, ulcerative genital ulcers are common venereal disease symptoms, while, pain and non-menstrual bleeding during intercourse is often indicative of undiagnosed venereal disease (Latif, 1989).

Furthermore, in this study HCV and HIV/HCV coinfection was marginally associated with the females irrespective of their history of STIs or venereal disease. Among the males there was no significant association between history of STIs and HCV or HIV/HCV coinfection. Some reports have advocated that skin tattoo a common ceremonial practice in some rural Gambian communities, because it sometimes involves skin piercing devices that may be used communally, could be a possible source of the transmissions of some blood borne infections including HIV and possibly HCV (Ndinya-achola *et al,* 1986). In this study 19 persons admitted to have tattooed their skin in the past. Antibodies to HIV-2 were detected in 1 out of these 19 persons (5.3%). In a similar manner, blood oath a primitive practice still in use in some Gambia communities to ensure allegiance was not found to have any significant association with HIV or HCV prevalence among those who admitted the act. Some studies have indicated that scarification ritual, tattooing, male and female circumcision could be incriminated in the spread of HIV (Ndinya-achola *et al,* 1986). However, no documented laboratory based study has investigated this.

An interesting finding is the low level of alcohol consumption among all the subjects, the increasing number of drinking bars in most parts of the big cities notwithstanding. In this study only 16 males out of the 1500 subjects admitted to drinking alcohol. None of the females admitted alcohol consumption. The finding of this low level of alcohol consumption may be due to the high Muslim population in the country. The Muslim religion forbids the drinking of alcohol by all. Generally, alcohol consumption and drug use has been has been associated with high-risk sexual activity among both homosexuals and heterosexuals (Stalls *et al,* 1986). This may be due to reduced sexual inhibition when drinking or to the fact that substance abuse and high-risk sex are common in the same social circles. However, in this study no HIV antibody was detected in any of the 16 persons who admitted alcohol consumption.

6.11.4 Sex during menses, use of contraceptive pills.

The findings of the study reveals that sex during menses, and use of contraceptive pills are not popular practices in the Gambia. In this study the few females who admitted the practice of these acts were not significantly associated with high HIV or HCV prevalence. Woman on contraceptive pills are known to be more vulnerable to sex than their counterparts who are not on contraceptives (Hudson, 1988). A few studies have reported some increased risk of HIV infection related to intercourse during menstruation (Berer and Ray, 1993) but other factors such as trauma were also involved. Traditionally, the Muslim religion forbids sexual relations during menstruation.

6.11.5 Knowledge and use of condoms.

Condoms are globally recognised as an effective way for the prevention of HIV and other STIs (UNAIDS, 2003). In this study even though there was a high level of condom awareness between the male and females only an insignificant number of the males used it. However, HIV, HIV and HIV/ HCV coinfection rates among all the study subjects were independent of their level of either their condom awareness or its use by the males. Even though there was an insignificant statistical association, the finding calls for an intervention programme to promote a greater use of condoms not only as a means of controlling HIV but other STDs.

CHAPTER SEVEN

7.0 SUMMARY, CONCLUSION AND CONTRIBUTION TO KNOWLEDGE

7.1 Summary

1. The determined overall prevalence of HIV in this study was 6.7% (101/1500) (95% CI, 5.6-8.2). The rates for HIV-1 was 4.3% (65/1500) (95% CI, 3.4-5.5), HIV-2 was 1.9 % (28/1500) (95% CI, 1.2-2.7) and HIV-D was 0.5 % (8/1500) (95% CI, 0.2-1.0).

2. The HIV rates for apparently normal subjects based on its value in male blood donors and females in monogamous marriage were determined to be 3.5 % (23/664). This rate is three fold the reported national prevalence for the country; the reasons for this disparity are unknown.

3. Females had a significantly higher ($p<0.001$) HIV prevalence (8.4 %, 78/928) (CI, 6.7-10.4) than their male counterparts 4.0 % (23/57 CI, 2.8-6.0); this finding confirms

WHO/UNAIDS (2002) report of twice as many young females than as men infected in sub-Sahara Africa.

4. This study showed that among the Gambian males HIV rates progressively increased with increasing age with the peak of infection in men aged 55 years and above (6.9% CI, 0.8-22.8); an infection pattern that corroborates the finding of Rehan (2003), but differs from what is found in most developed countries. This uncommon pattern of HIV epidemiology could be targeted in an intervention programme aimed at behavioural change.

5. HIV-1 prevalence but not HIV-2 or HIV-D was significantly associated ($p<0.05$) (OR 2.32; 95% CI, 1.25-4.58) with the females. No such association was found with the males.

6. This study is also the first documented extensive work that has evaluated the distribution of HCV among the apparently healthy and patients in the Gambia. The determined overall HCV prevalence rates for this study population were 2.1% (31/1500) (95% CI, 1.4-2.9). Its prevalence among non-HIV infected population was 1.6% (22/1399) (OR: 0.16; CI: 0.08-0.37) and 8.9% (9/101) (OR: 6.123; CI: 2.68- 11.98) in HIV infected population. The men accounted for 71% (22/31) of the HCV infection detected with a comparatively higher prevalence rate than females (3.8% CI, 2.4-5.8 versus 1.0% CI, 0.4-1.8). A higher statistical significant association was found between HCV prevalence and the males than the females ($p<0.0001$) (OR: 4.08, 95% CI, 1.83-8.55) versus p= (OR: 0.24; 95% CI, 0.1167-0.54). This rate is below the estimated worldwide prevalence of 3% and below the estimated value for the West African region (WHO, 2004) and also below the rate of 3.0% found by Kirk *et al*, (2004) in the country a year ago. The comparatively lower HCV prevalence found in this study notwithstanding this data highlights the potential public health hazard of HCV infection among HIV-infected persons in the Gambia, where anti-HCV testing is generally not performed in HIV-infected populations, other patients or among blood donors. This data therefore shall serve as a significant justification for an embracing intervention programme, that should justify provision of HCV testing facilities and screening of all potential blood donors, HIV infected persons and initiation of treatment for the infected.

7. This study indicated that co-infection between HIV and HCV co-infection was present in the Gambia and the infection rate was relatively low. The determined baseline

prevalence of this co-infection was 0.6%, (9/1500) among all study participants and 8.9% among HIV infected patients. The study also found a comparatively higher prevalence between HIV-1/HCV and HIV-2/ HCV (0.5%, 7/1500; 95% CI, 0.2-1.0 versus 0.1 % 2/1500; 95% CI, 0.0-0.5) with the men accounting for 77.7 percent (7/9) of the cases. The Study also showed that age and male gender factors were all significant ($p<0.05$) risk factors for HIV-1/HCV co-infection but not with HIV2/HCV co-infection. This finding also reveals the significant risk HIV and HCV co-infection poses to the public at large especially in the area of blood transfusion and also makes need for an intervention programme.

8. This study is the first documented work in the Gambia that has concurrently evaluated CD4+ count among blood donors, pregnant females and patients. Most documented study in the area is on patients. The determined mean CD4+ count of pregnant females was 461 cells/μl and 484 cells/μl for blood donors. The rates for anti-HIV positive persons were 310 cells/μl and 306 cells/μl and 365 cells/μl for HIV-1/HCV and HIV-2/HCV co-infections respectively. The findings confirms a highly significant ($p<0.0001$) statistical association between HIV and CD4+ counts and showed that determined CD4+ counts are within the accepted ranges but comparatively lower than that reported among the Caucasians. This finding has provided an important baseline data that serves as a guide for treatment of HIV infected persons. It also makes need for the development of a CD4+ count staging based on the local population.

9. This study indicates that the CD4+ count at diagnosis of persons with HIV and HCV co-infection was lower than of persons infected with HIV alone. It also indicates that the rate of decline in CD4+ in HIV and HCV co-infected persons was significantly higher than in HIV alone infected persons. These findings confirm the effect of HCV on HIV infection and corroborate similar reports. The implication of this finding is that patients co-infected with HIV and HCV could commence antiretroviral therapy with a higher CD4+ count than those with mono-infection.

10. This study is also the first documented extensive work on the distribution of HCV genotypes in the Gambia among the apparently healthy population and among HIV infected persons. It revealed that hepatitis C serotype 2 is the most prevalent type among Gambians and was significantly associated with age but not gender, while co-infection with HIV-1 was

predominantly associated with HCV serotype 1. The data suggest that serotype 2 spread earlier than genotypes 1 and 3 and corroborates a similar finding in Ghana, another country in the West African region. While, the finding of a comparatively higher level of HCV serotype 1 co-infection with HIV-1 which is associated with more severe end-stage liver disease (ESLD) has serious health implications most especially in terms of treatment and makes need for the provision of facilities not only for the detection of HCV infected individuals but also for the identification of its serotypes and treatment.

11. The determined HIV and HCV prevalences rates for pregnant females in this study was 4% (95% CI, 0.29-0.68) and 0.9% (6/693) (CI, 0.11-0.67) respectively, while their HIV/HCV co-infection rate was of 0.3% (2/693) (95% CI, 018-1.16). The females were significantly associated ($p<0.001$) with HIV and HCV prevalence but not with HIV/HCV co-infection. Similarly an HIV and HCV prevalence of 2.4 percent (11/460) (95% CI, 0.14-0.51) and 1.1 percent (5/460) (95% CI, 0.16-1.12) respectively was found among blood donors. These rates found in the low risk group are comparatively lower than the findings in most countries within the region, thus corroborating reports suggesting a low level of these infections in the region. These low levels not withstanding, the findings also have serous public health implication most especially in the area of blood transfusion and also adds to the need for provision of facilities for screening of donated blood for transfusion.

12. This study indicates that patients other than those with a history of HCC seen at RVTH within the study period had a significantly higher HIV, HCV and HIV/HCV co-infection rates 19.1% (53/277), 6.5% (18/277) (95% CI, 3.03-12.3), 1.8% respectively. These comparatively higher HIV rates found among patients, some of whom were in WHO clinical stage 3 or 4, could be suggestive of an emerging AIDS epidemic. When these individuals are added to the apparently healthy pool of infected persons found in this study, it could be suggestive of an impending major health crisis, in a country already saddled with inadequate health facilities and a fragile economy. This finding therefore makes need for early planning and emphases the need for early provision to be made to accommodate the envisaged additional health burden.

13. This study also found a comparatively higher prevalence of HBsAg among patients diagnosed with HCC than HCV (38.5%; 5/13 versus 7.7%; 1/13). The few HCC

patients tested in this study not withstanding. This finding corroborates reports of a high prevalence of HBV in the region and supports the suggestion of a minor role of HCV as compared to HBV in HCC in the region. In this study the possible attributable fraction of HCC due to HBV or HCV was 46%, thus suggestive of the involvement of other factors. In some countries HCC has been associated with chronic exposure to toxins originating from Aspergillus infected grains and peanuts (Montalto *et al*, 2002). Other associated risk factors include cigarette smoking and prolonged abuse of alcohol (Sun *et al*, 2003, Ogimoto *et al*, 2004) in addition to some hereditary factors (Montalto *et al*, 2002). Although these factors were not evaluated in this study or are their contributory role as causative agents of HCC in the Gambia known, however grains are the country's most staple food while cigarette smoking and groundnut consumption are very common habits in the Gambia. There is therefore need for studies to evaluate the possible involvement of non- viral factors in HCC in the country.

14. This study has contributed to our understanding of the distribution and risk factors for HIV, HCV and HIV/HCV co-infection in the Gambia and found age, gender , and history of sexual transmitted disease, income and recent change of town of residence as significant risk factors for HIV, HCV and HIV/HCV co-infection transmission. Similarly, it found HIV prevalence independent of the educational status of the study subjects, while it indicates that other risk factors such as female circumcision, use of contraceptives, knowledge of condom by both male and females, use of alcohol, sex during menses, skin tattoo, blood taking oath and wife inheritance and history of blood transfusion plays a minor role in the transmission of HIV, HCV and co-infection between HIV and HCV in the country. The finding of increasing HIV prevalence with income and independent of the educational status of the participants corroborates earlier reports suggesting the low level of behavioural change in the country. Thus there is need to deviate from the traditional behavioural change campaign and design an intervention programme with inputs from the end- beneficiaries. The method could address the lapses of previous ones and tailor driven to meet the target group or community needs.

15. The comparatively higher HIV prevalence found in this study in individuals involved in polygamous relation as against those in monogamous marriages corroborates Mboto and Epoke (2000) report and reveals the need for the redefinition of HIV surveillance studies in sub-Saharan African countries especially in a highly polygamous setting. Thus

persons involved in polygamous relationship should be defined as a high risk group and be excluded from participation as blood donors.

16. The study has also revealed a significant prevalence of HIV and HCV among the low risk groups such as blood donors and pregnant women and have highlighted the significance and implications of these findings.

17. The unusual pattern of HIV epidemiology found in this study in males 48 years and above is suggestive of the inadequacies associated with surveillance studies based on extrapolating data on studies conducted among pregnant women to indicate its level among men. This data can therefore serve a baseline for designing an intervention programme for this age group among others

18.. The finding of an HIV incidence rate of 1.44% (3/222) among the females and 1.5% (1/66) among the males throughout the 18 months duration of the follow-up study and the incrimination of STDs as the major associated risk factor for the incidence reveals the actual pace of HIV spread in the country and is suggestive of the dominating role of STDs in the spread of the infection in the region. This finding is particular important in designing an intervention programmes. It also makes need for adequate diagnosis and proper treatment of all STDs cases as a prelude to reducing HIV epidemics.

7.2 CONCLUSION AND CONTRIBUTION TO KNOWLEDGE

7.2.1 Distribution of HIV, HCV and HIV/HCV co-infection in the Gambia.

1. The determined overall prevalence of HIV in this study was 6.7% (101/1500) (95% CI, 5.6-8.2).The HIV rates for apparently normal subjects based on its value in male blood donors and females in monogamous marriage were determined to be 3.5% (23/664). This rate is three folds the reported national prevalence for the country. The reasons for this disparity are unknown. A significantly higher proportion of females than males were infected at a comparatively lower age than their male counterpart (8.4 %; 78/928) (CI, 6.7-10.4) versus 4.0 % (23/57 CI, 2.8-6.0). This finding confirms WHO/UNAIDS (2002) report of twice as many young females than as men infected in sub-Sahara Africa and is line with HIV epidemiology in the region.

2. This study is also the first documented extensive work that has evaluated the distribution of HCV among the apparently healthy and patients in the Gambia. The determined overall HCV prevalence rates for this study population were 2.1% (31/1500). Its prevalence among non-HIV infected population was 1.6% (22/1399) CI: and 8.9% (9/101). Infected males were comparatively older and thrice the number of infected females (3.8% CI, 2.4-5.8 versus 1.0% CI, 0.4-1.8). This rate is below the estimated worldwide prevalence of 3% and below estimated value for the West African region (WHO, 2004) and also below the rate of 3.0% found by Kirk *et al*, (2004) in the country a year ago. The comparatively lower HCV prevalence found in this study notwithstanding these data highlights the potential public health hazard of HCV infection among HIV-infected persons in the Gambia, where anti-HCV testing is generally not performed in HIV-infected populations, other patients or nor among blood donors. This data therefore shall serve as a significant justification for an embracing intervention programme, which should include provision of HCV testing facilities and screening of all potential blood donors, HIV infected persons and initiation of treatment for the infected.

