HUMAN IMMUNODEFICIENCY VIRUS AMONG ELDERLY MEDICAL PATIENTS AT THE KORLE BU TEACHING HOSPITAL, ACCRA, GHANA

THIS THESIS IS SUBMITTED TO THE UNIVERSITY OF GHANA, LEGON

BY

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DECLARATION

I do hereby declare that except for references to the work of other investigators which I duly acknowledge, the work presented in this thesis is original. It was carried out by me under the guidance of Prof. Andrew A. Adjei and Professor Robert K. Gyasi (Pathology Department, University of Ghana Medical School of the College of Health Sciences) and Dr Francis Ofei (Department of Medicine, Korle Bu Teaching Hospital). No part of this thesis has been previously submitted for a degree or any other qualification.

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DEDICATION

I dedicate this work to the memory of Dr Dr Daniel Osei

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LIST OF ABBREVIATIONS

AIDS	Acquired immunodeficiency syndrome
ART	Antiretroviral Therapy
CCR5	Chemokine Receptor type 5
CD4	T4 Helper HIV receptor cells
CDC	Centre for Disease Control
cDNA	Complementary Deoxyribose Nucleic Acid
CSW	Commercial Sex Workers
CXCR4	Chemokine Receptor type 4
DNA	Deoxyribose Nucleic Acid
ELISA	Enzyme-Linked Immunosorbent Assay
ECLIA	Electrochemilluminiscence Assay
EIA	Enzyme Immunoassay
GHS	Ghana Health Service
HAART	Highly Active Antiretroviral Therapy
HEV	High Endothelial Venules
HIV	Human Immunodeficiency Virus
HIVAN	HIV-Associated Nephropathy
IDU	Intravenous Drug Use
KBTH	Korle Bu Teaching Hospital
MSM	Men-having-Sex-with-Men

NACP	National AIDS/STI Control Program
NGO	Non-Governmental Organization
NNRTIs	Non-Nucleoside Reverse Transcriptase Inhibitors
NRTI	Nucleoside Reverse Transcriptase Inhibitors
PCR	Polymerase Chain Reaction
PIs	Protease Inhibitors
QS	Quantitation Standard
RNA	Ribose Nucleic Acid
SIV	Simian Immunodeficiency Virus
SME	Surgical Medical Emergency
STD	Sexually Transmitted Diseases
STI	Sexually Transmitted Infections
TREC	T Cell Receptor Excision Circles
UNAIDS	United Nations Program on HIV/AIDS
WHO	World Health Organization.

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ABSTRACT

Human immunodeficiency virus (HIV) has now spread to all parts of the world with rates particularly high in young people (15-49 years) than older people (>50 years). Despite the global attention being paid to the epidemic of infection with HIV, the rates among older people in the sub-Saharan Africa has been a neglected area of study.

This cross-sectional study carried out between the months of November, 2012 and January 2014 sought to determine the prevalence of HIV among elderly patients on admission at the Korle Bu Teaching Hospital and also the correlates of HIV infection among the patients (1,100 patients on admission at the SME and Medical Wards of KBTH). Of the total consenting elderly patients (aged 50 years and above) 60% were females and 40% were males.

The patients voluntarily completed a risk-factor questionnaire and provided blood specimen for HIV testing. The data was analyzed using univariate analysis and the median age of the patients was 63 years (range age 50-100 years). HIV sero-prevalence was 4.18% and the main determinants of HIV in the study population were, homosexuality (OR: 47.86; 95% CI: 4.27-537.91), long distance trading (OR 2.28; 95% CI 0.85-6.12), multiple sexual partners (OR: 1.25 95% CI: 0.69-2-28) and chewing tobacco (OR: 1.57; 95% CI 0.69-3.64). The knowledge and awareness of HIV transmission among the study population was also very low.

Consistent with the few studies worldwide, these results may suggest high prevalence of HIV infections among the elderly population in Ghana.

CHAPTER ONE

INTRODUCTION

1.1 BACKGROUND

Human immunodeficiency virus (HIV) has now spread to all parts of the world and the rates of infection are found to be particularly high in young people (15-49 years) than older people (>50 years) (UNAIDS, 2011, WHO, 2003, WHO/UNAIDS, 2009). Although 50 years is not the usual age threshold indicating elderly people, it remains an advanced age compared with the mean age observed in the early HIV pandemic, and so the Centre for Disease Control (CDC), U.S.A. and UNAIDS still use the age 50 years as a way of distinguishing older patients (CDC, 2006; CDC, 2003). As a result, most of the known epidemiological and clinical features of HIV and acquired immune deficiency syndromes (AIDS) were defined and continued to be defined using children and younger people than older people (UNAIDS, 2011, WHO, 2003, WHO/UNAIDS, 2009, CDC, 2006, CDC, 2003). Subsequently, most of the HIV/AIDS prevention efforts largely target the children and the younger people and little is known about the attitudes towards HIV and awareness of prevention, testing and treatment among older people. The traditional belief that sex is proper or mentioned among young people has become less valid as older people also remain sexually active and therefore remain at risk of HIV infection (Szwabo, 2003). Although sexual activity declined with age, Smith *et al.,* (2007) and Patel *et al.,* (2003) reported increased sexual activity among older people aged 60-94 years. Similar studies observed a wide range of sexual relationships, multiple relationships and relationships with people who are not well known among older people (Gott *et al.,* 2003, Cranston *et al.,* 1998) suggestive that older people remain at risk of HIV infection. In a related study,

Lindau *et al.,* (2006) documented that older women are most often not concerned about pregnancy risk, and as such the use of condoms and other safety methods of acquiring or preventing sexually transmitted infections (STIs) (such as HIV from a new partner are infrequently practiced. Studies from America, Africa and elsewhere have demonstrated that a growing number of people age 50 and older are living with HIV infection (Negin *et al.,* 2010, UNAIDS/WHO, 1999); and that documented risk factor for HIV infection among the elderly is heterosexual intercourse (Grabar *et al.,* 2006).

1.2 PROBLEM STATEMENT

Ghana, one of the growing economies in sub-Saharan Africa, has been experiencing a severe HIV/AIDS epidemic since the first Ghanaian sera found positive for HIV-1 antibodies was detected in 1986 (Mingle *et al.,*1986). Currently, the prevalence of HIV infection in Ghana is 1.37% (NACP, 2012). Sentinel studies conducted by the Ghana AIDS Commission has included most sub-populations thought to be at high-risk for HIV and STIs, but has excluded the elderly (>50 years old) (Szwabo, 2003). Recent reports from Africa and elsewhere have demonstrated that the diagnosis of HIV infection is recently occurring with increasing frequency in older people (CDC, 2006, Negin *et al.,* 2010, UNAIDS/WHO, 1999). The CDC reported that AIDS cases among American adults over 50 years of age quintupled during the last decade (CDC, 2003). Similar studies were reported in France (Lacerda *et al.,* 2008), Australia, Canada, United Kingdom (Nguyen *et al.,* 2008), Brazil (Lacerda *et al.,* 2008), Egypt (El-Sadr *et al.,* 1995), Tanzania (NACP, 1998), Kenya (Catherine *et al., 2009*), and Uganda (UNAIDS/WHO, 1999). Unfortunately, high numbers of older people present late during the course of HIV

infection and that most often older people are misdiagnosed as having age-related illness rather than HIV (Castilla *et al.,* 2002, Skiest *et al.,* 1996).

Undocumented reports from the Department of Medicine and Therapeutics, Korle Bu Teaching Hospital in 2012 indicated a rise in the number of older people (>50 years old) on admission at both the Surgical/Medical Emergency (SME) Ward and the Medical Wards, Department of Medicine, Korle Bu Teaching Hospital (KBTH), Accra, and among the significant findings, age-related death was the leading cause of death within the population. Although several studies have indicated that older patients with HIV infection presented with non-specific problems or signs and symptoms that mimic age-related illnesses (Grabar *et al.,* 2006), the diagnosis of HIV-related immunosuppression was unrecognized or overlooked and therefore the prevalence of HIV/AIDS and/or HIV/AIDS-related death among the elderly medical patients on admission at both Wards was not included in the report. In 1997, the Ministry of Health, Dar es Salaam, reported that AIDS was the fourth commonest cause of death among both males and females aged 60 years and above (Naylor *et al.,* 2005). Mtei and Pallangyo in 2001 observed that the overall sero-prevalence of HIV-infection among the elderly aged >55 years on admission at the Muhimbili Medical Center in Dar es Saalaam was 15%.