3. This study showed that Hepatitis C serotypes 2 is the most prevalent type among Gambians and was significantly associated with age but not sex, while co-infection with HIV-1 was predominantly associated with HCV serotype 1.The data suggest that serotype 2 spread earlier than genotypes 1 and 3 and corroborates a similar finding in the Ghana. The finding of a comparatively higher level of HCV serotype 1 co-infection with HIV-1 which is associated with more severe ESLD (Yoo *et al*, 2005) have serious health implications most especially in terms of treatment and makes need for the provision of facilities not only for the detection of HCV infected individuals but also for the identification of its serotypes, and for the initiation of an intervention programme.

4. This study indicated that co-infection between HIV and HCV co-infection was present in the Gambia and infection is relatively low. The determined baseline prevalence of this co-infection was 0.6%, (9/1500) among all study participants and 8.9% among HIV infected patients. Males accounted for more than three quarter of the infected persons at a comparatively higher age than their female counterparts. The finding of a comparatively higher level of HCV serotype 1 co-infection with HIV-1 which is associated with more severe ESLD has serious health implications most especially in terms of treatment and makes

need for the provision of facilities not only for the detection of HCV infected individuals but also for the identification of its serotypes. In addition there is need for the initiation of an intervention programme.

7.2.2 CD4+ count and trends in HIV, HCV, and HIV/HCV co-infected persons and apparently healthy persons.

This report is the first document study that has evaluated CD4+ count and its trends in HIV, HCV, and HIV/HCV co-infected persons and apparently healthy people in the country. The determined CD4+ count of apparently healthy Gambian males and females was 489 cells/μl and 496 cells/μl respectively. This rate is lower than that reported for Caucasians, but in agreement with the global range. Lower CD4+ counts were significantly associated with HIV or HIV/HCV co-infection. CD4+ decline was progressively higher in HIV/HCV co-infected persons than in persons infected with HIV or HCV alone. Decline rate was also higher in males than females. A significant progressive decline in CD4+ count was observed among apparently female control group. This finding is unclear and calls for a longitudinal study involving a cohort of pregnant and non- pregnant women. This finding underscores the need for early diagnosis of people at risk of HIV/HCV co-infection and supports reports suggesting the commencement of therapy at a comparatively higher CD4+ count in co-infected persons. It also calls for the development of CD4+ staging for use in the country based on Gambia population.

7.2.3 HCV versus HBV in HCC patients.

This study detected HBsAg (38.5%; 5/13 versus or HCV (7.7%; 1/13) in only 46% of the HCC patients, thus suggestive of the involvement of other factors. The few HCC patients tested in this study not withstanding. This finding corroborates report of a high prevalence of HBV in the region and supports the suggestion of a minor role of HCV as compared to HBV in HCC in the region. There is therefore a need for studies to evaluate the possible involvement of non- viral factors in HCC in the country. Such a study should investigate the contributory role of chronic exposure to toxins originating from Aspergillus infected grains and peanuts, cigarette smoking, (Sun *et al*, 2003, Ogimoto *et al*, 2004), all which are common habits in the Gambia in addition to investigation of some hereditary factors.

7.2.4 Sociodemographic and risk factors for HIV and HCV transmission

1. This study has contributed to our understanding of the distribution and risk factors for HIV, HCV and HIV/HCV co-infection in the Gambia and found age, gender, and history of sexual transmitted disease, income and recent change of town of residence as significant risk factors for HIV, HCV and HIV/HCV transmission. The finding of increasing HIV prevalence with income and independent of the educational status of the participants corroborates earlier reports suggesting the low level of behavioural change in the country. Thus there is need to deviate from the traditional behavioural change campaign and design an intervention programme with inputs from the end- beneficiaries. This method could address the lapses of previous ones and be tailor- driven to meet the target group or community.

2. The comparatively higher HIV prevalence found in these studies in individuals involved in polygamous relation as against those in monogamous marriages corroborates Mboto and Epoke (2000) report and reveals the need for the redefinition of HIV surveillance studies especially in a highly polygamous setting. Thus persons involved in polygamous relationship should be defined as a high risk group and be excluded from participation as blood donors.

3. The unusual pattern of HIV epidemiology found in this study in males 48 years and above is suggestive of the inadequacies associated with surveillance studies based on extrapolating data on studies conducted among pregnant to indicate its level among men. This data can therefore serve a baseline for designing an intervention programme.

7.2.5 Incidence of HIV and Associated Risk Factors

The finding of an HIV incidence rate of 1.44% (4/288) among the apparently healthy control group throughout the 18 months duration of the follow-up study and the incrimination of STDs as the major associated risk factor for the incidence reveals the actual pace of HIV spread in the country and is suggestive of the dominating role of STDs in the spread of the infection in the region. This finding is particular important in designing an intervention programme. It also makes need for adequate diagnosis and proper treatment of all STDs cases. It also helps to re-echo the need for more active condom promotion in the countr.

7.3 Recommendations and further Research

1. The findings in this study especially as regards HCV among blood donors are the potential danger posed by the non-screening of blood for transfusion for HCV or HBV. It is envisaged

for that this finding shall serve as a justification for ensuring that safer blood is made available to whomsoever.

2. There is need for the establishment of Hepatitis C surveillance unit not only in the Gambia but in all countries in sub-Saharan Africa. Such a unit should carry out routine surveillance studies aimed at identifying new cases and determining disease incidence and trends, determining risk factors for infection including traditional practices and disease transmission patterns; estimating disease burden; and identify infected persons who can be counselled and referred for medical follow-up. This is particularly necessary since regional distribution of HCV genotypes shall influence the configuration of diagnostic assays and vaccine design and also serve as an epidemiological tool for tracing sources of infection, especially since the route of transmission of HCV infection remains unknown in a significant number of infected individuals in the region. This shall go along way to preventing the emergence of a third epidemic HIV/HCV co-infection in the region in addition to the on-going two- HIV and HCV.

3. The high HIV and HCV prevalence's found among the males in this study has highlighted the incompleteness of HIV surveillance based on the use of pregnant women alone as done in the country among many other developing countries and makes need for the inclusion of men in the national sentinel surveillance studies. Furthermore the increasing prevalence of HCV in an HIV endemic environment makes need for the inclusion of HCV as an additional tests in the surveillance work to keep track of the trend in the epidemiology of these viruses and to have a very balanced data on both sexes. The financial burden of these especially to third world countries could be reduced by the design and production of a single test kit that can simultaneously detect HIV and HCV in blood or a more commonly expressed body fluid such as saliva or urine.

4. There is a need for the redefinition of HIV surveillance studies especially in a highly polygamous setting to consider persons involved in polygamous relationship as a high risk group and exclude them from such surveillance studies that tackles low risk group and blood donation exercises. This shall further help to reduce the risk of HIV transmission through blood transfusion.

5. The finding of a significant progressive decline in CD4+ among apparently healthy females as employed as control group in this study calls for the initiation a longitudinal study involving a cohort of pregnant and non- pregnant women. Such a study shall investigate the hormonal changes in pregnant and non pregnant women and correlate it with their CD4+ counts. This shall help to elucidate the dilemma found in this study.

6. Given the sizeable number of persons who are already infected with HCV, and HIV/HCV in the country and the region, there is an urgent need for the initiation of intervention programmes aimed at provision of effective antiviral therapeutics capable of inhibiting virus replication and stopping or delaying the progression of liver disease in those already infected and creation awareness and other prevention strategies to prevent an escalation of these infections.

7. HCV co-infection HIV clearly worsens the course of hepatitis C, but the reasons for this interaction are not very clear. Studies are therefore needed of the interaction between HCV and other infections, obesity, diabetes mellitus, iron, and possibly medication.

8. There is need for the development of a CD4+ staging system based on the Gambian population this thus calls for the provision of CD4+ enumeration facilities at least in the main referral hospital in the country

9. An intervention programme should be developed to educate the all groups concerning STDs and provision of facilities for adequate diagnosis and proper treatment of all STDs cases.

10. There is need for studies to evaluate the possible involvement of non- viral factors in HCC in the country. Such a study should investigate the contributory role of chronic exposure to toxins originating from Aspergillus infected grains and peanuts and cigarette smoking (Sun *et al*, 2003, Ogimoto *et al*, 2004) all which are common habits in the Gambia.

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APPENDIX 1: QUESTIONNAIRE

QUESTIONNAIRE

This questionnaire is a summary of the questions asked to all subjects who conceded to participate in the study after pre-test counselling. Interviews were on one to one basis with the researcher or trained nurse counsellors. Participants were told of the need for sincerity in answering the questions. They were also assured of the confidentiality of the information they provide and further informed that no one shall be identified at the end of the report. Most questions were explained in the local dialect were necessary.

SECTION ONE: Demographic characteristic

1. Date of birth or Age

2. Place of birth

3. Marital status

(a)Married

(b)Single

(c)Divorced/separated

(d)Widowed

4. How long have you been married?

(a) $\leq$3 years

(b) 4 – 6 years

(c) 7 – 9 years

(d) $\geq$ 10 years

5. How long have been living in your present city/town of residence?.

(a) $\leq$3 years

(b) 4 – 6 years

(c) 7 – 9 years

(d) ≥ 10 years

6. How many other wives does your husband have?

(Women only)

(a) None

(b) 2 others

(c) 3 others

(d) 4 or more others.

7. How many wives do you have?

(Men only)

1

2

3

4 or more

8. Tribe Please tick as applicable

(a) Mandinka

(b) Jola

(c) Fula

(d) Wolof

(e)Serahuli

(f) Serer

(g) Manjago

(h) Bambara

(i) Other Gambian tribe

(j) Senegalese

(h) Non-Gambia and Non-Senegalese

9. Religion

(a) Muslim

(b) Christian

(c) Others

(d) None

10. Number of people in household

(a) ≤ 5

(b) 5-8

(c) 9-12

(d) ≥13

11. Highest level of Education.

(a)Cannot read nor write

(b) Completed primary School

(c) Completed Secondary education

(d). Post secondary education.

12. Income per month (Dalais).

(a) ≤ 1,000

(b) 1000– 1999

(c) 2000 – 2999

(d) 3000-3999

(e) 4000-4999

(f) ≥5000

13. Duration of time in present abode (years)

(a) ≤ 5

(b) 6 – 10

(c) 11- 15

(d) ≥16

SECTION THREE: Personal/Behavioural Risk Factor

14. Type of Marriage

(a) Monogamous

(b) Polygamous

(c) Not applicable

15. Number of wives

(a) 1

(b) 2

(c) 3-4

(d) ≥ 5

(e) Not applicable.

16. I have other partners other my wife/wives or husband

(a) Yes

(b) No

(c) Not applicable

17. Last 5 years your physician has diagnosed venereal disease

(a) Yes, ulcerative VD

(b) Yes, Non-ulcerative VD

(c) No.

18. Have you ever been diagnosed of Hepatitis?

(a). Yes, Hepatitis A or B

(b) Yes, Hepatitis C

(c) No

19. Have you received blood transfusion since birth?

(a) Yes

(b) No

20. Have you ever shared injection or skin piercing device within the past ten years

(a) Yes

(b) No

21. I have taken a blood oath within the past ten years.

(a) Yes

(b) No

22. I am circumcised

(a) Yes

(b) No

23. I use contraceptive pills

(a) Yes, often

(b) Yes, occasionally

(c) Never

24. I have heard of male condom

(a) Yes

(b) No

25. I use condom

(a)Yes, always

(b) Yes, occasionally

(c) Never

26. I have heard of HIV/AIDS

(a) Yes

(b) Never

27. I know how HIV/AIDS is transmitted

(a)Yes

(b) No

28. Within the past 10 years I have experienced profuse vaginal (Females) or Urethral discharge (Males)

(a) Yes, always

(b) Yes, occasionally

(c) Never.

29. Within the past 10 years I have experienced pain during sexual intercourse (Females)

(a) Yes, always

(b) Yes, occasionally

(c)Never

30. I have had non-menstrual bleeding during sexual intercourse within the past ten years

(a) Yes

(b) No

31. I drink alcohol

(a) Often

(b) Sometimes

(c) Never

32. I have been involved in wife inheritance

(a) Yes

(b) No

33. I have tattooed my body within the past ten years

(a) Yes

(b) No

34. I have been involed in blood oath within the past ten years

(a) Yes

(b) No

35. I have accepted to participate in any follow-up tests and be contacted if necessary without prejudice to my right for privacy.

(a) Yes

(a) No

Thank you

APPENDIX 2: STATISTICAL ANALYSIS OF HIV, HCV AND HIV/HCV VARIABLES WITH REGARDS TO AGE AND SEX (SCREENING RESULT)

Screening Results

Age Range	# Screened	with HIV Ab #		Lo95%CI	Hi95%CI	
All Ages	1500	102	6.8%	5.6%	8.2%	
= 5	9	1	11.1%	0.3%	48.2%	
6-12	18	0	0.0%	0.0%	15.3%	The upper tail probability is 0.05, not 0.025, when n=0
13-19	211	9	4.3%	2.0%	7.9%	
20-26	431	29	6.7%	4.6%	9.5%	
27-33	376	49	13.0%	9.8%	16.9%	
34-40	179	6	3.4%	1.2%	7.2%	
41-47	150	3	2.0%	0.4%	5.7%	
48-54	83	1	1.2%	0.0%	6.5%	
= 55	43	5	11.6%	3.9%	25.1%	

Confirmatory Results

Sex	# Tested	With Ab's	Prevalence	Lo95%CI	Hi95%CI
Males	572	23	4.0%	2.6%	6.0%
Females	928	78	8.4%	6.7%	10.4%

Males - HIV-1

Age Range	# Males	with HIV-1 Ab	Prevalence	Lo95%CI	Hi95%CI
All Ages	572	14	2.4%	1.3%	4.1%
= 5	4	0	0.0%	0.0%	52.7%
6—12	7	0	0.0%	0.0%	34.8%
13-19	76	2	2.6%	0.3%	9.2%

20-26	116	3	2.6%	0.5%	7.4%
27-33	167	3	1.8%	0.4%	5.2%
34-40	58	1	1.7%	0.0%	9.2%
41-47	82	2	2.4%	0.3%	8.5%
48-54	33	1	3.0%	0.1%	15.8%
= 55	29	2	6.9%	0.8%	22.8%

Males - HIV-2

Age Range	# Males	with HIV-2 Ab	Prevalence	Lo95%CI	Hi95%CI
All Ages	572	8	1.4%	0.6%	2.7%
= 5	4	0	0.0%	0.0%	52.7%
6—12	7	0	0.0%	0.0%	34.8%
13-19	76	0	0.0%	0.0%	3.9%
20-26	116	2	1.7%	0.2%	6.1%
27-33	167	4	2.4%	0.7%	6.0%
34-40	58	0	0.0%	0.0%	5.0%
41-47	82	0	0.0%	0.0%	3.6%
48-54	33	0	0.0%	0.0%	8.7%
= 55	29	2	6.9%	0.8%	22.8%

Males - HIV-D

Age Range	# Males	with HIV-D Ab	Prevalence	Lo95%CI	Hi95%CI
All Ages	572	1	0.2%	0.0%	1.0%
= 5	4	0	0.0%	0.0%	52.7%
6—12	7	0	0.0%	0.0%	34.8%
13-19	76	0	0.0%	0.0%	3.9%
20-26	116	0	0.0%	0.0%	2.5%
27-33	167	0	0.0%	0.0%	1.8%
34-40	58	0	0.0%	0.0%	5.0%
41-47	82	0	0.0%	0.0%	3.6%
48-54	33	0	0.0%	0.0%	8.7%

= 55	29	1	3.4%	0.1%	17.8%

Females - HIV-1

Age Range	# Females	with HIV-1 Ab	Prevalence	Lo95%CI	Hi95%CI
All Ages	928	51	5.5%	4.1%	7.2%
= 5	5	1	20.0%	0.5%	71.6%
6—12	11	0	0.0%	0.0%	23.8%
13-19	135	5	3.7%	1.2%	8.4%
20-26	315	16	5.1%	2.9%	8.1%
27-33	209	26	12.4%	8.3%	17.7%
34-40	121	2	1.7%	0.2%	5.8%
41-47	68	1	1.5%	0.0%	7.9%
48-54	50	0	0.0%	0.0%	5.8%
= 55	14	0	0.0%	0.0%	19.3%

Females - HIV-2

Age Range	# Females	with HIV-2 Ab	Prevalence	Lo95%CI	Hi95%CI
All Ages	928	20	2.2%	1.3%	3.3%
= 5	5	0	0.0%	0.0%	45.1%
6—12	11	0	0.0%	0.0%	23.8%
13-19	135	0	0.0%	0.0%	2.2%
20-26	315	6	1.9%	0.7%	4.1%
27-33	209	12	5.7%	3.0%	9.8%
34-40	121	2	1.7%	0.2%	5.8%
41-47	68	0	0.0%	0.0%	4.3%
48-54	50	0	0.0%	0.0%	5.8%
= 55	14	0	0.0%	0.0%	19.3%

Females - HIV-D

Age Range	# Females	with HIV-D Ab	Prevalence	Lo95%CI	Hi95%CI
All Ages	928	7	0.8%	0.3%	1.5%
= 5	5	0	0.0%	0.0%	45.1%
6—12	11	0	0.0%	0.0%	23.8%
13-19	135	1	0.7%	0.0%	4.1%
20-26	315	2	0.6%	0.1%	2.3%
27-33	209	3	1.4%	0.3%	4.1%
34-40	121	1	0.8%	0.0%	4.5%
41-47	68	0	0.0%	0.0%	4.3%
48-54	50	0	0.0%	0.0%	5.8%
= 55	14	0	0.0%	0.0%	19.3%

Males - HCV

Age range	# Males	HCV Pos	Prevalence	Lo95%CI	Hi95%CI
All Ages	572	22	3.8%	2.4%	5.8%
= 5	4	0	0.0%	0.0%	52.7%
6—12	7	0	0.0%	0.0%	34.8%
13-19	76	0	0.0%	0.0%	3.9%
20-26	116	6	5.2%	1.9%	10.9%
27-33	167	3	1.8%	0.4%	5.2%
34-40	58	4	6.9%	1.9%	16.7%
41-47	82	7	8.5%	3.5%	16.8%
48-54	33	1	3.0%	0.1%	15.8%
= 55	29	1	3.4%	0.1%	17.8%

Females – HCV

Age range	# Females	HCV Pos	Prevalence	Lo95%CI	Hi95%CI
All Ages	928	9	1.0%	0.4%	1.8%
= 5	5	0	0.0%	0.0%	45.1%
6—12	11	0	0.0%	0.0%	23.8%
13-19	135	0	0.0%	0.0%	2.2%

20-26	315	4	1.3%	0.3%	3.2%
27-33	209	1	0.5%	0.0%	2.6%
34-40	121	2	1.7%	0.2%	5.8%
41-47	68	2	2.9%	0.4%	10.2%
48-54	50	0	0.0%	0.0%	5.8%
= 55	14	0	0.0%	0.0%	19.3%

All – HCV

Age range	# Patients	HCV Pos	Prevalence	Lo95%CI	Hi95%CI
All Ages	1500	31	2.1%	1.4%	2.9%
= 5	9	0	0.0%	0.0%	28.3%
6—12	18	0	0.0%	0.0%	15.3%
13-19	211	0	0.0%	0.0%	1.4%
20-26	431	10	2.3%	1.1%	4.2%
27-33	376	4	1.1%	0.3%	2.7%
34-40	179	6	3.4%	1.2%	7.2%
41-47	150	9	6.0%	2.8%	11.1%
48-54	83	1	1.2%	0.0%	6.5%
= 55	43	1	2.3%	0.1%	12.3%

All HIV-1

Age range	# Patients	HIV-1 Pos	Prevalence	Lo95%CI	Hi95%CI
All Ages	1500	65	4.3%	3.4%	5.5%
= 5	9	1	11.1%	0.3%	48.2%
6—12	18	0	0.0%	0.0%	15.3%
13-19	211	7	3.3%	1.3%	6.7%
20-26	431	19	4.4%	2.7%	6.8%
27-33	376	29	7.7%	5.2%	10.9%
34-40	179	3	1.7%	0.3%	4.8%
41-47	150	3	2.0%	0.4%	5.7%
48-54	83	1	1.2%	0.0%	6.5%

= 55	43	2	4.7%	0.6%	15.8%

All HIV-2

Age range	# Patients	HIV-2 Pos	Prevalence	Lo95%CI	Hi95%CI
All Ages	1500	28	1.9%	1.2%	2.7%
= 5	9	0	0.0%	0.0%	28.3%
6—12	18	0	0.0%	0.0%	15.3%
13-19	211	2	0.9%	0.1%	3.4%
20-26	431	6	1.4%	0.5%	3.0%
27-33	376	16	4.3%	2.5%	6.8%
34-40	179	1	0.6%	0.0%	3.1%
41-47	150	1	0.7%	0.0%	3.7%
48-54	83	1	1.2%	0.0%	6.5%
= 55	43	1	2.3%	0.1%	12.3%

All HIV-D

Age range	# Patients	HIV-D Pos	Prevalence	Lo95%CI	Hi95%CI
All Ages	1500	8	0.5%	0.2%	1.0%
= 5	9	0	0.0%	0.0%	28.3%
6—12	18	0	0.0%	0.0%	15.3%
13-19	211	0	0.0%	0.0%	1.4%
20-26	431	1	0.2%	0.0%	1.3%
27-33	376	4	1.1%	0.3%	2.7%
34-40	179	1	0.6%	0.0%	3.1%
41-47	150	1	0.7%	0.0%	3.7%
48-54	83	0	0.0%	0.0%	3.5%
= 55	43	1	2.3%	0.1%	12.3%

HIV-1 & HCV

Age range	# Patients	HIV-1 & HCV Pos	Prevalence	Lo95%CI	Hi95%CI
All Ages	1500	7	0.5%	0.2%	1.0%
= 5	9	0	0.0%	0.0%	28.3%

6—12	18	0	0.0%	0.0%	15.3%
13-19	211	0	0.0%	0.0%	1.4%
20-26	431	1	0.2%	0.0%	1.3%
27-33	376	3	0.8%	0.2%	2.3%
34-40	179	1	0.6%	0.0%	3.1%
41-47	150	1	0.7%	0.0%	3.7%
48-54	83	0	0.0%	0.0%	3.5%
= 55	43	1	2.3%	0.1%	12.3%

HIV-2 & HCV

Age range	# Patients	HIV-2 & HCV Pos	Prevalence	Lo95%CI	Hi95%CI
All Ages	1500	2	0.1%	0.0%	0.5%
= 5	9	0	0.0%	0.0%	28.3%
6—12	18	0	0.0%	0.0%	15.3%
13-19	211	0	0.0%	0.0%	1.4%
20-26	431	0	0.0%	0.0%	0.7%
27-33	376	1	0.3%	0.0%	1.5%
34-40	179	0	0.0%	0.0%	1.7%
41-47	150	0	0.0%	0.0%	2.0%
48-54	83	1	1.2%	0.0%	6.5%
= 55	43	0	0.0%	0.0%	6.7%

Comparison of Males vs. Females

	Males	Females	Odds Ratio (F/M)	Lo95%CI	Hi95%CI	p-value
Total #	572	928				
With HIV-1	14	51	2.32	1.25	4.58	0.006
With HIV-2	8	20	1.55	0.65	4.10	0.332
With HIV-D	1	7	4.34	0.55	196	0.165
With Any HIV	23	78	2.19	1.34	3.67	0.001

With HCV	22	9	0.24	0.10	0.56	<0.001

APPENDIX 3: SUMMARY OF SELECTED SUBJECTS VARIABLES IN RELATION TO THEIR *P*-VALUE, CONFIDENCE INTERVAL (CI) AND ODDS RATIO (OR)

Variable	Prevalence rate (%)	*P*- value	CI:	OR
ILLITERATES (n=510)				
HIV	9.4 (48/510)	0.00121	1.20-2.55	1.83
HCV	3.5 (18/510)	0.00319	1.32-5.44	2.75
HIV&HCV coinfection	0.8 (4/510)	0.211	0,41-5.75	1.56
EDUCATED (n=990)				
HIV	5.3 (53/990)	0.0012	0.39-0.82	0.54
HCV	1.3(13/990)	0.0032	0.18-0.75	0.36
HIV&HCV coinfection	0.5(5/990)	0.211	0.17-2.3	
EMPLOYED (n=493)				
HIV	5.3 (53/990)	0.0012	0.39-0.82	0.54
HCV	1.3(13/990)	0.0032	0.18-0.75	0.36
HIV and HCV coinfection	0.5(5/990)	0.211	0.17-2.3	
UN-EMPLOYED (n=1003)				
HIV	8.1(40/493)	0.028	0.95-1.54	1.37

HCV	1.6(8/493)	0.113	0.32-1.58	0.71
HIV&HCV coinfection	0.6(3/393)	0.273	0.25-4.07	1.02
INCOME ≤ D1000 monthly (n=949)				
HIV	7.0 (66/949)	0.0777	0.7367-1.62	1.102
HCV	2.4 (23/949)	0.0871	0.7001-3.45	1.567
HIV&HCV coinfection	0.6(6/949)	0.2916	0.2916-4.624	1.162
INCOME ≥ D1000 monthly (n=551)				
HIV	6.4(35/551)	0.0777	0.614-1.357	0.9075
HCV	1.5 (8/551)	0.0871	0.2698-1.3301	0.5932
HIV&HCV coinfection	0.5(3/551)	0.2916	0.2162-3.42	0.8604

APPENDIX 4: KNOWLEDGE OF HIV/AIDS AND CONDOM OF STUDY SUBJECTS IN RELATION TO THEIR HIV ANTIBODY STATUS, *P*-VALUE, OR, AND CI

Variable	# in category (%) Males (523); Females (844)	# HIV-1 positive (%)(Males=13 Females=47)	# HIV-2 positive (%) (Males=7 Females=16	# HIV-D positive (Males=1 Females=7	Total # HIV positive (p-value)	OR	95%
I know how HIV/AIDS is transmitted (Response from both sexes)	1121 (82.0%)	41(3.7)	19(1.7)	6(0.5%)	66 (p=0.1161)	0.9634	0.560 1.662
I do not know how HIV/AIDS is transmitted.(Response from both sexes)	230(16.8)	8(3.4)	4(1.7)	1(0.4)	13 (p=0.1213)	0.9435	0.532 1.684
I have never heard of HIV/AIDS. (Response from both sexes)	16(1.2)	1(6.25)	0	1(6.25)	2 (p=0.1799)	2.3002	0.574 7.953
I have heard of condoms (males)	501(95.7)	13(2.6)	6(1.2)	1(0.2)	20 (p=0.3825)	0.8732	0.123 6.259
I have never heard of condoms (Males)	22(4.3)	0	1(4.5)	0	1 (p=0.3825)	1.1452	0.16- 8.103
I have heard of condoms (females)	662(78.4)	35(5.3)	13(2.0)	6(0.9)	54 (p=0.1138)	0.9215	0.544 1.581
I have never heard of condoms (females)	182(21.6)	12(6.6)	3(1.6)	1(0.5)	1 (p=0.11382)	1.0852	0.632 1.836

APPENDIX 5: BEHAVIOURAL RISK FACTORS OF STUDY SUBJECTS IN RELATION TO THEIR HIV ANTIBODY STATUS, *P*-VALUE, OR, CI

Variable	# in category (%) Males (523); Females (844)	# HIV-1 Positive (%) (Males=13 Females=47)	# HIV-2 Positive (%) (Males=7 Females=1	# HIV-D Positive (Males=1 Females=7	Total # HIV positive (*p*-value)	OR	95% CI,
Use of Condom							
I use condoms always (Males)	12(2.3)	0	0	0	0	N/A	N/A
I use condoms occasionally (Males)	54(10.3)	1(1.8)	1(1.8)	0	2 (p=0.2891)	0.9494	0.2277 3.9738
I have never use condoms (Males)	457(87.3)	12(2.6)	6(1.3)	1(0.2)	1 (p=0.2609)	1.3861	0.327-5.7562
Sex during menses							
I have Sex during my menses	11(1.3)	0	0	0	0	0	0
I have never had sex during menses	833(98.7)	47(5.6)	16(1.9)	7(0.8)	70 (p=0.3835)	0	0
Use of contraceptive pills							
I use contraceptive pills regularly	10(1.2)	0	0	0	0	N/A	N/A
I use contraceptive pills occasionally	28(3.3)	1(3.5)	1(3.5)	0	2 (p=0.27751)	0.8462	0.211-3.3222
I never use contraceptive pills	806(95.5)	46(5.7)	15(1.9)	7(0.9)	68 (p=0.2707)	1.25	0.3149 4.81

APPENDIX 6: HISTORY OF STIS/STDS IN RELATIONSHIP TO SUBJECTS HIV ANTIBODY STATUS, *P*-VALUE, OR, AND CI

Variable	No. in category (%) Males (523); Females (844)	No, HIV-1 Positive (%) (Males=13 Females=47)	No. HIV-2 Positive (%) (Males=7 Females=16	No. HIV-D Positive (Males=1 Females=7)	Total No. HIV positive (p-value)	OR	95% CI,
Had non- menstrual bleeding after intercourse within the past five years							
Yes	26(3.1)	2(7.7)	0	1(3.8)	3 (P=0.2056)	1.462	0.4742 - 4.1848
No	818(96.9)	45(5.5)	16(2.0)	6(0.7)	67 (p=0.0205)	0.684	0.239-2.1087
Had painful intercourse after within the past five years							
Yes	50(5.9)	4(8.0)	0	1(2.0)	5 (p=0.1741)	1.2462	0.5151 -2.897
No	794(94.1)	43(5.4)	16(2.0)	6(0.8)	65 (p=0.1741)	1.2462	0.5151 -2.897
Within the past five years I have been treated for venereal disease (women subjects only)(n=844)							
Yes, ulcerative VD	11(1.3)	2(18.0)	1(1.3)	1(1.3)	4; (p=0.0081)	6.64	2.0308 - 10.3719
Yes, Non-ulcerative	68(8.1)	7(10.3)	2(2.9)	1(1.5)	10 (p=0.02696)	2.0575	1.0212 - 3.5423
Never	765(90.6)	38(5.0)	13(1.7)	5(0.7)	56 (p=0.0022)	0.3667	0.2412 - 0.7074
Within the past five years I have been treated for venereal disease (men subjects only) (n=523)							