In Ghana, HIV infection among older people has largely been ignored over the years with little or no research on HIV-related knowledge and attitudes among the older people. Some of the factors that may explain the lack of interest and the late diagnosis of HIV infection in the older people include less common routine screening, failure of physicians to consider the possibility of HIV infection in the elderly, and confusion between symptoms of opportunistic infections and those of frequent co-morbid conditions associated with ageing.

Policies and programs targeting the elderly in most sub-Saharan African countries seem missing and the goals of most health policies are towards the traditionally vulnerable, women and children. Besides, older people are usually excluded from HIV studies conducted by the Ghana AIDS Commission. Consequently, there is no controlled data on epidemiological and clinical features of HIV on this age group.

For financial reasons, population surveys cannot be conducted, and therefore sentinel studies are the only means for providing information regarding the transmission of infections such as HIV among older people as well as monitoring the changes over time. This study therefore investigates the prevalence of HIV and risk factors among older people on admission at the SME and Medical Wards at KBTH, Accra, Ghana.

1.3 OBJECTIVES

The specific objectives of the proposed project are:

1. To determine the prevalence of HIV among the older people on admission at the Medical Wards and SME Wards of the KBTH.
2. To define the risk factors associated with the transmission of HIV among the elderly.
3. To determine the immune status of elderly patients using CD4/CD8 profiles
4. To collect and collate useful epidemiological data which will serve as a future reference for health and research purposes on HIV/AIDS risk factors among the elderly.

1.4 JUSTIFICATION

In developed countries, various measures have been put in place to address specific issues relating to HIV infection in patients aged 50 and above. This has become necessary because several reports suggest that HIV-infected elderly subjects are an emerging patient category in the fight against HIV/AIDS pandemic (Doueck *et al.*, 1998, Ye *et al.*, 2004). With the introduction of highly active antiretroviral therapy (HAART) and anti-HIV therapeutic strategies, survival following HIV diagnosis has increased dramatically among HIV-infected patients. However, in Africa, very little or no such measures have been put in place to address specific issues relating to HIV infection in patients over 50 years of age. In Ghana, very little is known about the HIV status of the elderly particularly those on admission at the various district and regional hospitals throughout the country. Therefore, screening and investigating HIV-related knowledge and attitudes among patients on admission over the age of 50 years is important for a number of reasons. Older people remain sexually active, mostly play the role as caregivers, and involved in taking care of sick young adults and children and therefore remain at risk of HIV infection or act as bridge populations who may spread the infections to their dependents. Moreover, older people play critical roles as educators in the Ghanaian setting and remain influential community members and leaders. Yet, very little research has been done on HIV-related knowledge and attitudes among older people in Ghana. The study proposal aims at a better understanding of the management, treatment, prevention, attitudes and behavior, and risk of HIV among elderly medical patients. The relation between the elderly and the high transmission of HIV is an interesting field of research that has not been explored. Research into this area in more detail would provide useful information and aid in identifying older

people who are carriers or sero-positives of HIV. The in-depth study of HIV-infected elderly medical patients may provide a better understanding of the risk factors for the transmission of HIV in the country. Any changes in the risk factor profile of older people will be known and thus campaigns targeting older people with HIV would be intensified. This may lead to further description of the older population as high, medium or low risk population; and thus the information gathered will help their health care givers, particularly clinicians/physicians that will ensure timely and early diagnosis of HIV infection and ensure antiretroviral initiation. Ultimately, health professionals and the general population will become more aware of the risks of HIV associated with the elderly, it is possible that the risk factor profile of older people will change and that campaigns targeting older people against HIV would be intensified.

PLANNED USE OF EXISTING RESEARCH DATA IF APPROPRIATE

Currently, no research data exist in Ghana on the prevalence of HIV among the elderly to the best of my knowledge. It is hoped that the results of this study would act as the basis for setting up systems and put in place measures to monitor and control the spread of HIV/STIs among the elderly. Various institutions including the Ghana AIDS Commission, Ghana Health Service, Ministry of Health and Non-governmental Organisations (NGO's) may use the results in their daily activities. Data from this study would also form the basis for vigorous advocacy and hopefully policy formulation to include the elderly in the epidemiological and clinical features of HIV and AIDS. It would also galvanize policy on counseling and screening/testing for HIV among the elderly so that the appropriate

preventative measures would be instituted to prevent the onward transmission by those with the infection to families and sexual partners. Indeed, this study would provide information that would be invaluable to health planners and policy makers in Ghana.

BENEFICIARIES/PLAN FOR UTILIZATION OF THE STUDY RESULTS

The beneficiaries of the study will be primarily the Ghana AIDS Commission, Clinicians and Consultants, Ghana Health Service, the Ministry of Health, Non-governmental organizations, policy makers and the Ghanaian population at large.. The citizens of Ghana will benefit by having more accurate information on the attitudes towards HIV and awareness of prevention, testing and treatment among the elderly.

CHAPTER TWO

LITERATURE REVIEW

2.1 The Human Immunodeficiency Virus (HIV)

An epidemic of unexplained cases of immunodeficiency reported in the western world during the early years of 1980 led to the discovery of HIV. Sexual or blood contact or both were the suggested routes of transmission and in 1984, a retrovirus known as the Human Immunodeficiency Virus (HIV) was confirmed as the causative agent. (Sasadeusz *et al.*, 2008, Weiss, 2008).

2.1.1 Origin of HIV

Lentiviruses have been associated with a disease process called simian AIDS in many primate species. These primate lentiviruses are similar to HIV but unlike HIV, the viral genome is not integrated into the chromosomal DNA (Emerman *et al.,* 1998).

Molecular epidemiologic data suggest that HIV type 1 (HIV-1), the most common subtype of HIV that infects humans, was derived from the simian immunodeficiency virus, called SIVcpz, of the *Pan troglodytes troglodytes* subspecies of chimpanzee. The lentivirus strain SIVcpz is highly homologous with HIV-1, and another form of simian immunodeficiency virus found in *sooty mangabeys* (SIVsm) has molecular similarities and likely generated HIV-2 *(*Heeney *et al., 2006).*

There are four main subtypes of HIV-1 groups; M, N, O, and P. Group M is the pandemic form of HIV-1 that has spread widely to infect millions of persons worldwide. There is

molecular epidemiologic evidence for multiple cross-species transmissions of SIVcpz to humans occurring in the first half of the 20th century to establish group M. Based on the biology of these retroviruses, transmission to humans likely occurred through cutaneous or mucous membrane exposure to infected primate blood and/or body fluids. Such exposures occur most commonly in the context of hunting. Group O was discovered in 1990 and represents less than 1% of global HIV-1 infections; it is mainly in Cameroon, Gabon, and neighboring. Group N was identified in 1998 and so far, only 13 cases have been documented, all in persons living in Cameroon. Group P was discovered in 2009 in two persons from Cameroon *(*Sharp *et al.,* 2011*).*

One additional major human retrovirus, called HIV-2, has more similarity to simian immunodeficiency virus (SIV) than to HIV-1. HIV-2 is mostly found in West Africa, with its highest prevalence rates recorded in Guinea-Bissau and Senegal *(*Sharp *et al.,* 2011*).*

Zoonotic infection of humans may have occurred long in the past, but only in the late 20th century did demographic and social conditions change significantly to permit HIV to spread more rapidly. Zoonotic infection of man with retroviruses is possible, as documented by infection of primate handlers with simian foamy retroviruses *(*Heneine *et al.,* 1998*).*

Retrospective studies performed on frozen sera have shown evidence for HIV in patients in Africa prior to 1960.(Mack *et al.,* 2003) Reports in the early 1980's referred to the agent causing AIDS as either human T- lymphocytotropic virus, type III (HTLV-III) or as lymphadenopathy associated virus (LAV) *(* Levy *et al., 2009).*

2.0.2 General HIV Disease Process

Human immunodeficiency virus is an enveloped retrovirus belonging to the lentiviridae. HIV transmission occurs when the virus enters the blood stream by direct contact or penetration of mucosal surfaces. HIV infection begins with its attachment to CD4 and other co-receptors such as CCR5 and CXCR4 on the surface of the cell. Once it gains access to the cell, viral RNA is transcribed to DNA by the HIV reverse transcriptase. Viral DNA then integrates into the host cell genome and uses host cell machinery to produce viral progeny. HIV specifically targets and infects CD4+ cells, which includes T-helper lymphocytes and other mononuclear cells, ultimately leading to the destruction of these important mediators of the immune system (Ivan*et al.*, 2006).