Yes, urethral discharge	8(1.5)	2(25.0)	0	0	2; p=0.03285	8.9474	1.9375 - 25.0046
Yes, genital ulcers	2(0.4)	1(50.0)	0	0	1; p=0.07722	22.05	3.0521 - 55.5846
Never	513(98.1)	10(1.9)	7(1.4)	1(0.2)	18; p=0.0052	0.0354	0.0409 -03342
Within the past five years I have been treated for venereal disease (women subjects only)(n=844)							
Yes, ulcerative VD	11(1.3)	2(18.0)	1(1.3)	1(1.3)	4; (p=0.0081)	6.64	2.0308 - 10.3719
Yes, Non-ulcerative	68(8.1)	7(10.3)	2(2.9)	1(1.5)	10 (p=0.0266)	2.0575	1.0212 - 3.5423
Never	765(90.6)	38(5.0)	13(1.7)	5(0.7)	56 (p=0.0022)	0.3667	0.2412 - 0.7074
Within the past five years I have been treated for venereal disease (men subjects only) (n=523)							
Yes, urethral discharge	8(1.5)	2(25.0)	0	0	2; p=0.03285	8.9474	1.9375 - 25.0046
Yes, genital ulcers	2(0.4)	1(50.0)	0	0	1; p=0.07722	22.05	3.0521 - 55.5846
Never	513(98.1)	10(1.9)	7(1.4)	1(0.2)	18; p=0.0052	0.0354	0.0409 -03342

APPENDIX 7: KNOWLEDGE OF HIV/AIDS AND CONDOM OF STUDY SUBJECTS IN RELATION TO THEIR ANTI-HCV AND HIV/HCV COINFECTION STATUS, *P*-VALUE, OR AND CI

Variable	No. in category (%) Males (523); Females (844)	Number HCV positive (%)(Males=22 Females=9	P-value Odds Ratio 95% Confidence interval	No.HIV & HCV Positive) (%)(Males=7, Females=2)	P-value Odds Ratio Confidence interval
I know how HIV/AIDS is transmitted (Response from both sexes)	1121 (82.0%)	25(2.2)	P=0.1774 OR: 0.9124 CI, 0.3792-2.205	7(0.6)	P=0.2917 OR: 0.766 CI, 0.1605-3.675
I do not know how HIV/AIDS is transmitted.(Response from both sexes)	230(16.8)	5(2.1)	P=0.1925 OR: 0.9496 CI, 0.3689-2.4497	2(0.9)	P=0.2816 OR: 1.416 CI, 0.3877-4.5192
I have never heard of HIV/AIDS. (Response from both sexes)	16(1.2)	1(6.3)	P=0.0198 OR: 0.1611 CI, 0.0226-1.1203	0	NA
I have heard of condoms (males)	501(95.7)	21(4.2)	P=0.3839 OR: 0.9188 CI, 0.1299-65478	7(1.4)	P=0.7389
I have never heard of condoms (Males)	22(4.3)	1(4.5)	P=0.3839 OR: 1.0884 CI, 0.1527-7.699	0	NA
I use condoms always (Males)	12(2.3)	0	NA	0	NA

I use condoms occasionally (Males)	54(10.3)	2(3.7)	P=0.2836 OR: 0.8635 CI,, 0.2087-3.6149	1(1.9)	P=0.3789 OR: 1.456 CI,, 0.1770-11.7998
I have never use condoms (Males)	457(87.3)	19(4.2)	P=0.2439 OR: 0.911 CI,, 0.2783-3.0066	6(1.3)	P=0.39611 OR: 0.8647 CI, 0.106-7.085
I have heard of condoms (females)	662(78.4)	7(1.1)	P=0.3074 OR: 0.9618 CI, 0.2016-4.5925	1(0.2)	P=0.3386 OR: 0.2738 CI, 0.0173-4.3743
I have never heard of condoms (females)	182(21.6)	2(1.1)	P=0.3076 OR: 1.0254 CI, 0.2148-4.892	1(0.5)	P=0.3386 OR: 3.6519 CI, 0.2286-57.8735

APPENDIX 8: BEHAVIOURAL RISK FACTORS AND HISTORY OF STIS/STDS OF STUDY SUBJECTS IN RELATION TO THEIR ANTI-HCV AND HIV/HCV COINFECTION STATUS, *P*-VALUE, OR AND CI

Variable	No. in category (%) Males (523); Females (844)	No. HCV positive (%)(Males=22 Females=9)	P-value, Odds Ratio 95% Confidence interval	No. HIV&HCV Positive) (%)(Males=7 Females=2)	P-value Odds Ratio Confidence interval
I have Sex during my menses	11(1.3)	1(9.1)	P=0.1066 OR: 10.3 CI, 1.2898	0	P=0.1066 OR: 0.0971 CI, 0.0144-0.7953

I have never had sex during menses	833(98.7)	8(1.0)	P=0.106689 OR: 0.0971 CI, 0.0144-0.7953	2(0.4)	P=0.97488
History of ulcerative VD (females)	11(1.3)	2(18.2)	P=0.00516 OR: 26.2222 CI, 5.0521-92.6601	1(9.1)	P=0.025 OR: 83.2 CI, 5.0524-1135.0
History of non-ulcerative (females)	68(8.1)	2(2.9)	P=0.129908 OR: 3.329 CI, 0.6908-15.3898	1(1.5)	P=0.14830 OR: 11.567 CI,, 0.7217-189.43
No VD (females)	765(90.6)	5(0.7)	P=0.005643 OR: 0.1234 CI, 0.3401-1.0954	0	P=0.15034 OR: 0.089 CI, 0.0056-1.408
History of venereal disease (men)(n=523)					
History of urethral discharge	8(1.3)	1(12.5)	P=0.2439 OR: 3.5357 CI, 0.4896-21.1593	1(12.5)	P=0.1112 OR: 10.36 CI, 1.2754
History genital ulcers	2(0.4)	1(50%)	P=0.0772 OR: 25.05 CI, 3.0521-55.5846	0	N/A
No. VD (males)	513(98.1)	20(3.9)	P=0.281714 OR: 0.3651 CI, 3.0521-55.5846	6(1.2)	P=0.012 OR: 0.1065 CI, 0.015-0.8838

APPENDIX 9:CD4+dx OF HIV INFECTED PERSONS ACCORDING TO AGE AND SEX

S/N	Age(yrs)	Sex	DX	CD4+ count cells /μl
1	19	M	HIV-1	184
2	28	F	HIV-1	378
3	19	M	HIV-1	199
4	20	F	HIV-1	198
5	20	F	HIV-1	586
6	26	F	HIV-1	285
7	57	M	HIV-1	574
8	28	F	HIV-1	395
9	23	M	HIV-1	485
10	24	F	HIV-1	177
11	16	F	HIV-1	384
12	13	F	HIV-1	389
13	26	M	HIV-1	187
14	20	F	HIV-1	365
15	48	M	HIV-1	190
16	17	F	HIV-1	199
17	22	F	HIV-1	393
18	27	F	HIV-1	280
19	24	M	HIV-1	358
20	47	M	HIV-1	458

21	25	F	HIV-1	262
22	21	F	HIV-1	273
23	26	F	HIV-1	256
24	26	F	HIV-1	190
25	28	F	HIV-1	180
26	33	F	HIV-1	376
27	23	F	HIV-1	189
28	28	F	HIV-1	483
29	32	F	HIV-1	334
30	26	F	HIV-1	286
31	16	F	HIV-1	352
32	26	F	HIV-1	375
33	32	M	HIV-1	525
34	31	F	HIV-1	321
35	33	M	HIV-1	196
36	28	F	HIV-1	154
37	28	F	HIV-1	170
38	30	F	HIV-1	588
39	30	F	HIV-1	190
40	3	F	HIV-1	183
41	20	F	HIV-1	132
42	42	F	HIV-1	98
43	28	F	HIV-1	185
44	32	F	HIV-1	168

45	28	F	HIV-1	178
46	28	F	HIV-1	187
47	33	F	HIV-1	368
48	30	F	HIV-1	179
49	29	F	HIV-1	450
50	41	M	HIV-1	168
51	34	M	HIV-1	474
52	22	F	HIV-1	579
53	29	F	HIV-1	291
54	38	F	HIV-1	181
55	32	F	HIV-1	173
56	28	F	HIV-1	358
57	28	F	HIV-1	366
58	27	F	HIV-1	198
59	56	M	HIV-1	199
60	31	F	HIV-1	198
61	33	F	HIV-1	357
62	28	F	HIV-1	198
63	32	M	HIV-1	472
64	33	F	HIV-1	198
65	35	F	HIV-1	475
66	27	F	HIV-2	190
67	31	F	HIV-2	564
68	28	M	HIV-2	177

69	27	F	HIV-2	158
70	20	F	HIV-2	280
71	55	M	HIV-2	305
72	32	F	HIV-2	165
73	30	F	HIV-2	190
74	29	M	HIV-2	189
75	56	M	HIV-2	388
76	22	F	HIV-2	169
77	36	F	HIV-2	425
78	32	F	HIV-2	367
79	30	M	HIV-2	504
80	28	F	HIV-2	551
81	27	F	HIV-2	580
82	28	F	HIV-2	176
83	33	M	HIV-2	178
84	32	F	HIV-2	223
85	33	F	HIV-2	140
86	25	M	HIV-2	483
87	22	F	HIV-2	276
88	20	F	HIV-2	547
89	24	F	HIV-2	189
90	40	F	HIV-2	564
91	26	M	HIV-2	172
92	27	F	HIV-2	255

93	34	F	HIV-2	554
94	27	F	HIV-D	280
95	20	F	HIV-D	168
96	67	M	HIV-D	103
97	27	F	HIV-D	474
98	34	F	HIV-D	187
99	14	F	HIV-D	168
100	23	F	HIV-D	277
101	29	F	HIV-D	198
Mean	29.1			299

APPENDIX 10: CD4+ TRENDS IN HIV-1 INFECTED MALES (CASE SUBJECTS)(ALL PARTICIPANTS) CD4+Dx

(cells /μl)

S/N	Age	Sex	(n=7)	1st count (n=4)	2nd count (n=3)	3rd count (n=2)
1	19	M	199	N/A	N/A	N/A
2	57	M	574	536	243	N/A
3	47	M	458	405	372	307
4	32	M	525	478	N/A	N/A
5	33	M	196	N/A	N/A	N/A
6	56	M	199	N/A	N/A	N/A
7	32	M	472	436	370	324
Average	39.4		375	464	328	316

APPENDIX 11:CD4+ TRENDS IN HIV-1 INFECTED FEMALES (ALL PARTICIPANTS)

(cells /μl)

S/N	Age n=49	Sex	CD4+ Dx n=49	1st count n=25	2nd count n=18	3rd count n=10
1	28	F	378	345	270	N/A
2	20	F	198	N/A	N/A	N/A
3	20	F	586	522	476	401
4	26	F	285	211	N/A	N/A
5	28	F	395	336	248	N/A
6	24	F	177	N/A	N/A	N/A
7	16	F	384	345	271	201
8	13	F	389	345	256	188
9	20	F	365	323	265	201
10	17	F	199	N/A	N/A	N/A
11	22	F	393	345	292	224
12	25	F	262	222	N/A	N/A
13	21	F	273	226	N/A	N/A
14	26	F	256	N/A	N/A	N/A
15	26	F	190	N/A	N/A	N/A
16	33	F	376	322	264	204
17	23	F	189	N/A	N/A	N/A
18	28	F	483	435	368	295
19	32	F	334	250	N/A	N/A
20	26	F	286	N/A	N/A	N/A
21	16	F	352	300	226	N/A
22	26	F	375	324	250	N/A
23	31	F	321	254	N/A	N/A
24	28	F	154	N/A	N/A	N/A
25	28	F	170	N/A	N/A	N/A
26	30	F	588	542	482	421

27	30	F	190	N/A	N/A	N/A
28	3	F	183	N/A	N/A	N/A
29	20	F	132	N/A	N/A	N/A
30	42	F	98	N/A	N/A	N/A
31	28	F	185	N/A	N/A	N/A
32	32	F	168	N/A	N/A	N/A
33	28	F	178	N/A	N/A	N/A
34	28	F	187	N/A	N/A	N/A
35	33	F	368	320[b]	N/A	N/A
36	30	F	179	N/A	N/A	N/A
37	29	F	450	390	246	N/A
38	22	F	579	523	468	408
39	29	F	291	246	N/A	N/A
40	38	F	181	N/A	N/A	N/A
41	32	F	173	N/A	N/A	N/A
42	28	F	358	306	234	N/A
43	28	F	366	317	237	N/A
44	27	F	198	N/A	N/A	N/A
45	31	F	198	N/A	N/A	N/A
46	33	F	357	299	213	N/A
47	28	F	198	N/A	N/A	N/A
48	33	F	198	N/A	N/A	N/A
49	35	F	475	433	378	303
AV.	26.5		291	339	303	285

b HCV sero-conversion

APPENDIX 12:CD4+ TRENDS IN HIV-2 INFECTED MALE PATIENTS (cells /μl)

S/N	Age (Yrs)	Sex	CD4+ Dx	1st count	2ND COUNT	3rd count
			n=7	n=3	n=1	n=1
1	55	M	305	237[a]	N/A	N/A
2	29	M	189[a]	N/A	N/A	N/A
3	30	M	504	443	328	269
4	33	M	178[a]	N/A	N/A	N/A
5	25	M	483	420	N/A	N/A
6	26	M	172[a]	N/A	N/A	N/A
7	28	M	177[a]	N/A	N/A	N/A
Average	32.3		287	345	259	269

[a] Referred for antiretroviral therapy

APPENDIX 13: CD4+ TRENDS IN HIV-2 INFECTED FEMALE STUDY PATIENTS

(cells /μl)

S/N	Age (Yrs)	Sex	CD4+ Dx	1st count	2nd count	3rd count
			n=17	n=10	n=7	n=5
1	27	F	190[a]	N/A	N/A	N/A
2	31	F	564	496	407	341
3	20	F	280	207[a]	N/A	N/A
4	32	F	165[a]	N/A	N/A	N/A
5	30	F	190[a]	N/A	N/A	N/A
6	22	F	169[a]	N/A	N/A	N/A
7	36	F	425	336	213[a]	N/A
8	32	F	367	271	N/A	N/A
9	28	F	551	485	417	342
10	27	F	580	508	442	N/A
11	28	F	176[a]	N/A	N/A	N/A
12	32	F	223[a]	N/A	N/A	N/A
13	22	F	276	196[a]	N/A	N/A
14	20	F	547	492	423	340
15	24	F	189[a]	N/A	N/A	N/A
16	40	F	564	508	427	341
17	34	F	554	504	468	410
Average	28.5		354	403	400	355

[a] Referred for antiretroviral therapy

APPENDIX 14:CD4+ TRENDS IN HIV-D STUDY PATIENTS (MALE AND FEMALES)

(cells /μl)

S/N	AGE	Sex	CD4+ Dx	1st Count	2nd count	3rd Count
	n=8		N=7	n=3	n=1	n=1
1	27	F	280	151[a]	N/A	N/A
2	20	F	168[a]	N/A	N/A	N/A
3	67	M	103[a]	N/A	N/A	N/A
4	27	F	474	398	398	258
5	34	F	187[a]	N/A	N/A	N/A
6	14	F	168[a]	N/A	N/A	N/A
7	23	F	277	178[a]	N/A	N/A
8	29	F	198[a]	N/A	N/A	N/A
Average	30.1		232	242	398	258

[a] Referred for antiretroviral therapy

APPENDIX 15: SUMMARY OF CD4+ TRENDS IN ALL HIV-INFECTED PATIENTS WHO PARTICIPATED FULLY IN THE FOLLOW-UP STUDY (cells /μl)

S/N	Age	Sex	CDdx	1st count	2nd count	3rd count
1	20	F	586	522	476	401
2	16	F	384	345	271	201
3	13	F	389	345	256	188
4	20	F	365	323	265	201
5	22	F	393	345	292	224
6	28	F	483	435	368	295
7	30	F	588	542	482	421
8	22	F	579	523	468	408

9	35	F	475	433	378	303
10	33	F	376	322	264	204
11	47	M	458	405	372	307
12	32	M	472	436	370	324
13	31	F	564	496	407	341
14	28	F	551	485	417	342
15	20	F	547	492	423	340
16	40	F	564	508	427	341
17	34	F	554	504	468	410
18	30	M	504	443	328	269
19	27	F	474	398	398	258
Mean	27.8		490	437	375	304

APPENDIX 16: CD4+ CELL COUNT OF HCV SEROPOSITIVE MALE SUBJECTS (cells /μl)

S/N	Age	Sex	CD4+ dx n=15	1st count n=14	2nd count n=14	3rd count n=12
1	47	M	478	524	516	N/A
2	26	M	501	467	512	524
3	46	M	404	521	498	472
4	33	M	452	456	455	432
5	45	M	444	N/A	N/A	N/A
6	22	M	534	612	590	560
7	45	M	476	433	480	466
8	45	M	432	412	460	N/A
9	32	M	500	478	512	478
10	32	M	412	453	466	490
11	24	M	555	488	472	455
12	25	M	456	478	472	502
13	40	M	552	434	455	430
14	52	M	539	470	512	494
15	22	M	550	512	476	489
Average	35.7		486	481	491	483

APPENDIX 17: CD4+ CELL COUNT OF HCV SEROPOSITIVE FEMALE PATIENTS (cells /μl)

S/N	Age	Sex	CD4+ Dx	1st count	2nd count	3rd count
			n=7	n=6	n=6	n=5
1	45	F	606	554	580	556
2	26	F	542	490	478	460
3	38	F	410	380	424	450
4	24	F	522	508	516	N/A
5	44	F	564	N/A	N/A	N/A
6	20	F	521	506	488	514
7	22	F	506	545	562	516
AV	31.2		524	497	508	499

APPENDIX 18: CD4+ TRENDS IN HIV-1/HCV COINFECTED STUDY PATIENTS (MALE AND FEMALES) (cells /μl)

S/N	Age	Sex	CD4+ Dx	1st count	2nd count	3rd count
	n=7	n=7	n=7	n=4	n=2	n=2
1	27	F	280	232[a]	N/A	N/A
2	41	M	168[a]	N/A	N/A	N/A
3	48	M	190[a]	N/A	N/A	N/A
4	34	M	474	370	251	160
5	26	M	187[a]	N/A	N/A	N/A
6	24	M	358	272	N/A	N/A
7	23	M	485	407	293	182
Average	31.9		306	320	272	171

[a] Referred for antiretroviral therapy

APPENDIX 19: CD4+ TRENDS IN HIV-2/HCV COINFECTED PATIENTS (MALE AND FEMALE) (cells /μl)

S/N	Age	Sex	CD4+ Dx	1st count	2nd count	3rd count
	n=2	n=2	n=2	n=2	n=1	n=0
1	36	F	425	348	N/A	N/A
2	55	M	305	245	N/A	N/A
Average			365	297		

APPENDIX 20:CD4+ TRENDS IN HIV /HCV SERONEGATIVE MALE CONTROL SUBJECTS (cells /μl)

S/N	Age (Years)	CD4+dx (n=66)	1st count (n=66)	2nd count (n=60)	3rd count (n=60)
1	18	540	511	520	536
2	18	633	601	578	590
3	19	522	506	496	511
4	19	516	534	503	526
5	22	418	434	450	446
6	22	403	378	N/A	N/A
7	22	579	524	543	555
8	22	444	460	488	502
9	23	402	424	434	455
10	24	455	468	442	402
11	25	457	472	505	490
12	25	556	548	532	560
13	25	430	411	452	438
14	26	423	451	448	457
15	26	422	455	434	460
16	26	552	534	525	543
17	26	438	450	448	452
18	26	599	580	572	586
19	26	486	490	510	498
20	27	523	545	560	555
21	27	567	580	566	576
22	27	467	456	470	466
23	28	415	422	434	453
24	28	504	534	524	511
25	28	517	490	509	526
26	28	506	551	533	521
27	29	589	601	578	598

28	29	512	523	502	496
29	30	509	498	511	520
30	29	566	546	560	533
31	29	445	422	452	458
32	29	414	456	460	446
33	30	508	515	524	515
34	30	513	556	563	537
35	30	486	517	511	498
36	30	556	560	548	560
37	31	511	542	534	524
38	31	534	546	523	528
39	31	402	456	472	487
40	32	421	403	N/A	N/A
41	32	422	398	N/A	N/A
42	32	522	503	528	542
43	33	523	546	558	542
44	42	520	512	477	486
45	44	489	474	441	424
46	44	516	552	526	542
47	44	509	531	522	536
48	45	556	567	540	538
49	45	556	523	544	537
50	46	520	534	527	538
51	47	409	398	426	452
52	47	535	540	548	560
53	55	532	502	549	534
54	55	515	535	521	542
55	55	545	560	533	557
56	56	409	411	428	453
57	56	239	179	N/A	N/A
58	56	505	525	540	548
59	57	509	543	526	528

60	58	330	368	N/A	N/A
61	58	506	488	511	534
62	58	448	478	490	488
63	59	521	552	536	544
64	60	524	511	528	506
65	63	329	270	N/A	N/A
66	66	534	516	541	525
Mean	35.8	489	492	509	513

Key:

CD4+dx: Count taken on first diagnosis of HIV and HCV status

1st, 2nd and 3rd counts: Counts taken at 6 months intervals

SC=Seroconversion

N/A= Not applicable (neither the patient did not show up again or was excluded following seroconversion)

APPENDIX 21: CD4+ COUNT TRENDS IN HIV/HCV-SERONEGATIVE FEMALE CONTROL SUBJECTS

(cells /μl)

S/N	Age (Years)	CD4+dx (n=222)	1st count (n=220)	2nd count (n=216)	3rd count (n=215)
1	17	512	534	523	540
2	18	567	510	538	555
3	19	551	556	532	560
4	19	589	567	564	541
5	19	516	534	511	529
6	26	478	490	512	524
7	20	408	378	390	410
8	20	401	N/A	N/A	N/A
9	23	456	488	446	410
10	25	412	446	432	456
11	25	409	364	N/A	N/A
12	20	445	455	428	462
13	20	434	423	463	453
14	20	467	498	456	478
15	26	424	418	434	453
16	23	443	456	414	430
17	25	412	448	423	450
18	25	467	455	478	501

19	25	456	467	438	478
20	21	478	445	458	432
21	21	498	460	477	467
22	22	443	455	468	440
23	23	467	452	480	455
24	25	467	512	478	456
25	26	487	514	490	478
26	23	434	456	476	455
27	24	423	416	456	468
28	24	412	N/A	N/A	N/A
29	23	432	452	479	456
30	21	456	472	490	515
31	22	467	488	442	462
32	26	456	478	490	466
33	24	454	448	467	470
34	26	467	478	490	486
35	24	456	412	444	467
36	22	437	463	412	467
37	23	434	455	467	453
38	26	423	436	454	478
39	26	412	436	434	489
40	25	467	455	490	466
41	26	456	479	432	490
42	24	478	462	437	417

43	24	498	443	442	497
44	24	409	412	366	378
45	26	456	423	456	472
46	24	467	487	454	443
47	25	434	423	416	441
48	21	489	501	498	522
49	26	401	387	366	356
50	24	434	456	464	429
51	22	456	398	346	N/A
52	24	478	492	456	422
53	25	445	427	489	413
54	26	489	514	499	507
55	25	407	434	411	452
56	30	409	378	386	396
57	30	434	454	434	467
58	33	423	445	432	443
59	32	412	435	418	456
60	29	423	423	433	423
61	27	406	413	452	490
62	27	411	453	478	409
63	30	438	478	434	453
64	28	409	423	401	443
65	31	456	410	387	467
66	31	412	435	455	421

67	29	565	552	578	487
68	27	576	390	N/A	N/A
69	29	434	456	423	434
70	32	433	467	444	453
71	33	401	432	420	443
72	31	434	467	455	424
73	29	456	434	456	412
74	27	405	412	440	434
75	27	445	476	468	418
76	29	401	434	421	366
77	32	407	378	323	N/A
78	28	434	453	426	409
79	32	345	389	355	303
80	30	454	410	443	425
81	32	414	425	465	412
82	29	427	433	412	456
83	30	478	532	564	432
84	30	418	452	441	467
85	32	408	390	423	423
86	33	434	423	456	452
87	31	412	434	423	468
88	32	423	438	443	456
89	33	453	467	442	434
90	30	407	423	436	454

91	31	422	448	456	478
92	29	437	458	423	403
93	29	423	445	478	443
94	31	449	467	489	445
95	32	403	444	468	434
96	32	432	452	441	435
97	33	454	412	372	489
98	29	443	433	N/A	409
99	29	423	456	410	434
100	27	409	428	452	433
101	32	434	401	436	456
102	31	412	442	434	478
103	27	434	456	467	454
104	29	456	421	443	423
105	33	510	463	410	433
106	32	532	524	554	456
107	29	567	578	534	489
108	27	519	546	544	545
109	33	544	567	565	567
110	31	534	498	423	543
111	27	580	523	570	509
112	29	523	512	546	544
113	27	521	591	567	576
114	27	507	534	523	512

115	33	514	459	N/A	544
116	33	508	544	513	526
117	32	527	512	546	534
118	32	545	563	578	534
119	30	523	567	548	544
120	32	512	544	522	578
121	32	550	523	538	576
122	32	515	490	515	544
123	31	545	578	567	455
124	28	567	589	548	465
125	29	509	534	512	534
126	32	549	516	544	528
127	33	523	556	567	546
128	32	521	545	578	545
129	31	545	578	533	564
130	29	534	567	546	554
131	27	512	523	578	532
132	29	534	567	523	509
133	30	543	589	544	504
134	30	545	512	569	512
135	33	507	477	513	544
136	32	511	545	543	534
137	29	534	555	532	512
138	28	542	578	567	534

139	28	521	510	546	565
140	28	502	534	546	501
141	29	543	558	578	545
142	33	543	514	538	565
143	32	589	604	561	508
144	30	509	488	462	498
145	32	545	534	522	545
146	31	502	521	542	523
147	32	534	578	567	544
148	32	512	534	546	566
149	31	534	567	534	512
150	30	543	534	578	533
151	30	518	455	407	480
152	30	590	572	546	578
153	32	507	533	544	533
154	32	555	478	423	388
155	31	522	546	551	501
156	29	545	559	578	534
157	29	534	498	532	544
158	33	567	534	534	512
159	32	589	564	543	532
160	31	554	524	578	541
161	32	551	567	556	520
162	32	545	578	545	502

163	29	534	523	578	554
164	28	512	467	534	512
165	29	545	567	512	534
166	29	556	589	545	566
167	33	578	545	578	523
168	32	564	532	589	534
169	27	580	557	545	502
170	27	555	520	567	513
171	28	509	534	590	565
172	32	523	556	545	512
173	31	545	578	512	532
174	33	566	590	543	508
175	33	518	545	567	544
176	27	509	567	589	567
177	29	545	589	543	502
178	30	523	503	566	509
179	32	545	467	509	454
180	31	554	578	456	402
181	33	567	548	512	466
182	30	567	534	545	512
183	32	589	564	523	544
184	32	509	534	512	566
185	28	565	520	567	524
186	29	578	545	545	523

187	25	578	557	567	543
188	25	543	567	587	544
189	26	512	534	567	511
190	22	545	486	598	564
191	23	567	523	564	516
192	21	556	523	564	510
193	21	589	546	435	464
194	21	523	548	456	N/A
195	20	545	578	567	523
196	23	567	602	578	533
197	25	545	505	578	523
198	26	578	552	534	567
199	24	507	538	545	512
200	25	523	567	543	534
201	22	545	589	535	567
202	34	428	445	490	468
203	34	554	578	530	509
204	34	505	567	523	478
205	39	558	534	545	510
206	38	545	523	523	534
207	34	533	578	566	533
208	39	545	545	534	567
209	40	523	523	567	503
210	40	567	534	590	534

211	36	545	537	503	534
212	36	554	578	564	534
213	36	590	545	512	522
214	34	564	523	544	514
215	35	545	567	523	501
216	34	565	523	545	533
217	34	576	544	567	534
218	34	509	512	548	512
219	35	567	523	564	523
220	36	565	534	567	543
221	37	564	567	509	533
222	38	534	512	547	523
Average	28.6	496	503	499	491

APPENDIX 22: CD4+dx COUNT OF ALL MALES WHO PARTICIPATED IN THE STUDY. (cells /μl)

S/N	Age	CD4+dx	Status
1	19	184	HIV-1
2	19	199	HIV-1
3	57	379	HIV-1
4	26	526	HIV-1
5	25	198	HIV-1
6	50	672	HIV-1
7	26	392	HIV-1
8	44	454	HIV-1
9	30	163	HIV-1
10	33	185	HIV-1
11	42	172	HIV-1
12	34	471	HIV-1
13	56	199	HIV-1
14	31	475	HIV-1
15	28	177	HIV-2
16	55	305	HIV-2
17	29	189	HIV-2
18	56	388	HIV-2
19	30	504	HIV-2
20	33	178	HIV-2
21	25	483	HIV-2

22	26	172	HIV-2
23	67	103	HIV-D
24	47	478	HCV
25	26	501	HCV
26	46	404	HCV
27	33	452	HCV
28	45	444	HCV
29	22	534	HCV
30	45	476	HCV
31	45	432	HCV
32	32	500	HCV
33	32	412	HCV
34	24	555	HCV
35	25	456	HCV
36	40	552	HCV
37	52	539	HCV
38	22	550	HCV
39	26	501	HCV cont
40	46	404	HCV cont
41	33	452	HCV cont
42	45	444	HCV cont
43	22	534	HCV cont
44	45	476	HCV

			cont
45	45	432	HCV cont
46	32	500	HCV cont
47	32	412	HCV cont
48	24	555	HCV cont
49	25	456	HCV cont
50	40	552	HCV cont
51	52	539	HCV cont
52	22	550	HCV cont
53	18	633	All Neg
54	1	502	All Neg
55	18	507	All Neg
56	19	590	All Neg
57	15	578	All Neg
58	18	540	All Neg
59	19	522	All Neg
60	56	489	All Neg
61	12	517	All Neg
62	12	632	All Neg
63	16	530	All Neg
64	17	555	All Neg