2.1.3 Aging and HIV Infection on the Immune System

The thymus in mammals is bilobed organ in the thoracic cavity overlying the heart and major blood vessels (Ivan *et al.*, 2006)**.** Each lobe is organized into lobules separated from each other by connective tissue trabeculae. The main blood vessels that regulate cell traffic in the thymus are high endothelial venules (HEVs) at the corticomedullary junction on the thymic lobules. It is through these veins that T cell progenitors formed in the fetal liver and bone marrow enter the epithelial anlage and migrate towards the cortex of the thymus.

T cell education (differentiation and proliferation of T cell progenitors) processes take place in the cortex of the thymus that lead to the formation and generation of mature T cells through a corticomedullary gradient of migration.

The thymus is, therefore, an important organ involved in the human immune system development and serves as the primary location for T lymphocyte maturation. The genetic diversity of T lymphocytes is recognized in its function as naïve T cells responding to new antigenic exposures or as memory T cells responding to antigens to which the body has previously been exposed to (Nguyen *et al.,* 2008).

In particular, the activation of CD4+ T-helper cells triggers an immune response through T cell differentiation and proliferation; activation of B cells resulting in antibody development and secretion; stimulation of other effector cells, such as CD8+ cytotoxic T cells and macrophages, through cytokine release; and/or delayed-type hypersensitivity.

The increasing ratio of ageing population poses new challenges to healthcare systems. The elderly seldom suffer from severe illnesses. Vaccination could offer some protection against some of these infections, but can be very effective if cells and molecules of the immune system are still in the repertoire. Immunosenescence affect both the innate and the adaptive immunity.

As an individual ages, involution of the thymus occurs, and resultant thymic volumes are significantly lower in persons 45 years and older as compared to younger persons. (Kaleyjian *et al.,* 2003) Moreover, the production of naïve T cells declines with increasing age and thymic output is only minimal after age 55 (Naylor *et al.,* 2005). Increased age is further associated with diminished T cell functionality, reduced memory T cell populations, and fewer numbers of properly functioning CD8+ cytotoxic T cells (Effros, 2004). These and many other factors could account for the reasons why elderly persons are more prone to new and opportunistic infections, exhibit less than optimal immune response to

immunizations, or manifest senescence to skin tests such as with purified protein derivative (PPD) (Effros, 2004). Changes, such as inhibition of thymic function and naïve T cells production caused by HIV infection in the immune system resembles ageing effects. This renders the progression of HIV infection in the elderly more pronounced (Douek *et al.*, 1998). There is now unequivocal evidence of both thymic infection and the disruption of thymopoiesis by HIV-1. Clinical and morphologic studies of HIV-infected children and adults indicate that the thymus is affected by HIV.

In vitro information of thymic organ culture, thymic epithelial cell culture, the SCID-hu mouse system and SHIV infection of primates have supported HIV-induced thymic damage. The mechanisms underlying this could be many, including direct thymocyte killing by the virus, apoptosis, or disruption of thymic stromal architecture. T cell receptor excision circles (TREC) have been developed as a marker of new thymic emigrants. Decreases in TREC concentrations have been found in both HIV-infected pediatric and older patients. Mathematical models have suggested that thymic infection in children is more severe than in adults, particularly during infection with strains that use CXCR4 as coreceptor. However, thymic infection is more severe in adults than younger patients particularly during infection with strains that use CCR5 as a coreceptor. Research has shown that thymic recovery may be achieved in some patients as a result of potent antiretroviral therapy. Extensive thymic damage may, however, hamper immune reconstitution (Ye *et al.*, 2004).

CD4 cell counts do not only show low significant levels in HIV-infected young and older adults as compared to their aged-matched controls, but HIV-infected elderly show the lowest counts according to Kalayjian *et al.*,2003. Also, in both HIV-infected and non-HIV-

infected older persons, the functional $CD8^+$ T cells was lower compared to the younger subjects, but the impact was most among HIV-infected older subjects. Since CD8+ cytotoxic T cells are important in the containment of HIV replication, reduction in their number and functionality associated with HIV infection and aging, coupled with loss of CD4 cells, may explain the accelerated progression of HIV infection in older adults.

Finally, as a person ages, there are changes in T cell receptor expression such as increased expression of the CCR5 co-receptor, which is plays an important role in HIV infection pathogenesis could greatly influence disease progression (Kaleyjian *et al.*, 2003, Yung *et al.*, 2003). Thus effects of ageing could be compounded or synergistic with the adverse effects of HIV infection on the human immune system.

Two reasons could account for the ageing population infected with HIV: 1) Very good HIV drugs are prolonging life expectancies (beyond 50 years) of many patients on therapy and 2) the increasing rate phenomena of the elderly being infected with HIV even though most new infections occur in younger population.

Though there has been improvement in HIV therapy that allows most HIV positive persons to live healthy live and long, living and ageing with HIV is different as compared to ageing with HIV negative. Some medical conditions of the aged appear to occur earlier and progresses faster in HIV-infected people. HIV infection compromises the immune system's ability to fight off infection, allowing opportunistic infections to occur. The cells of the immune system are always activated because they are always struggling to fight the virus in HIV-infected person. After many years of continuous activation, the immune system show signs of pre-mature ageing in people with HIV (Roit *et al.*, 2006).

Several studies have shown age as an independent predictor of clinical progression in HIV. Egger and colleagues, 2002 identified in a study to evaluate the prognosis of HIV infection in treatment-naïve patients starting on HAART, 50 years and older as an independent prognostic factor influencing the clinical progression of HIV infection to AIDS or death (Egger *et al.*, 2002)

Shorter survival periods were associated with the time of HIV diagnosis of age 60 years and older as revealed by Butt *et al.*, 2001. Also, Babiker and colleagues (2001) determined that increasing age at time of seroconversion was associated with lower survival rates among HIV-infected patients, particularly in the years prior to the use of HAART. Adjusted for general aging effects on mortality, the median survival for those who were infected at ages 25 to 34 years old was 11 years, higher as compared to 6.6 years and 4.4 years in those who were infected at 55 to 64 years old and 65 years and older, respectively. The researchers also revealed that for every 10-year increase in age at time of HIV infection, the overall mortality rate increased by 43%. Aside from age at infection, the time since infection was positively correlated with increased mortality in older persons (ages 45 to 55 years old) who had the highest death rate as compared to younger persons less than 45 years old. Rapid progression to AIDS and decreased survival in older HIV-infected patients has also been confirmed in French and Spanish cohorts (Grabar *et al.*, 2004, Nogueras *et al.*, 2006).

2.2 Epidemiology

HIV-infected elderly are an increasing and emerging category of patients worldwide. This means that clinicians will encounter an increasing number of older HIV-infected patients in the coming years. Epidemiological and research data indicates that the HIV-infected population is ageing in parallel with the use of effective antiretroviral drugs in many rich countries, and that newly infected or newly diagnosed older patients are increasing in number than in younger patients (Grabar *et al.,* 2004, Castilla *et al.,* 2002, Skiest *et al.,*1996).