65	19	272	All Neg
66	13	333	All Neg
67	19	341	All Neg
68	15	364	All Neg
69	17	322	All Neg
70	16	445	All Neg
71	19	475	All Neg
72	18	426	All Neg
73	17	402	All Neg
74	16	428	All Neg
75	76	442	All Neg
76	16	408	All Neg
77	16	443	All Neg
78	15	462	All Neg
79	19	418	All Neg
80	13	460	All Neg
81	18	446	All Neg
82	17	410	All Neg
83	13	534	All Neg
84	16	556	All Neg
85	15	578	All Neg
86	9	245	All Neg
87	56	239	All Neg
88	58	371	All Neg
89	10	566	All Neg
90	16	208	All Neg
91	56	204	All Neg

92	19	536	All Neg
93	18	567	All Neg
94	14	589	All Neg
95	16	507	All Neg
96	17	533	All Neg
97	19	513	All Neg
98	55	132	All Neg
99	16	567	All Neg
100	17	589	All Neg
101	19	509	All Neg
102	16	550	All Neg
103	18	568	All Neg
104	17	520	All Neg
105	17	538	All Neg
106	17	513	All Neg
107	5	260	All Neg
108	19	567	All Neg
109	15	543	All Neg
110	16	517	All Neg
111	17	540	All Neg
112	18	519	All Neg
113	6	504	All Neg
114	14	539	All Neg
115	18	502	All Neg
116	19	512	All Neg
117	55	432	All Neg
118	15	568	All Neg

119	16	536	All Neg
120	17	516	All Neg
121	25	390	All Neg
122	20	323	All Neg
123	61	104	All Neg
124	15	534	All Neg
125	18	540	All Neg
126	14	536	All Neg
127	18	514	All Neg
128	56	154	All Neg
129	20	387	All Neg
130	17	517	All Neg
131	18	580	All Neg
132	19	556	All Neg
133	16	562	All Neg
134	17	633	All Neg
135	17	567	All Neg
136	16	543	All Neg
137	14	520	All Neg
138	25	190	All Neg
139	25	124	All Neg
140	22	173	All Neg
141	24	185	All Neg
142	20	198	All Neg
143	23	224	All Neg
144	24	256	All Neg
145	20	279	All Neg

146	26	209	All Neg
147	24	211	All Neg
148	24	235	All Neg
149	20	278	All Neg
150	24	298	All Neg
151	24	235	All Neg
152	23	289	All Neg
153	25	205	All Neg
154	26	226	All Neg
155	24	212	All Neg
156	20	276	All Neg
157	22	245	All Neg
158	24	265	All Neg
159	26	243	All Neg
160	25	267	All Neg
161	24	334	All Neg
162	20	356	All Neg
163	22	312	All Neg
164	21	390	All Neg
165	24	345	All Neg
166	24	367	All Neg
167	25	387	All Neg
168	26	323	All Neg
169	26	346	All Neg
170	22	378	All Neg
171	22	308	All Neg
172	24	345	All Neg

173	14	633	All Neg
174	18	567	All Neg
175	22	345	All Neg
176	22	321	All Neg
177	17	520	All Neg
178	26	345	All Neg
179	14	512	All Neg
180	21	339	All Neg
181	22	360	All Neg
182	26	450	All Neg
183	24	456	All Neg
184	25	406	All Neg
185	26	400	All Neg
186	24	422	All Neg
187	23	423	All Neg
188	20	403	All Neg
189	24	455	All Neg
190	24	456	All Neg
191	25	406	All Neg
192	22	423	All Neg
193	26	408	All Neg
194	20	410	All Neg
195	22	406	All Neg
196	26	497	All Neg
197	20	444	All Neg
198	26	423	All Neg
199	21	403	All Neg

200	22	423	All Neg
201	22	421	All Neg
202	24	422	All Neg
203	25	423	All Neg
204	26	416	All Neg
205	20	490	All Neg
206	25	432	All Neg
207	22	418	All Neg
208	26	423	All Neg
209	25	457	All Neg
210	22	444	All Neg
211	26	422	All Neg
212	25	430	All Neg
213	22	403	All Neg
214	26	486	All Neg
215	24	455	All Neg
216	23	402	All Neg
217	26	438	All Neg
218	26	599	All Neg
219	25	556	All Neg
220	22	579	All Neg
221	26	552	All Neg
222	23	534	All Neg
223	24	535	All Neg
224	25	515	All Neg
225	20	502	All Neg
226	22	547	All Neg

227	26	581	All Neg
228	24	582	All Neg
229	25	580	All Neg
230	26	556	All Neg
231	20	520	All Neg
232	22	579	All Neg
233	25	552	All Neg
234	22	507	All Neg
235	26	546	All Neg
236	21	570	All Neg
237	20	502	All Neg
238	25	548	All Neg
239	33	224	All Neg
240	31	267	All Neg
241	29	214	All Neg
242	30	280	All Neg
243	27	234	All Neg
244	29	256	All Neg
245	28	276	All Neg
246	32	278	All Neg
247	29	242	All Neg
248	31	256	All Neg
249	33	278	All Neg
250	31	231	All Neg
251	27	226	All Neg
252	32	522	All Neg
253	31	534	All Neg

254	33	309	All Neg
255	33	413	All Neg
256	32	432	All Neg
257	30	486	All Neg
258	29	414	All Neg
259	30	513	All Neg
260	28	506	All Neg
261	28	533	All Neg
262	13	534	All Neg
263	28	415	All Neg
264	27	467	All Neg
265	29	445	All Neg
266	31	402	All Neg
267	32	421	All Neg
268	32	422	All Neg
269	33	423	All Neg
270	32	408	All Neg
271	32	543	All Neg
272	30	556	All Neg
273	29	589	All Neg
274	28	504	All Neg
275	27	523	All Neg
276	27	567	All Neg
277	28	567	All Neg
278	30	598	All Neg
279	33	523	All Neg
280	33	553	All Neg

281	32	555	All Neg
282	29	512	All Neg
283	30	509	All Neg
284	30	502	All Neg
285	55	545	All Neg
286	30	575	All Neg
287	31	540	All Neg
288	27	541	All Neg
289	28	509	All Neg
290	58	576	All Neg
291	27	504	All Neg
292	33	505	All Neg
293	31	506	All Neg
294	30	537	All Neg
295	30	514	All Neg
296	29	612	All Neg
297	31	511	All Neg
298	22	311	All Neg
299	28	606	All Neg
300	27	544	All Neg
301	28	756	All Neg
302	30	544	All Neg
303	28	542	All Neg
304	29	517	All Neg
305	30	554	All Neg
306	27	578	All Neg
307	33	543	All Neg

308	33	541	All Neg
309	32	532	All Neg
310	30	580	All Neg
311	29	543	All Neg
312	28	602	All Neg
313	27	505	All Neg
314	30	511	All Neg
315	28	534	All Neg
316	30	551	All Neg
317	28	569	All Neg
318	28	570	All Neg
319	27	602	All Neg
320	27	505	All Neg
321	28	556	All Neg
322	30	557	All Neg
323	29	558	All Neg
324	30	559	All Neg
325	32	560	All Neg
326	30	561	All Neg
327	27	562	All Neg
328	28	568	All Neg
329	30	569	All Neg
330	29	555	All Neg
331	30	556	All Neg
332	32	572	All Neg
333	30	508	All Neg
334	33	532	All Neg

335	32	544	All Neg
336	30	567	All Neg
337	29	532	All Neg
338	28	523	All Neg
339	27	545	All Neg
340	28	534	All Neg
341	28	536	All Neg
342	30	547	All Neg
343	28	568	All Neg
344	28	597	All Neg
345	27	589	All Neg
346	33	578	All Neg
347	31	528	All Neg
348	29	553	All Neg
349	30	543	All Neg
350	28	504	All Neg
351	27	590	All Neg
352	32	540	All Neg
353	31	542	All Neg
354	29	534	All Neg
355	30	512	All Neg
356	28	532	All Neg
357	30	543	All Neg
358	29	503	All Neg
359	30	522	All Neg
360	27	544	All Neg
361	30	587	All Neg

362	33	598	All Neg
363	32	512	All Neg
364	10	512	All Neg
365	30	572	All Neg
366	33	363	All Neg
367	30	334	All Neg
368	27	378	All Neg
369	12	563	All Neg
370	31	580	All Neg
371	30	543	All Neg
372	28	506	All Neg
373	33	512	All Neg
374	33	588	All Neg
375	33	532	All Neg
376	30	536	All Neg
377	28	567	All Neg
378	28	590	All Neg
379	32	521	All Neg
380	31	546	All Neg
381	27	544	All Neg
382	30	587	All Neg
383	28	590	All Neg
384	59	521	All Neg
385	60	534	All Neg
386	30	533	All Neg
387	27	512	All Neg
388	33	543	All Neg

389	33	566	All Neg
390	30	589	All Neg
391	28	534	All Neg
392	28	589	All Neg
393	36	190	All Neg
394	36	137	All Neg
395	38	267	All Neg
396	34	256	All Neg
397	36	289	All Neg
398	38	290	All Neg
399	34	212	All Neg
400	38	278	All Neg
401	36	346	All Neg
402	38	321	All Neg
403	38	338	All Neg
404	36	374	All Neg
405	34	467	All Neg
406	38	486	All Neg
407	40	414	All Neg
408	35	412	All Neg
409	37	432	All Neg
410	34	488	All Neg
411	38	410	All Neg
412	38	417	All Neg
413	38	486	All Neg
414	40	443	All Neg
415	35	467	All Neg

416	38	457	All Neg
417	34	445	All Neg
418	34	402	All Neg
419	35	432	All Neg
420	36	412	All Neg
421	37	444	All Neg
422	37	540	All Neg
423	39	626	All Neg
424	40	598	All Neg
425	34	532	All Neg
426	34	512	All Neg
427	38	564	All Neg
428	34	589	All Neg
429	38	555	All Neg
430	34	534	All Neg
431	40	512	All Neg
432	34	534	All Neg
433	34	543	All Neg
434	38	548	All Neg
435	37	600	All Neg
436	60	590	All Neg
437	29	566	All Neg
438	40	522	All Neg
439	34	576	All Neg
440	36	508	All Neg
441	36	606	All Neg
442	34	544	All Neg

443	34	501	All Neg
444	40	523	All Neg
445	40	543	All Neg
446	35	504	All Neg
447	35	587	All Neg
448	44	114	All Neg
449	47	178	All Neg
450	42	143	All Neg
451	44	109	All Neg
452	47	145	All Neg
453	43	167	All Neg
454	44	187	All Neg
455	42	134	All Neg
456	46	123	All Neg
457	42	176	All Neg
458	41	153	All Neg
459	42	208	All Neg
460	47	254	All Neg
461	44	298	All Neg
462	44	212	All Neg
463	46	278	All Neg
464	47	330	All Neg
465	43	345	All Neg
466	44	378	All Neg
467	47	342	All Neg
468	45	367	All Neg
469	48	444	All Neg

470	44	456	All Neg
471	47	478	All Neg
472	46	456	All Neg
473	44	489	All Neg
474	47	409	All Neg
475	47	535	All Neg
476	44	509	All Neg
477	41	556	All Neg
478	42	520	All Neg
479	43	579	All Neg
480	44	599	All Neg
481	45	556	All Neg
482	46	520	All Neg
483	47	579	All Neg
484	44	552	All Neg
485	47	520	All Neg
486	44	563	All Neg
487	44	544	All Neg
488	47	521	All Neg
489	44	521	All Neg
490	45	587	All Neg
491	41	566	All Neg
492	47	511	All Neg
493	45	509	All Neg
494	46	567	All Neg
495	44	523	All Neg
496	46	633	All Neg

497	46	564	All Neg
498	43	589	All Neg
499	45	590	All Neg
500	47	554	All Neg
501	41	502	All Neg
502	44	543	All Neg
503	42	567	All Neg
504	44	534	All Neg
505	45	523	All Neg
506	46	533	All Neg
507	47	567	All Neg
508	47	587	All Neg
509	43	584	All Neg
510	44	522	All Neg
511	45	512	All Neg
512	41	521	All Neg
513	47	552	All Neg
514	45	548	All Neg
515	43	578	All Neg
516	44	514	All Neg
517	46	576	All Neg
518	42	589	All Neg
519	48	267	All Neg
520	50	330	All Neg
521	49	444	All Neg
522	54	511	All Neg
523	50	556	All Neg

524	54	590	All Neg
525	48	582	All Neg
526	48	512	All Neg
527	48	535	All Neg
528	54	515	All Neg
529	53	531	All Neg
530	27	542	All Neg
531	54	582	All Neg
532	29	573	All Neg
533	33	574	All Neg
534	50	542	All Neg
535	51	530	All Neg
536	49	579	All Neg
537	54	552	All Neg
538	50	507	All Neg
539	3	538	All Neg
540	48	507	All Neg
541	54	533	All Neg
542	48	548	All Neg
543	48	543	All Neg
544	54	523	All Neg
545	50	578	All Neg
546	51	529	All Neg
547	54	588	All Neg
548	53	508	All Neg
549	55	128	All Neg
550	56	122	All Neg

551	55	167	All Neg
552	58	189	All Neg
553	34	528	All Neg
554	34	504	All Neg
555	57	509	All Neg
556	29	378	All Neg
557	48	581	All Neg
558	30	321	All Neg
559	63	329	All Neg
560	19	532	All Neg
561	19	516	All Neg
562	15	498	All Neg
563	58	448	All Neg
564	55	532	All Neg
565	30	543	All Neg
566	55	515	All Neg
567	28	334	All Neg
568	48	567	All Neg
569	5	511	All Neg
570	49	525	All Neg
571	54	546	All Neg
572	56	572	All Neg
Average	31.9	460	

APPENDIX 23: CD4+dx COUNT OF ALL FEMALES WHO PARTICIPATED IN THE STUDY. (Cells /μl)

AGE	CD4+dx	Result
28	378	HIV-1
20	198	HIV-1
20	586	HIV-1
26	285	HIV-1
28	395	HIV-1
24	177	HIV-1
16	384	HIV-1
13	389	HIV-1
20	365	HIV-1
17	199	HIV-1
22	393	HIV-1
33	272	HIV-1
25	262	HIV-1
21	273	HIV-1
26	256	HIV-1
26	190	HIV-1
28	180	HIV-1
27	384	HIV-1
23	189	HIV-1
28	483	HIV-1
32	334	HIV-1
26	286	HIV-1
16	352	HIV-1
26	375	HIV-1

31	321	HIV-1
28	154	HIV-1
28	170	HIV-1
30	588	HIV-1
30	190	HIV-1
3	183	HIV-1
20	132	HIV-1
42	98	HIV-1
28	185	HIV-1
32	168	HIV-1
28	178	HIV-1
28	187	HIV-1
33	368	HIV-1
30	179	HIV-1
29	450	HIV-1
22	579	HIV-1
29	291	HIV-1
38	181	HIV-1
32	173	HIV-1
28	358	HIV-1
28	366	HIV-1
27	198	HIV-1
31	198	HIV-1
33	357	HIV-1
28	198	HIV-1
33	198	HIV-1
35	475	HIV-1

27	190	HIV-2
31	564	HIV-2
27	158	HIV-2
20	280	HIV-2
32	165	HIV-2
30	190	HIV-2
22	169	HIV-2
36	425	HIV-2
32	367	HIV-2
28	551	HIV-2
27	580	HIV-2
28	176	HIV-2
32	223	HIV-2
33	140	HIV-2
20	547	HIV-2
24	189	HIV-2
40	564	HIV-2
27	255	HIV-2
34	554	HIV-2
27	280	HIV-D
20	168	HIV-D
27	474	HIV-D
34	187	HIV-D
14	168	HIV-D
23	277	HIV-D
29	198	HIV-D
45	606	HCV

26	542	HCV
38	410	HCV
24	522	HCV
44	564	HCV
20	521	HCV
22	506	HCV
26	542	All Neg
38	410	All Neg
24	522	All Neg
44	564	All Neg
3	216	All Neg
3	506	All Neg
5	511	All Neg
3	552	All Neg
9	421	All Neg
11	543	All Neg
9	523	All Neg
9	556	All Neg
9	589	All Neg
9	509	All Neg
10	517	All Neg
12	509	All Neg
12	534	All Neg
12	504	All Neg
10	543	All Neg
16	108	All Neg
16	202	All Neg

18	423	All Neg
16	421	All Neg
17	226	All Neg
18	523	All Neg
13	528	All Neg
14	532	All Neg
16	218	All Neg
17	508	All Neg
19	345	All Neg
17	312	All Neg
15	589	All Neg
16	534	All Neg
19	416	All Neg
18	336	All Neg
16	544	All Neg
18	307	All Neg
18	567	All Neg
19	551	All Neg
14	251	All Neg
13	432	All Neg
19	421	All Neg
17	512	All Neg
13	534	All Neg
18	213	All Neg
19	589	All Neg
19	516	All Neg
15	564	All Neg

16	529	All Neg
17	534	All Neg
19	309	All Neg
16	413	All Neg
17	456	All Neg
19	432	All Neg
17	401	All Neg
14	478	All Neg
16	448	All Neg
18	456	All Neg
19	432	All Neg
19	456	All Neg
19	432	All Neg
19	421	All Neg
17	449	All Neg
18	514	All Neg
19	589	All Neg
19	543	All Neg
17	534	All Neg
13	523	All Neg
19	437	All Neg
17	445	All Neg
17	440	All Neg
13	521	All Neg
19	423	All Neg
16	518	All Neg
18	508	All Neg

18	453	All Neg
17	543	All Neg
16	446	All Neg
16	543	All Neg
19	432	All Neg
17	564	All Neg
18	424	All Neg
18	543	All Neg
16	543	All Neg
19	412	All Neg
19	509	All Neg
17	587	All Neg
16	567	All Neg
18	478	All Neg
19	486	All Neg
19	578	All Neg
15	412	All Neg
18	428	All Neg
19	423	All Neg
19	576	All Neg
16	513	All Neg
19	321	All Neg
14	567	All Neg
18	509	All Neg
17	329	All Neg
17	406	All Neg
16	545	All Neg

16	556	All Neg
16	472	All Neg
18	578	All Neg
18	432	All Neg
18	523	All Neg
14	543	All Neg
19	411	All Neg
18	567	All Neg
19	513	All Neg
19	543	All Neg
19	556	All Neg
16	433	All Neg
16	590	All Neg
16	506	All Neg
17	534	All Neg
18	543	All Neg
16	454	All Neg
18	227	All Neg
15	456	All Neg
13	589	All Neg
19	587	All Neg
18	543	All Neg
18	414	All Neg
18	515	All Neg
19	543	All Neg
19	512	All Neg
18	534	All Neg

15	328	All Neg
15	567	All Neg
18	565	All Neg
19	238	All Neg
19	543	All Neg
19	567	All Neg
18	422	All Neg
19	565	All Neg
17	543	All Neg
18	523	All Neg
19	543	All Neg
16	543	All Neg
18	545	All Neg
17	312	All Neg
17	411	All Neg
16	534	All Neg
16	509	All Neg
17	208	All Neg
19	534	All Neg
17	543	All Neg
23	523	All Neg
21	267	All Neg
26	545	All Neg
26	409	All Neg
23	545	All Neg
22	554	All Neg
21	567	All Neg

21	517	All Neg
23	522	All Neg
22	250	All Neg
21	487	All Neg
23	545	All Neg
25	534	All Neg
25	467	All Neg
24	551	All Neg
23	545	All Neg
20	403	All Neg
22	514	All Neg
24	245	All Neg
24	504	All Neg
26	509	All Neg
26	543	All Neg
24	521	All Neg
23	217	All Neg
20	467	All Neg
23	518	All Neg
22	245	All Neg
20	354	All Neg
21	478	All Neg
25	224	All Neg
21	564	All Neg
23	545	All Neg
24	567	All Neg
20	278	All Neg

22	202	All Neg
24	367	All Neg
25	345	All Neg
26	389	All Neg
26	424	All Neg
23	443	All Neg
25	412	All Neg
22	554	All Neg
25	456	All Neg
21	367	All Neg
24	516	All Neg
20	309	All Neg
24	323	All Neg
21	345	All Neg
22	555	All Neg
24	578	All Neg
21	545	All Neg
25	345	All Neg
22	534	All Neg
22	567	All Neg
21	321	All Neg
24	589	All Neg
25	554	All Neg
26	478	All Neg
22	536	All Neg
23	567	All Neg
26	321	All Neg

20	523	All Neg
26	332	All Neg
22	550	All Neg
21	503	All Neg
25	412	All Neg
20	508	All Neg
22	232	All Neg
20	445	All Neg
20	434	All Neg
26	512	All Neg
20	534	All Neg
23	512	All Neg
24	534	All Neg
26	487	All Neg
25	411	All Neg
24	567	All Neg
24	589	All Neg
20	234	All Neg
25	527	All Neg
26	546	All Neg
22	348	All Neg
22	389	All Neg
23	434	All Neg
20	555	All Neg
20	509	All Neg
24	267	All Neg
22	467	All Neg

20	507	All Neg
22	551	All Neg
23	434	All Neg
26	423	All Neg
24	312	All Neg
22	523	All Neg
24	545	All Neg
25	566	All Neg
23	501	All Neg
26	509	All Neg
26	412	All Neg
25	467	All Neg
26	456	All Neg
24	478	All Neg
24	498	All Neg
24	409	All Neg
26	456	All Neg
20	109	All Neg
21	156	All Neg
21	460	All Neg
21	445	All Neg
21	412	All Neg
25	467	All Neg
26	409	All Neg
26	565	All Neg
24	367	All Neg
25	509	All Neg

21	498	All Neg
22	256	All Neg
22	524	All Neg
23	576	All Neg
23	544	All Neg
26	223	All Neg
25	449	All Neg
20	445	All Neg
26	434	All Neg
23	412	All Neg
24	423	All Neg
26	453	All Neg
26	467	All Neg
25	454	All Neg
26	478	All Neg
23	367	All Neg
25	289	All Neg
22	545	All Neg
23	523	All Neg
22	545	All Neg
21	556	All Neg
26	389	All Neg
24	409	All Neg
25	434	All Neg
26	423	All Neg
24	234	All Neg
25	523	All Neg

22	378	All Neg
26	543	All Neg
21	332	All Neg
21	589	All Neg
21	523	All Neg
20	545	All Neg
23	567	All Neg
26	578	All Neg
24	507	All Neg
20	445	All Neg
23	467	All Neg
24	476	All Neg
22	354	All Neg
24	531	All Neg
22	578	All Neg
26	554	All Neg
26	523	All Neg
21	556	All Neg
23	498	All Neg
23	443	All Neg
24	420	All Neg
20	456	All Neg
25	545	All Neg
20	408	All Neg
20	543	All Neg
20	523	All Neg
24	558	All Neg

21	456	All Neg
24	478	All Neg
22	321	All Neg
20	518	All Neg
24	542	All Neg
22	365	All Neg
24	367	All Neg
24	422	All Neg
22	509	All Neg
24	545	All Neg
24	232	All Neg
21	309	All Neg
21	589	All Neg
23	509	All Neg
25	512	All Neg
26	534	All Neg
20	562	All Neg
26	512	All Neg
23	250	*All Neg*
22	545	All Neg
26	490	All Neg
21	512	All Neg
21	609	All Neg
20	453	All Neg
22	432	All Neg
21	564	All Neg
20	256	All Neg

21	554	All Neg
24	467	All Neg
21	477	All Neg
24	434	All Neg
24	243	All Neg
25	412	All Neg
26	434	All Neg
23	456	All Neg
20	523	All Neg
20	543	All Neg
21	327	All Neg
20	543	All Neg
20	401	All Neg
23	456	All Neg
24	533	All Neg
20	589	All Neg
20	564	All Neg
22	256	All Neg
23	567	All Neg
24	423	All Neg
24	412	All Neg
23	432	All Neg
21	456	All Neg
23	289	All Neg
26	456	All Neg
22	512	All Neg
26	467	All Neg

24	456	All Neg
22	437	All Neg
25	408	All Neg
26	409	All Neg
24	267	All Neg
23	434	All Neg
26	488	All Neg
20	529	All Neg
25	207	All Neg
22	487	All Neg
24	134	All Neg
25	467	All Neg
25	534	All Neg
22	256	All Neg
24	467	All Neg
25	434	All Neg
21	489	All Neg
26	401	All Neg
24	434	All Neg
22	456	All Neg
24	478	All Neg
25	445	All Neg
26	489	All Neg
25	407	All Neg
23	434	All Neg
23	345	All Neg
25	454	All Neg

26	454	All Neg
26	467	All Neg
26	478	All Neg
26	256	All Neg
23	511	All Neg
29	427	All Neg
32	414	All Neg
23	467	All Neg
25	543	All Neg
26	207	All Neg
22	545	All Neg
25	409	All Neg
21	342	All Neg
22	443	All Neg
23	323	All Neg
26	512	All Neg
24	454	All Neg
21	504	All Neg
23	536	All Neg
20	409	All Neg
21	322	All Neg
23	580	All Neg
21	550	All Neg
24	301	All Neg
21	512	All Neg
20	408	All Neg
26	512	All Neg

21	478	All Neg
30	478	All Neg
30	418	All Neg
26	243	All Neg
24	543	All Neg
23	578	All Neg
26	353	All Neg
21	590	All Neg
25	454	All Neg
24	234	All Neg
26	534	All Neg
22	543	All Neg
27	307	All Neg
32	512	All Neg
32	550	All Neg
29	523	All Neg
29	401	All Neg
32	407	All Neg
30	267	All Neg
32	545	All Neg
33	523	All Neg
28	502	All Neg
31	534	All Neg
32	589	All Neg
32	589	All Neg
27	412	All Neg
27	406	All Neg

27	519	All Neg
32	408	All Neg
33	454	All Neg
27	278	All Neg
32	527	All Neg
24	534	All Neg
25	578	All Neg
32	445	All Neg
30	454	All Neg
26	534	All Neg
23	534	All Neg
27	338	All Neg
31	545	All Neg
30	446	All Neg
28	231	All Neg
30	545	All Neg
33	507	All Neg
30	409	All Neg
30	434	All Neg
31	545	All Neg
28	267	All Neg
27	411	All Neg
27	512	All Neg
29	534	All Neg
30	543	All Neg
33	423	All Neg
29	534	All Neg

32	511	All Neg
29	534	All Neg
30	518	All Neg
30	590	All Neg
27	405	All Neg
27	445	All Neg
30	234	All Neg
28	542	All Neg
28	434	All Neg
32	507	All Neg
32	555	All Neg
31	522	All Neg
29	545	All Neg
29	534	All Neg
29	456	All Neg
30	267	All Neg
33	434	All Neg
31	412	All Neg
29	543	All Neg
32	423	All Neg
32	589	All Neg
30	509	All Neg
32	545	All Neg
31	502	All Neg
32	534	All Neg
33	543	All Neg
32	432	All Neg

29	224	All Neg
29	443	All Neg
32	515	All Neg
29	534	All Neg
28	512	All Neg
29	545	All Neg
27	434	All Neg
29	456	All Neg
33	510	All Neg
32	532	All Neg
29	567	All Neg
33	216	All Neg
33	544	All Neg
31	534	All Neg
27	580	All Neg
31	233	All Neg
27	521	All Neg
27	507	All Neg
33	514	All Neg
33	508	All Neg
33	326	All Neg
28	245	All Neg
32	412	All Neg
32	545	All Neg
31	554	All Neg
31	412	All Neg
32	434	All Neg

33	567	All Neg
29	509	All Neg
32	549	All Neg
32	228	All Neg
32	521	All Neg
29	423	All Neg
31	449	All Neg
32	403	All Neg
27	509	All Neg
29	545	All Neg
30	523	All Neg
29	423	All Neg
27	409	All Neg
28	567	All Neg
27	406	All Neg
28	521	All Neg
29	212	All Neg
32	545	All Neg
30	523	All Neg
29	423	All Neg
31	545	All Neg
27	354	All Neg
33	566	All Neg
31	456	All Neg
32	512	All Neg
29	256	All Neg
30	543	All Neg

31	464	All Neg
33	423	All Neg
32	564	All Neg
29	434	All Neg
32	433	All Neg
31	412	All Neg
31	434	All Neg
33	567	All Neg
27	278	All Neg
31	554	All Neg
32	551	All Neg
32	209	All Neg
28	565	All Neg
29	578	All Neg
28	403	All Neg
27	555	All Neg
30	438	All Neg
33	518	All Neg
27	580	All Neg
31	235	All Neg
29	565	All Neg
27	576	All Neg
28	509	All Neg
30	414	All Neg
31	523	All Neg
27	543	All Neg
29	267	All Neg

30	407	All Neg
29	556	All Neg
33	578	All Neg
33	401	All Neg
30	567	All Neg
33	213	All Neg
32	509	All Neg
28	409	All Neg
27	513	All Neg
29	554	All Neg
33	453	All Neg
30	208	All Neg
31	422	All Neg
29	437	All Neg
32	523	All Neg
34	412	All Neg
34	567	All Neg
36	487	All Neg
39	456	All Neg
36	545	All Neg
39	558	All Neg
34	564	All Neg
34	456	All Neg
37	564	All Neg
34	465	All Neg
35	212	All Neg
38	422	All Neg

40	326	All Neg
39	427	All Neg
35	434	All Neg
40	523	All Neg
34	543	All Neg
35	545	All Neg
40	522	All Neg
34	336	All Neg
36	466	All Neg
37	245	All Neg
37	448	All Neg
34	421	All Neg
34	402	All Neg
35	423	All Neg
37	478	All Neg
38	411	All Neg
36	378	All Neg
40	443	All Neg
36	447	All Neg
37	468	All Neg
39	443	All Neg
35	453	All Neg
34	264	All Neg
34	456	All Neg
37	587	All Neg
38	478	All Neg
39	487	All Neg

34	408	All Neg
34	411	All Neg
38	456	All Neg
36	454	All Neg
36	347	All Neg
36	423	All Neg
39	456	All Neg
40	478	All Neg
40	487	All Neg
36	490	All Neg
36	178	All Neg
34	445	All Neg
34	467	All Neg
36	454	All Neg
37	434	All Neg
38	445	All Neg
34	286	All Neg
34	406	All Neg
37	476	All Neg
38	465	All Neg
38	487	All Neg
34	498	All Neg
36	409	All Neg
37	445	All Neg
38	443	All Neg
39	456	All Neg
35	476	All Neg

36	434	All Neg
34	222	All Neg
36	498	All Neg
38	409	All Neg
35	465	All Neg
36	431	All Neg
37	415	All Neg
34	445	All Neg
35	489	All Neg
34	428	All Neg
34	554	All Neg
34	505	All Neg
38	233	All Neg
38	545	All Neg
34	533	All Neg
39	545	All Neg
38	390	All Neg
40	567	All Neg
36	545	All Neg
36	554	All Neg
36	590	All Neg
40	231	All Neg
35	545	All Neg
34	565	All Neg
34	576	All Neg
36	270	All Neg
38	534	All Neg