The ageing HIV epidemic in many settings is fueled by the following phenomena: ART enables infected people to live longer and older and middle-aged individuals are becoming infected with the human immunodeficiency virus. In resource-rich countries like the USA and Britain, a 35-year-old infected individual with HIV who initiates ART at a CD4 count below 100 cells is expected to live to 62 years old: this same individual is expected to live an additional 10 years if ART is initiated at a CD4 count above 200 cells (Lancet, 2008). Sterne JA *et al.,* and Kitahata MM *et al.,* believe even longer life expectancy if this same individual were to start ART at CD4 counts above 350 or 500 cells (Sterne *et al.,* 2009, Kitahata *et al.,* 2009) This with many other predictions underscores the importance of early diagnosis and treatment with ART in order to achieve the benefit from its profound effect on ageing with HIV.

The gradual increase in age at both diagnosis of HIV infection and AIDS is noted worldwide. The introduction of HAART has led to advancement in diagnostic resources, antimicrobial therapy and prophylaxis and this intervention has led to a dramatic change in the natural history of HIV disease from an acute to a sub-acute disorder, invariably

progressing towards advanced AIDS stages and death or to a chronic, treatable infection. This has led to a marked decrease in morbidity and mortality rates (Palalla *et al.,* 1998, Powderly *et al.,* 1998, Whitman *et al.,* 2000)

Secondly, although there has been a general change of behavioural risks to HIV transmission, there has been continued risky sexual behavior and abuse of drugs, reduced attention to preventive measures and lack of very specific target education and information among the elderly (Roberto, 2002). Thirdly, late recognition of elderly HIV infected and even unaware of their HIV status is a debilitating contributing factor to the alarmingly increasing incidence of newly diagnosed HIV infection or AIDS among the elderly (Roberto, 2002).

A study (Gabor *et al.,* 1989), conducted at the Johns Hopkins Hospital Emergency Department to assess the impact of HIV epidemic, 152 (6.0%) of 2544 consecutive patients were diagnosed to have HIV infection. Infected patients were three times more likely to be admitted as sero-negative patients.

By 2015 according to Edward *et al.,* 2012, half of USA population of people living with HIV infection will be older than 50 years of age. This ageing phenomenon with HIV infection will be mirrored in developing countries like Ghana as ART coverage continues to cover more sentinel sites across the globe. Also, by the year 2050, the proportion of the elderly living in developing nations will increase to about 20% (UN, 2007). This indicates the elderly will increasingly form an integral proportion in numeric terms in developing countries.

In sub-Saharan Africa, ART or HAART has already decreased mortality rates, with about 320,000 (20%) fewer people dying of HIV-related causes in 2009 than in 2004 (UNAIDS, 2010). A study in Uganda reports HIV-infected people in their 40s who are on ART is expected to live well into their 60s (Mills *et al.,* 2011). This increased life expectancy of HIV-infected persons could lead to increases in HIV prevalence among older adults. Negin and colleagues in 2010 revealed that approximately 1 in 8 HIV-infected adults and I in 10 patients receiving ART in sub-Saharan Africa are older than 50 years of age. These ratios are likely to increase many folds in the coming decades.

South African data reveals that HIV prevalence among people older than 50 years of age will nearly double in the next 30 years and the absolute number of similarly aged HIV-infected patients could triple in the same period (Hontelez *et al.,* 2011). This ageing epidemic has very important consequences of health systems and administrations.

In Lancet, 2011, Mills et al suggested the need to prioritize HIV-infected elderly persons in the area of prevention and care. The Centers for Disease Control (CDC) estimated that about 29% of the entire population of persons living with HIV in the USA alone were aged over 50 years in 2008 and this is projected to double in about 5 years' time, indicating that more than half of all HIV-infected patients will be over 50 years of age (Effros *et al.,* 2008)

Although it is estimated in some sub-Saharan Africa countries that due to increasing access to ART, HIV-infected elderly population will rise, its magnitude has not been quantified and prepared for. In South Africa, it is estimated that, HIV prevalence in the population aged over 50 years is 9% (Negin *et al.,* 2010,Wallrauch *et al.,*2010), which means that they are most likely to be confronted with this shifting HIV epidemic.

The elderly are more vulnerable to HIV infection. Older adults at risk are approximately one-fifth as likely as younger adults at risk to test negative to HIV. Transfusions, which are more needed in the elderly, represent about 25% of the cases of those aged over 65 years higher than 1% in younger adults. This increases the potential transmission via this route. In theory, a donor who is infected but has not yet seroconverted will test negative to HIV antibody. This false seronegative "window" has been estimated to last a median of 2.1 months, with 95% of persons developing antibody within 5.8 months (Nelson *et al.*, 1992).

2.3 HIV Risk Behaviors among the Elderly

HIV is known to be transmitted mainly through sexual contact (commonly heterosexual or men-having-sex-with-men, MSM), perinatally from mother-to-child, blood contact (eg, blood transfusion, needle stick, or intravenous drug use, IDU).

Changing trends in risk factors has been recognized among persons 50 years and older at the time of HIV diagnosis. Blood transfusion was the primary route for transmission for this age group until the inception of screening blood supply IDU and MSM make up approximately 50% for risk factors currently associated with HIV infection in the older population **(**Hall *et al.*, 2007). Among MSM, Hall *et al.*, in 2007 estimated that, HIV diagnosis increased by 3.1% and 6.2% in persons 45 to 49 years old and 50 to 54 years old respectively in the US.

Among hospitalized patients 60 years and older at time of death and had no history of HIV or AIDS, a study revealed 6.2% of men and 8.9% of women were HIV-seropositive, and more than 60% of those patients who tested HIV positive had no documented or

identifiable risk factors for HIV(El-Sadr *et al.*, 1995) Misconceptions about HIV infection and who it affects, denial of risk factors, sense of hopelessness even with HIV-positive status known, and active drug use are among the factors which might hinder the elderly from seeking an HIV test (Lekas *et al.*, 2005)

Heterosexual contact (accounting for about 10% of HIV infection in the elderly) is on the increase, predominantly affecting more women. It is estimated that more than 50% of HIV infection in older women are reported to be due to heterosexual transmission against 15% IDU. According to the CDC, between 1999 and 2004, among heterosexually-acquired HIV/AIDS cases, 19% and 12% were men and women 50 years and older (Espinoza *et al.*, 2007).

Additionally, the estimated annual percentage in heterosexually-acquired HIV/AIDS cases recorded a significant increase among men 50-59 years old (+4.9%) and women 60 years and older (+4.1%). At the same period, diagnosis of HIV and AIDS was slightly higher in non-Hispanics White and Hispanics compared to non-Hispanic Blacks. (Espinoza *et al.*, 2007)

Older adults were observed to engage in a variety of sexual activities in a review of HIV risk factors in the elderly, although older men reportedly more sexually active than older women. Majority of the older women had little or no knowledge of personal risk and/or have not engaged in protection behaviors. Also, women who had partners (including married women) were less likely to use barrier contraceptive method. The use of condom appears to decrease with age, probably related to not being worried about getting pregnant or the undue perception of not being at risk for asexually transmitted diseases (STDs).

Some potential barriers to condom use was revealed to include difficulties in communication between partners, concern for lack of trust, and feelings that the male partner controls condom use (Zablotsky *et al.*, 2003)

Other factors which might increase the risk of HIV transmission among older women may include changing sexual relationships due to divorce or death and menopausal changes in vaginal mucosa increasing the likelihood for trauma and STDs (Shah and Mildvan 2006).Although drugs used to treat erectile dysfunction in older men have not been shown to result in increased HIV transmission in one study, this remains a possibility (Karlovsky *et al.*, 2004)

There remains a possibility of increased risk of HIV transmission in a study by Karlovsky *et al.*, 2004; Shah and Mildvan, 2006, in the elderly being treated with drugs for erectile dysfunction, though have not been shown. Late HIV testing and diagnosis may be the reason for an increased number of newly reported infection cases in the elderly. Majority of these infections are chronic, long standing HIV infection, not acute infections. Missed diagnosis of HIV infection in the elderly may include, among the following factors; poor understanding of HIV risk factors (including safe sex practices); routine HIV screening being uncommon among the elderly; failure of healthcare providers to consider HIV infection in this patient population; and confusion about HIV-specific or opportunistic infection symptoms and comorbidities with symptoms other diseases frequently related with older age (eg. Dementia, Alzheimer's**)** (CDC, 2007) Thus, it is recommended that HIV should be considered in all sexually active older patients even if they report being monogamous. Older patients' sexual orientation, discomfort regarding their own sexuality, or not comfortable with disclosing their sexual information they feel should be discrete.

2.4 HIV Testing

HIV can be transmitted through contaminated blood and blood products, through sexual contact or from a HIV infected mother to her child before, during or after birth.

Two type of HIV have been well identified up to date; HIV-1 and HIV-2. Various subtypes have been described, each of which has a different geographical distribution. HIV-1 can be divided into distantly related groups: group M (for main), group N (for non-M, non-O) and group O (for outlier). Based on the genetic relationship, at least 9 different subtypes or clades (A, B, C, D, F, G, H, J, and K) have been identified within HIV-1 group M (www.avert.org/hiv-types.htm). Recombinant HIV-1 viruses consisting of sequences of 2 or even more different subtypes exist and are spreading epidemically (Burke, 2004).

Antibodies to HIV proteins, indicating the presence of an HIV infection, can be found in serum usually 6–12 weeks after infection (Burke, 2004). Due to differences in the sequence of immunodominant epitopes, especially in the envelope proteins HIV-1 group M, HIV-1 group O and HIV-2, specific antigens are necessary to avoid failure in the detection of an HIV infection by immunoassays (Philips 2000). By detecting the HIV-1 p24 antigen in blood specimens of recently infected patients with a high viral load, HIV infection can be detected about 6 days earlier than with a traditional antibody assays (Cohen *et al.*, 2010). Anti-HIV antibodies and the HIV-1 p24 antigen can be detected simultaneously using a 4th generation HIV assay. This leads to improved sensitivity and, therefore, a shorter diagnostic window as compared to anti-HIV assays.

Rapid diagnostic tests, utilizing blood samples or oral secretions can yield results in as little as 15 or 30 minutes and can be performed in the clinic or office comfortably with minimal

equipment and training. Enzyme Immunoassay (EIA) is used to detect HIV antibodies, followed by a confirmatory test such as a Western blot of Immunofluorescence assay (IFA) if screening test is positive.

2.4.1 Rapid HIV Test

Rapid HIV tests are strip/cassette-based Immunochromatographic immunoassays whose physical principle relies on the migration of micro- or nanoparticles along a membrane to yield qualitative results. Microscopic particles are individually coated with HIV antigens and are adsorbed unto the reaction nitrocellulose membrane of a test cartridge to form test spots and an anti-human IgG control immobilized onto a nitrocellulose membrane the Control zones. Upon addition of specimen diluent, HIV antibodies present in the sample will bind to the coated antigens in the membrane. A conjugate or buffer solution is added to the test cartridge that binds to antigen-antibody complexes on the membrane and produces a colour in the test spots and the control spot with antibody bound. The appearance and location of colour test spots determines if the sample is reactive for HIV-1, HIV-2, or both. Most rapid tests can also detect HIV-1 and HIV-2 *(*Phillips, *2000)* and may be performed using whole blood, plasma or serum.

A negative result means that the test does not detect any HIV antibodies. A positive result however should be considered "preliminary positive" and must be confirmed with a second test such as a Western blot or a second rapid test from a different manufacturer. In Ghana, OraQuick is the recommended rapid confirmatory test. (NACP, 2010)

2.4.2 The Oraquick® Advance Rapid HIV-1/2 Antibody Test

The OraQuick® Advance Rapid HIV-1/2 Antibody test is a qualitative detection of antibodies to HIV-1 and HIV-2. It comprises a single use test device and a vial containing a pre-measured amount of a buffered developer solution. It is performed manually using blood or oral fluid and visually read after 20 minutes.

The OraQuick® Advance rapid test utilizes lateral flow immunoassay procedure. The device plastic housing holds an assay test strip comprised of several materials that provide the matrix for the immunochromatography of the specimen and the platform for indication of the test results.

The assay test strip contains synthetic peptides representing the HIV envelope region and a goat anti-human IgG procedural control immobilized onto a nitrocellulose membrane in the Test (T) zone and the Control (C) zone, respectively.

Following the addition of specimen into the vial of developer solution and insertion of the test strip, specimen migrates up the strip. If a specimen containing antibodies encounters the T zone, it reacts with the antigens immobilized on the nitrocellulose membrane and a reddish-purple line appears, qualitatively indicating the presence of antibodies to HIV-1 and/or HIV-2 in the specimen. The intensity of the line color is not directly proportional to the amount of antibody present in the specimen.

Further up the assay strip the C zone is a procedural control that serves to demonstrate that a specimen was added to the vial and that the fluid has migrated adequately through the test device. A reddish-purple line will appear in the C zone during the performance of

all valid tests, whether or not the sample is positive or negative for antibodies to HIV-1 and/or HIV-2.

2.4.3 Enzyme Immunoassays (EIA)

They are qualitative and quantitative assays used to measure the concentration of an antibodies or antigen in solution. Its principle is based on separation of specific and non-specific interactions that occurs via serially binding ag-ab to a polystyrene multiwell plate and reading the optical densities with a spectrophotometer.

2.4.3.1 The Genscreen™ Ultra HIV Ag-Ab

The Genscreen™ Ultra HIV Ag-Ab (Bio-Rad Laboratories, Hercules, USA.) is an enzyme immunoassay based on the principle of the sandwich technique for the detection of HIV antigen and of the various antibodies associated with HIV-1 and/or HIV-2 virus in human serum or plasma. The solid phase is coated with: monoclonal antibodies against p24 HIV-1 antigen and purified gp160 recombinant protein, a synthetic peptide mimicking a totally artificial HIV-1 group O-specific epitope and a peptide mimicking the immunodominant epitope of the HIV-2 envelop protein.

The conjugates are based on the use of: biotinylated polyclonal antibodies to HIV Ag; streptavidin and HIV antigens – peroxidase conjugate (gp41 and gp36 peptides mimicking the immunodominant epitopes of the HIV-1 and HIV-2 envelop glycoproteins and the same synthetic peptide mimicking a totally artificial HIV-1 group O-specific epitope used for the solid phase)

According to the CDC, current HIV-1 EIAs' can accurately identify infections with nearly all non-B subtypes and many infections with group O HIV subtypes" (MMWR, 2001). HIV antibodies detectable by enzyme immunoassay typically appear after seroconversion or within 3 weeks of infection (Cohen *et al.*, 2010).

2.4.3.2 The Electrochemiluminescence Immunoassay (ECLIA)

With the Elecsys HIV Combi PT assay, the HIV-1 p24 antigen and antibodies to HIV-1 and HIV-2 can be detected simultaneously within one determination (4th generation HIV assay). The assay uses recombinant antigens (gp41 including group O, gp36, reverse transcriptase ((RT)) derived from the *env* and *pol* regions of HIV-1 (including group O) and HIV-2 to determine HIV-specific antibodies (IgG and IgM). For the detection of HIV-1 p24 antigen, specific monoclonal antibodies are used (Gűrtler *et al.*,., 1998, Weber *et al.*, 2001).

This employs a sandwich principle. The first incubation is the pretreatment of 40ul of sample with a detergent agent. Biothylated monoclonal anti-p24 antibodies/HIV-specific recombinant antigens/HIV-specific peptides and monoclonal anti-p24 antibodies/HIV-specific recombinant antigens/HIV-specific peptides labeled with a ruthenium complex react to form a sandwich complex as the second incubation period.

After addition of streptavidin-coated microparticles, the complex becomes bound to the solid phase via interaction of biotin and streptavidin. The reaction mixture is aspirated into the measuring cell where the microparticles are magnetically captured onto the surface of the electrode. Unbound substances are then removed with a ProCell/ProCell M.

Application of a voltage to the electrode then induces chemiluminescent emission which is measured by a photomultiplier. Results are determined automatically by the software by comparing the electrochemiluminescence signal of the cutoff value previously obtained by calibration.