34	543	All Neg
34	344	All Neg
34	566	All Neg
37	506	All Neg
36	410	All Neg
38	545	All Neg
35	567	All Neg
35	321	All Neg
34	578	All Neg
34	598	All Neg
36	247	All Neg
37	545	All Neg
36	256	All Neg
45	567	All Neg
34	524	All Neg
35	345	All Neg
40	578	All Neg
34	509	All Neg
35	567	All Neg
36	565	All Neg
42	533	All Neg
41	544	All Neg
46	309	All Neg
46	476	All Neg
45	546	All Neg
43	543	All Neg
44	534	All Neg

43	289	All Neg
42	233	All Neg
45	578	All Neg
44	534	All Neg
41	523	All Neg
46	533	All Neg
42	126	All Neg
44	470	All Neg
42	567	All Neg
47	195	All Neg
42	545	All Neg
45	567	All Neg
44	432	All Neg
42	446	All Neg
45	457	All Neg
44	116	All Neg
47	445	All Neg
44	475	All Neg
45	445	All Neg
46	108	All Neg
38	534	All Neg
39	545	All Neg
46	509	All Neg
42	180	All Neg
44	545	All Neg
43	544	All Neg
44	375	All Neg

43	512	All Neg
42	534	All Neg
43	423	All Neg
45	126	All Neg
42	546	All Neg
42	345	All Neg
44	523	All Neg
42	546	All Neg
41	412	All Neg
45	567	All Neg
46	589	All Neg
43	567	All Neg
45	456	All Neg
46	545	All Neg
42	545	All Neg
45	112	All Neg
45	567	All Neg
45	580	All Neg
44	534	All Neg
47	567	All Neg
45	545	All Neg
41	432	All Neg
41	523	All Neg
48	422	All Neg
45	578	All Neg
44	523	All Neg
46	523	All Neg

44	544	All Neg
45	567	All Neg
42	334	All Neg
44	567	All Neg
48	125	All Neg
48	307	All Neg
50	432	All Neg
52	578	All Neg
48	545	All Neg
49	512	All Neg
53	545	All Neg
50	451	All Neg
54	520	All Neg
52	245	All Neg
52	567	All Neg
50	555	All Neg
50	527	All Neg
50	512	All Neg
48	480	All Neg
48	422	All Neg
52	513	All Neg
48	598	All Neg
49	540	All Neg
49	523	All Neg
48	517	All Neg
51	178	All Neg
48	527	All Neg

48	506	All Neg
48	432	All Neg
50	522	All Neg
48	534	All Neg
51	528	All Neg
53	443	All Neg
49	445	All Neg
48	562	All Neg
49	531	All Neg
50	508	All Neg
48	534	All Neg
48	412	All Neg
48	507	All Neg
50	523	All Neg
49	409	All Neg
49	489	All Neg
51	521	All Neg
48	502	All Neg
48	514	All Neg
53	467	All Neg
54	520	All Neg
48	543	All Neg
48	523	All Neg
49	509	All Neg
48	520	All Neg
57	167	All Neg
55	537	All Neg

62	507	All Neg
55	132	All Neg
58	213	All Neg
61	422	All Neg
55	168	All Neg
56	541	All Neg
49	436	All Neg
55	545	All Neg
55	534	All Neg
56	533	All Neg
56	159	All Neg
55	401	All Neg
55	564	All Neg

AV=28.7 447

Key: CD4+dx= CD4+ count at the diagnosis of HIV or HCV or HIV/HCV status

All Neg-HIV and HCV seronegative

HIV-1=HIV-1
positive

HIV-2=HIV-2
positive

HIV-D=HIV-D
positive

HCV=HCV positive

APPENDIX 24: LINEAR REGRESSION ANALYSIS OF CD4+ TRENDS IN ALL HIV INFECTED MALES AND FEMALES WHO PARTICIPATED FULLY IN THE FOLLOW-UP STUDY(AGE VERSUS COUNTS)

Test: Linear regression

Fit: Age vs CD4+dx, 1st, 2nd and 3rd counts

(CD4 count = cells /μl)

n	19
R^2	0.15
Adjusted R^2	-0.10
SE	8.8425

Term	Coefficient	SE	p	95% CI of Coefficient
Intercept	27.4825	18.0202	0.1495	-11.1669 to 66.1320
CDdx	0.1523	0.2744	0.5876	-0.4362 to 0.7408
1st count	-0.2382	0.3201	0.4691	-0.9249 to 0.4484
2nd count	-0.0387	0.1433	0.7910	-0.3461 to 0.2687
3rd count	0.1458	0.1612	0.3810	-0.1999 to 0.4915

Source of variation	SSq	DF	MSq	F	p
Due to regression	186.496	4	46.624	0.60	0.6712
About regression	1094.662	14	78.190		
Total	1281.158	18			

APPENDIX 25: LINEAR REGRESSION ANALYSIS OF CD4+ TRENDS IN ALL HIV INFECTED MALES AND FEMALES WHO PARTICIPATED FULLY IN THE FOLLOW-UP

Test: Linear regression

Fit: CD4+dx vs 1st, 2nd and 3rd counts

(CD4 count = cells /μl)

n	19

R^2	0.99
Adjusted R^2	0.99
SE	8.3211

Term	Coefficient	SE	p	95% CI of Coefficient	
Intercept	0.2425	16.9575	0.9888	-35.9016	to 36.3866
1st count	1.1063	0.0957	<0.0001	0.9023	to 1.3104
2nd count	0.3134	0.1079	0.0109	0.0834	to 0.5433
3rd count	-0.3665	0.1185	0.0074	-0.6191	to -0.1139

Source of variation	SSq	DF	MSq	F	p
Due to regression	109260.541	3	36420.180	525.99	<0.0001
About regression	1038.617	15	69.241		
Total	110299.158	18			

APPENDICX 26: LINEAR REGRESSION ANALYSIS OF CD4+ TRENDS IN HCV INFECTED MALES AND FEMALES WHO PARTICIPATED FULLY IN THE FOLLOW-UP

Test: Linear regression

Fit: Age vs CD4+dx, 1st, 2nd and 3rd counts

(CD4 count = cells /μl)

n	17

R^2	0.39
Adjusted R^2	0.18
SE	9.2233

Term	Coefficient	SE	p	95% CI of Coefficient	
Intercept	60.7954	31.6127	0.0785	-8.0828	to 129.6736
CDdx	-0.0194	0.0457	0.6795	-0.1190	to 0.0803
1st count	-0.1863	0.0838	0.0463	-0.3690	to -0.0036
2nd count	0.2901	0.1262	0.0403	0.0152	to 0.5650
3rd count	-0.1475	0.1123	0.2134	-0.3922	to 0.0971

Source of variation	SSq	DF	MSq	F	p
Due to regression	641.058	4	160.265	1.88	0.1780
About regression	1020.824	12	85.069		
Total	1661.882	16			

APPENDICX 27: LINEAR REGRESSION ANALYSIS OF CD4+ TRENDS IN HCV INFECTED MALES AND FEMALES WHO PARTICIPATED FULLY IN THE FOLLOW-

Test: Linear regression

Fit:CD4+dx vs 1st, 2nd and 3rd counts

(CD4 count = cells /μl)

n	17

R^2	0.25
Adjusted R^2	0.08
SE	55.9192

Term	Coefficient	SE	p	95% CI of Coefficient	
Intercept	244.8347	179.2305	0.1951	-142.3693	to 632.0387
1st count	0.2042	0.5051	0.6926	-0.8870	to 1.2955
2nd count	0.6517	0.7433	0.3965	-0.9541	to 2.2575
3rd count	-0.3417	0.6741	0.6207	-1.7980	to 1.1147

Source of variation	SSq	DF	MSq	F	p
Due to regression	13594.513	3	4531.504	1.45	0.2739
About regression	40650.428	13	3126.956		
Total	54244.941	16			

APPENDIX 28: LINEAR REGRESSION ANALYSIS OF CD4+ TRENDS IN ALL HIV AND HCV SERONEGATIVE MALE CONTROL SUBJECTS WHO PARTICIPATED FULLY IN THE FOLLOW-UP STUDY

Test: Linear regression

Fit: Age vs CD4+dx, 1^{st}, 2^{nd} and 3 rd counts

(CD4 count = cells /μl)

n	60

R^2	0.03
Adjusted R^2	-0.04
SE	13.3231

Term	Coefficient	SE	p	95% CI of Coefficient	
Intercept	10.7203	21.6491	0.6224	-32.6654	to 54.1060
CDdx	-0.0619	0.0806	0.4458	-0.2234	to 0.0996
1st count	-0.0239	0.0982	0.8085	-0.2206	to 0.1728
2nd count	0.0907	0.1369	0.5101	-0.1835	to 0.3650
3rd count	0.0416	0.1131	0.7145	-0.1851	to 0.2683

Source of variation	SSq	DF	MSq	F	p
Due to regression	326.117	4	81.529	0.46	0.7652
About regression	9762.733	55	177.504		
Total	10088.850	59			

APPENDIX 29: LINEAR REGRESSION ANALYSIS OF CD4+ TRENDS IN ALL HIV AND HCV SERONEGATIVE FEMALECONTROL SUBJECTS WHO PARTICIPATED FULLY IN THE FOLLOW-UP

Test: Linear regression

Fit: Age vs CD4+dx, 1st, 2nd and 3rd counts

(CD4 count = cells /μl)

n	214

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ORIGINAL

R^2	0.06
Adjusted R^2	0.04
SE	4.3999

Term	Coefficient	SE	p	95% CI of Coefficient	
Intercept	19.6605	3.0163	<0.0001	13.7143	to 25.6067
CDdx	0.0133	0.0111	0.2304	-0.0085	to 0.0352
1st count	0.0032	0.0116	0.7839	-0.0197	to 0.0260
2nd count	0.0053	0.0104	0.6135	-0.0153	to 0.0259
3rd count	-0.0036	0.0099	0.7149	-0.0231	to 0.0159

Source of variation	SSq	DF	MSq	F	p
Due to regression	242.773	4	60.693	3.14	0.0157
About regression	4046.092	209	19.359		
Total	4288.864	213			

APPENDIX 30: LINEAR REGRESSION ANALYSIS OF CD4+ TRENDS IN ALL HIV ANI HCV SERONEGATIVE FEMALE CONTROL SUBJECTS WHO PARTICIPATED FULL IN THE FOLLOW-UP STUDY

Test: Linear regression

Fit: CD4+dx vs 1st, 2nd and 3rd counts

(CD4 count = cells /μl)

n	214
R^2	0.78
Adjusted R^2	0.78
SE	27.3943

Term	Coefficient	SE	p	95% CI of Coefficient	
Intercept	14.8356	18.7516	0.4297	-22.1299	to 51.8010
1st count	0.5867	0.0597	<0.0001	0.4689	to 0.7044
2nd count	0.2141	0.0633	0.0009	0.0893	to 0.3388
3rd count	0.1657	0.0604	0.0066	0.0466	to 0.2847

Source of variation	SSq	DF	MSq	F	p
Due to regression	560209.121	3	186736.374	248.83	<0.0001
About regression	157593.556	210	750.446		
Total	717802.678	213			

APPENDIX 31: LINEAR REGRESSION ANALYSIS OF CD4+ TRENDS IN HCV INFECTED MALES AND WHO PARTICIPATED FULLY IN THE FOLLOW-UP STUDY

Test: Linear regression

Fit: Age vs CD4+dx, 1st, 2nd and 3rd counts

(CD4 count = cells /μl)

n	12
R^2	0.56

Adjusted R^2	0.31				
SE	7.8906				
Term	Coefficient	SE	p	95% CI of Coefficient	
Intercept	92.0415	31.2441	0.0215	18.1610	to 165.9220
CDdx	-0.4312	0.4037	0.3209	-1.3858	to 0.5234
1st count	-0.1390	0.3621	0.7125	-0.9951	to 0.7172
2nd count	0.3793	0.3973	0.3715	-0.5602	to 1.3188
3rd count	0.1958	0.3132	0.5517	-0.5449	to 0.9365
Source of variation	SSq	DF	MSq	F	p
Due to regression	561.169	4	140.292	2.25	0.1640
About regression	435.831	7	62.262		
Total	997.000	11			

APPENDIX 32: PUBLICATIONS ARISING FROM THIS THESIS

Peer reviewed journals

Mboto CI, Davies AJ, Fielder M, Jewell, AP. **2006** CD4+ lymphocyte values and trends in individuals infected with Human Immunedeficency Virus and or co-infected with Hepatitis C Virus in the Gambia. *In preparation.*

Mboto CI, Davies AJ, Fielder M, Jewell, AP. **2006** Distribution of Hepatitis C virus serotypes in HIV and non-HIV-infected persons in The Gambia. *East African Medical Journal.* Submitted

Mboto CI, Davies AJ, Fielder M, Jewell, AP. **2006** Co-infection with HIV and Hepatitis C in The Gambia. *West African Journal of Medicine* in press

Mboto CI, Davies AJ, Fielder M, Jewell, AP. **2005** Prevalence and risk factors for hepatitis C antibodies in asymptomatic first time blood donors in The Gambia. *British Journal of Biomedical Science* ***62,*** 89-91.

Mboto CI, Davies AJ, Fielder M, Jewell, AP. **2005** Hepatitis B and Hepatitis C virus infection in patients with Hepatocellular Carcinoma in The Gambia. *Internal Seminars in Surgical Oncology* **4,** 20

Abstracts submitted

Mboto CI, Davies AJ, Fielder M, Jewell, AP. **2006** Socio-demographic and risk factors associated with viral hepatitis among first time blood donors in The Gambia. *International Society for Blood Transfusion, Cape Town*

Mboto CI, Davies AJ, Fielder M, Jewell, AP. **2005** Prevalence and risk factors for Hepatitis C antibodies in asymptomatic first time blood donors in the Gambia. *European Association for the Study of the Liver, Paris*

Mboto CI, Davies AJ, Fielder M, Jewell, AP. **2005 Co**-infection with HIV and Hepatitis C in The Gambia. *European Association for the Study of the Liver, Paris*

Mboto CI, Davies AJ, Fielder M, Jewell, AP. **2005** Human immunodeficiency virus and Hepatitis C co-infection in sub-Saharan West Africa. *European Association for the Study of the Liver, Paris*

Mboto CI, Davies AJ, Fielder M, Jewell, AP. **2005** Hepatitis B and Hepatitis C virus infection in patients with Hepatocellular Carcinoma in The Gambia. *European Association for the Study of the Liver, Paris*

Mboto CI, Davies AJ, Fielder M, Jewell, AP. **2005** Human immunodeficiency virus and Hepatitis C co-infection in Western Saharan Africa: An emerging epidemic. *Association of Medical Laboratory Scientists of Nigeria. Kano*

Mboto CI, Davies AJ, Fielder M, Jewell, AP. **2005** Viral Hepatitis associated with first time blood donors in The Gambia. *Association of Medical Laboratory Scientists of Nigeria. Kano*

Book Chapters

Mboto CI, Jewell AP **2006** Health care in The Gambia. Pocket Guide To Cultural Health Assessment 3rd edition. **Mosby**