2.4.4 Polymerase Chain Reaction (PCR)

2.4.4.1 The CobasAmpliprep/CobasTaqmanHIV-1 Test - PCR

The COBASAmpliPrep/COBASTaqManHIV-1 Test is a nucleic acid amplification test for the quantitation of Human Immunodeficiency Virus Type 1 (HIV-1) RNA in human plasma. Specimen preparation is automated using the COBASAmpliPrep Instrument with amplification and detection automated using the COBASTaqManAnalyzer or the COBASTaqMan48 Analyzer.

The COBASAmpliPrep/COBASTaqManHIV-1 Test is based on three major processes: (1) specimen preparation to isolate HIV-1 RNA; (2) reverse transcription of the target RNA to generate complementary DNA (cDNA), and (3) simultaneous PCR amplification of target cDNA and detection of cleaved dual-labeled oligonucleotide probe specific to the target.

The COBASAmpliPrep/COBASTaqManHIV-1 Test permits automated specimen preparation followed by automated reverse transcription, PCR amplification and detection of HIV-1 target RNA and HIV-1 Quantitation Standard (QS) Armored RNA. The Master Mix reagent contains primers and probes specific for both HIV-1 RNA and HIV-1 QS RNA. The Master Mix has been developed to ensure equivalent quantitation of group M subtypes of HIV-1. The detection of amplified DNA is performed using a target-specific

and a QS-specific dual-labeled oligonucleotide probe that permit independent identification of HIV-1 amplicon and HIV-1 QS amplicon.

The quantitation of HIV-1 viral RNA is performed using the HIV-1 QS. It compensates for effects of inhibition and controls the preparation and amplification processes, allowing a more accurate quantitation of HIV-1 RNA in each specimen. The HIV-1 QS is a non-infectious Armored RNA construct that contains HIV sequences with identical primer binding sites as the HIV-1 target RNA and a unique probe binding region that allows HIV-1 QS amplicon to be distinguished from HIV-1 target amplicon.

The HIV-1 QS is added to each specimen at a known copy number and is carried through the specimen preparation, reverse transcription, PCR amplification and detection steps of cleaved dual-labeled oligonucleotide detection probes. The COBASTaqManAnalyzer or COBASTaqMan48 Analyzer calculates the HIV-1 RNA concentration in the test specimens by comparing the HIV-1 signal to the HIV-1 QS signal for each specimen and control.

Written informed consent, as well as pre- and post-test counseling is required in most cases. Current CDC HIV testing guidelines suggest routine, voluntary HIV screening in all persons age 13–64 years old in health care settings, regardless of risk; repeat HIV screening at least annually in persons with known risk factors; and opt out HIV screening with the opportunity to ask questions and the option to decline testing (Branson *et al.,* 2008).

2.5 HIV Treatment in the Elderly

Antiretroviral therapy (ART) in HIV treatment is administration of a minimum of two or three different agents. Currently, there are six(6) classes of antiretroviral agents; nucleoside reverse transcriptase inhibitors(NRTI) block the viral RNA to DNA transcription process by substituting in chain-terminating nucleosides (or nucleotides) in the DNA chain; non-nucleoside reverse transcriptase inhibitors (NNRTIs) change the conformation of the reverse transcriptase enzyme, rendering the enzyme dysfunctional; protease inhibitors (PIs) inhibit the protease enzyme which cleaves viral proteins into functional components prior to packaging into new HIV particles; fusion inhibitors which block the fusion of HIV with the host cell at the initial point of contact preventing HIV infection; entry inhibitors which block the entry of HIV into the host cell by blocking the cell surface co-receptor CCR5; and integrase inhibitors prevent the integration of the viral DNA into the host genome (Riot *et al.,* 2006)

There are no clear specific treatment guidelines currently available that focus on management of the HIV-infected elderly. There is also limited information on the efficacy and safety of selected antiretroviral regimens for the elderly patients. Immune reconstitution (CD4 cell recovery) is perceived to be limited in elderly HIV-infected patients with antiretroviral initiation. Grabar and colleagues, 2004 reported that, 13% HIV-infected elderly patients 50 years and older among 3,015 HIV-infected patients, the meant CD4 count increases were significantly higher in younger patients as compared to older patients when stratified by baseline HIV viral load and CD4 cell counts; though the CD4 cell count increased significantly within the first six months of ARV therapy.

Manfredi and Chiodo (2000) demonstrated similar results where patients 55 years and older. They estimated a significantly blunted CD4 cell count response in the elderly compared to the response seen in patients 35 years and younger. Another study showed that initial CD4 cell count response in older patients was slower during the initial phase of HAART therapy, but after 3 years of ARV therapy, the CD4 cell counts were not significantly different from that of younger patients (Silverberg *et al.,* 2007)

Studies show contrast and varied relationship between CD4 cell count rise and virologic responses in older and younger HIV-infected patients (Wellons *et al.,* 2002, UNAIDS, 1999). Age-associated decrease in thymic function and possibly a better medical adherence account for the contrast.

Exposure to adverse side effects from AR therapy is less well documented. Medication side infects in general tend to be higher in older patients (Wellons *et al.,* 2002) and this may be associated to age-related declines in hepatic and renal functions. For instance, HIV can decrease renal function by specifically infecting the kidney leading to a condition known as HIV-associated nephropathy (HIVAN) (Herman *et al.*, 2003). HIVAN is also more likely to occur in those who are Black, have AIDS, and are not on ARV treatment (Lucas *et al.*, 2004)

Changes in body composition due to age may also influence drug pharmacokinetics by altering drug volume of distribution (Bressler *et al.,* 2003) Decreases in body weight and in total body water content can lead to more concentrated drug levels in blood and tissues, and can result in enhanced drug effects and toxicity. On the other hand, increases in body fat, which acts as a depot for lipid soluble drugs, can result in decreased serum drug

concentrations and may initially lower drug effects. With continuous time and dosing, accumulation of lipid-soluble drugs in body fat may lead to toxicity. Slower gastrointestinal absorption rate may lead to delayed onset of drug effects. Drugs that are highly-protein bound may produce enhanced effects as a person ages and protein concentrations decline (Bressler *et al.*, 2003, Kinirons *et al.*, 2004).

2.6 Co-Morbid Disease Conditions

A study reported hospitalization rates increase, among HIV-infected patients 50 years while hospitalization rates for those 18 to 30 years old decreased dramatically between 1996 and 2000 (Gebo *et al.*, 2005).

Heart disease, diabetes, and cancers are among the most prevalent chronic medical conditions affecting older adults and account for some of the leading causes of death in the US population age 65 years and above (Gebo *et al.*, 2005).

Other conditions such as hypertension, hyperlipidemia, cerebrovascular diseases, and declining renal function also become more prevalent among the ageing population. Among HIV-infected patients, a higher percentage of older patients have diabetes, chronic respiratory disorders, hypertension, and hyperlipidemia, as well as other cardiac conditions, such as coronary heart disease and heart failure (Butt *et al.*, 2004, Palacios *et al.*, 2006)

A study, Shah *et al.*, 2002, reported among HIV-infected persons 55 years and older, 89% had one or more co-morbidities, with an average of 2.4 co-morbid conditions per patient. The effects of these co-morbid conditions on the natural history of HIV infection in older

patients, the compound toxicities from HIV therapy, and morbidity and mortality in these patients needs to be determined.

Declining CD4 cell counts in HIV infection is associated with increased risk for certain types of malignancy. The CDC's AIDS case definition includes Kaposi's sarcoma (KS), lymphomas, such as Burkitt's lymphoma and non-Hodgkin's lymphoma (NHL), and invasive cervical carcinoma (CDC 1992). Cancer risk also increases with age in non-HIV-infected persons, but very limited data is available evaluating the impact of HIV infection on cancer risk in older persons and vice versa. Studies have suggested that women with HIV infection are at greater risk for osteopenia and osteoporosis, which can be further accentuated when taking certain ARV regimens (Dolan *et al.,* 2004, Pan *et al.,* 2006, Anastos *et al.*, 2007). HIV-infected older males also manifest decreased bone mineral density compared to uninfected males (Arnsten *et al.*, 2007).

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Study design

This cross sectional study was carried out between the months of November 2012 and January 2014 among elderly people (> 50 years old) on admission at the Surgical Medical Emergency (SME) and Medical Wards, Department of Medicine and Therapeutics KBTH, situated in Accra. There was no selection of patients from a larger cohort of elderly Medical cases, all elderly patients aged 50 years and above on admission at the SME and Medical Wards were recruited. The protocol for the study was submitted to the Ethical and Protocol Review Committee (EPRC) of the UGMS for approval.

3.2 Study Site Description

KBTH is a tertiary hospital and the major referral centre in the country. It also serves as the teaching hospital of the University of Ghana Medical School (UGMS). The SME and Medical Wards are the biggest tertiary care centres in the hospital, seeing over 60% of the total cases in the country that are processed through the Department of Medicine and Therapeutics. The patients in this study originated from various social and ethnic groups as well as geographically distinct areas from the vast territory of the Greater Accra region of Ghana and other regions.

3.2.1 Sample Size Determination

The sample size for this survey was determined based on achieving a 90% power to detect major risk factors of HIV among elderly patients on admission at the SME Ward and the Medical Ward with relative risk (RR) in the range of 2-3 and significance level of 95% (P= 0.05). An estimated 5% HIV sero-prevalence among the older people was used based on the peak prevalence of 3.6% in Accra (The HIV sero-prevalence of this city is as reported in the HIV Sentinel Surveillance Report 2010 of the National AIDS/STI Control Programme of the Ghana Health Service). A total of 1,100 elderly medical patients were selected (based on the HIV sero-prevalence among elderly people (5%) and that among the general community (Accra 3.6%) to detect a RR of 2.0, and then 167 older people to detect a RR of 3.0.

3.2.2 Sampling Strategy

A total of 1,100 elderly male and female, patients on admission were selected from the two study sites. Prior to their recruitment, the patients were adequately informed about the aims of the study at the appropriate literacy levels (Appendix A). The informed consent process, interviews and specimen collection were all conducted privately in each of the study sites. Patients underwent individual pretest counseling sessions before specimen collection.

3.2.3 Questionnaire

All the 1,100 consenting patients completed a structured questionnaire (Appendix B) assessing socio-demographic, sexual and histories of HIV/AIDS related knowledge and

STI symptoms, and risk factors of HIV seropositivity. Where it was not possible to interview patient's, information were extracted from the patient's hospital records and the attending relatives.Anonymity and confidentiality of results/outcomes were strongly emphasized to encourage patients to be truthful in answering sensitive questions on sexual behaviour, alcohol, etc.

3.2.4 Inclusion criteria

Male and female patients aged 50 years and above who were on admission at the KBTH (SME and Medical block) and did not know their HIV status.

3.2.5 Exclusion criteria

Patients on admission at KBTH who were less than 50 years old or knew their HIV status were not qualified to participate in the study.

3.3 Sample Collection, Processing and Storage.

Blood samples (about 5 ml) were collected from each of the 1,100 consenting elderly patients into EDTA tubes and transported to the Pathology Department, University of Ghana Medical School in an ice-chest. The samples were centrifuged at 1600 x g for 20 minutes at room temperature within 12 hours after collection and the serum/plasma kept at -20°C in a sterile 2.0 mL polypropylene screw cap tube (starstedt 72.694.006) until analyzed.

Plasma were screened for anti-HIV (1 & 2) antibodies with First response HIV Card Test 1-2.0 (PMC Medical, India). Non-reactive samples from the first test were retested for antibodies/antigens with a COBAS e411 immunoassay (ROCHE diagnostics, GmbH, Sandhofer strasse 116, D-68305 Manheim, Germany) in accordance with the manufacturer's instructions. Positive specimens were considered where both tests were reactive. Indeterminate samples were further tested with Genscreen^{TM} ULTRA HIV Ag-Ab (Bio-Rad Laboratories, Hercules, USA.) at the Public Health Refernce Laboratory, KBTH and real-time polymerase chain reaction, RT-PCR (ROCHE diagnostics, GmbH, Sandhofer strasse 116, D-68305 Manheim, Germany) at the Immunology and Cell Biology Department, Central Laboratory, KBTH. The guidelines for the classification of HIV/AIDS in adults from the CDC, U.S.A. was applied.

3.4 Laboratory Analysis

HIV serial testing algorithm was employed.

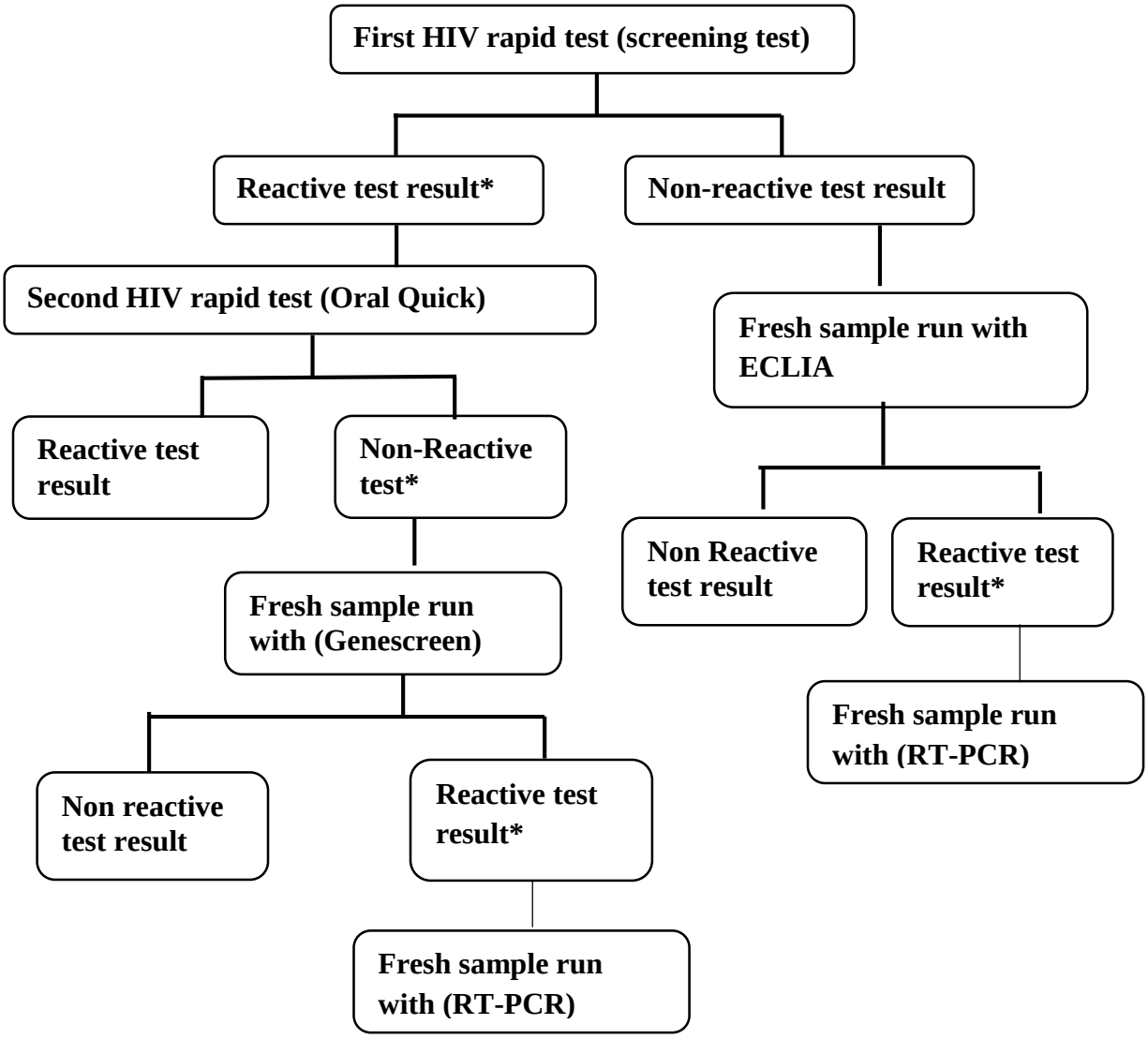

Fig. 1. HIV testing algorithm used in the study.

3.4.1 First Response HIV Card Test 1-2.0

The rapid detection test kit used for the initial analysis was First Response HIV Card test 1–2.0 (PMC Medical India Pvt Ltd). To carry out the test, 20µl to 30µl of serum was added to the sample well of the test kit. It was then incubated and the results read immediately after 20 minutes. The positive samples had two or three purple bands one at the control and the other(s) at the test region (one each for HIV-1, HIV-2 or both) while the negative samples had only one purple band at the control. All the test procedures were in accordance with the manufacturer's instructions.

3.4.2 Oraquick® Advance Rapid HIV-1/2 Antibody Test (Orasure Technologies, Inc. USA)

All reactive samples from the First Response HIV Card 1–2.0 test were tested with OraQuick® Advance Rapid HIV-1/2 Antibody Test (OraSure Technologies, Inc. USA) for confirmation. To perform the test, 5ul of serum was added to the sample well of the test kit and the results read immediately after 20 minutes. The positive samples had two rose-pink bands one at the control and the other at the test region while the negative samples had only one rose-pink band at the control. All the test procedures were in accordance with the manufacturer's instructions.

3.4.3 Genscreen™ Ultra HIV Ag-Ab (Bio-Rad Laboratories, Hercules, CA)

The Genscreen™ ULTRA HIV Ag-Ab was used to detect HIV p24 antigen and antibodies to HIV-1 (groups M and O) on the indeterminate sera samples.

All reagents were allowed to reach room temperature (18-30°C) and reconstituted for use. 25ul of conjugate 1 (biotinylated polyclonal antibodies to p24 HIV 1) was first added to each well followed by 75ul of HIV Ag positive control (Purified and inactivated HIV 1 antigen) in well A1. 75ul of HIV Ab positive control (Heat inactivated human plasma positive for anti-HIV antibodies) was added in well B1, 75ul of negative control (Heat inactivated human plasma negative for HIV antigen, anti-HIV-1, anti-HIV-2) in well C1, D1 and E1 and 75ul of specimen (serum from patients) was added in subsequent wells. After adding the sample, the conjugate 1 turns yellow-green to blue. The mixture was homogenized the by at least 3 aspirations or by shaking the microplate after the pipetting step and incubated for an hour. The plate was then washed three times with wash buffer (diluted from the concentrated buffer to 1:20), and blotted dry. 100 ul of conjugate 2 (peroxidase labelled Streptavidin and purified HIV1&2 antigens + skimmed milk solution) was added to each microwell, incubated for 30 minutes and washed five times. 80ul of freshly prepared substrate solution (Peroxidase substrate buffer + chromogen) was quickly dispensed into each well), after which the reaction was allowed reaction to develop in the dark for 30 minutes at room temperature (18-30oC). This resulted in the development of pink colour in some wells that had the samples added to them while the wells without samples remained colourless. The reaction was then stopped by adding 100 µl of the stop solution (1N H2SO4). After the addition of the stopping solution the pink coloration of the substrate disappears (for the negative samples) or turns from blue to yellow (for the positive samples). The optical density (OD) of the wells were determined at 450/620-700nm using a plate reader (spectrophotometer) within 30minutes of stopping the reaction the cut off value was used to determine if a sample was positive or negative.

Those wells with OD below the cut-off value of the microtitre plate were considered non-reactive and therefore negative while those with optical densities above the cut-off value were regarded as reactive and therefore confirmed positive. All samples were tested in duplicates, incubation periods had an allowable ±4 minutes and the room temperature was maintained between 18-30^{o}C.

3.4.4 The Electrochemiluminescence Immunoassay (ECLIA)

The test performance of ECLIA has been established and certified by a Notified Body according to the Common Technical Specifications (CTS) for diagnostic use and for screening of blood donations and research. HIV combi PT (HIV-1 antigen and total antibodies to HIV-1 and HIV-2) kit (ref: 05390095 190) was used on the automated COBAS e411 immunoassay analyser.

Briefly, reagents in the kit are ready for use (except for HIVCOMPT Cal1 and HIVCOMPT Cal2) and are supplied in bottles compatible with the system. HIVCOMPT Cal1 and HIVCOMPT Cal2: the contents are dissolved in one bottle by adding exactly 1.0 mL distilled water and allowed to stand for 15 minutes to reconstitute. The reconstituted calibrator is transferred into the supplied empty labelled snap-cap bottles.

Assay: briefly, 40 ul of sample was pretreated with a detergent. 60 ul of biotinylated monoclonal anti-p24 antibodies, 60 ul HIV-specific recombinant antigens, 60ul HIV-specific peptides were added. 60ul of monoclonal anti-p24 antibodies, 60ul of HIV-specific recombinant antigens, 60ul of HIV-specific peptides labeled with a ruthenium complex *Tris(2,2'-bipyridyl)ruthenium(II)-complex* ($Ru(bpy)_3^{2+}$)wereadded to form a sandwich

complex. 40ul of streptavidin-coated microparticles was added to the complex. Unbound substances are washed with 100ul of ProCell and ProCell M each, 3x. The reaction mixture was then aspirated into the measuring cell where the microparticles are magnetically captured onto the surface of the electrode. The analyzer automatically calculates the cutoff based on the measurement of HIVCOMPT Cal1 and HIVCOMPT Cal2. The result of the sample was given either as reactive or non-reactive as well as in the form of a cutoff index (signal sample/cutoff). Samples with a cutoff < 0.90 are non-reactive in the Elecsys HIV combi PT assay. These samples were considered as negative for HIV-1 Ag and HIV-1/2 specific antibodies. Samples

3.4.5 The Polymerase Chain Reaction (PCR)

COBASAmpliPrep Instrument Manual for use with the COBASTaqMan48 Analyzer and the AMPLILINK Software, Version 3.1.x Series was used.

The COBASAmpliPrep/COBASTaqManHIV-1 Test is an *in vitro* nucleic acid amplification test for the quantitation of Human Immunodeficiency Virus Type 1 (HIV-1) RNA in human plasma using the COBASAmpliPrep Instrument for automated specimen processing and COBASTaqManAnalyzer or COBASTaqMan48 Analyzer for automated amplification and detection.

This is based on four major processes: (1) sample pre-extraction and incubation, (2) sample preparation to isolate HIV-1 target nucleic acids by a generic silica-based capture technique; (3) reverse transcription of the target RNA to generate complementary DNA (cDNA), and (4) simultaneous amplification of target by Polymerase Chain Reaction

(PCR) and detection of cleaved dual-labeled oligonucleotide detection probe specific to the target.

Briefly, samples and controls are placed at room temperature until completely thawed and vortex for 3-5 seconds before use.

1. COBAS Ampliprep Instrument Set-up:

Part A. Maintenance and Priming

Part B. Loading of Reagent Cassettes

Part C. Loading of Disposables

Part D. Ordering and Loading of Specimens

Part E. Start of Cobas Ampliprep Instrument Run

Part F. End of COBASAmpliPrep Instrument Run and Transfer to COBASTaqMan48 Analyzer

2. Amplification and Detection (COBASTaqMan48 Analyzer set-up

Part G. Loading of Processed Specimens

Part H. start the COBASTaqMan48 Analyzer run

Part I. End of COBASTaqMan48 Analyzer run

3. Results

The COBASTaqMan48 Analyzer automatically determines the HIV-1 RNA concentration for the specimens and controls. The HIV-1 RNA concentration is expressed in copies (cp)/mL. The conversion factor between HIV-1 RNA copies/mL and HIV-1 International Units (IU)/mL is 0.6 cp/IU, using the WHO 1st International Standard for HIV-1 RNA for Nucleic Acid-Based Techniques (NAT) (NIBSC 97/656).

AMPLILINK Software:

- Determines the Cycle Threshold value (Ct) for the HIV-1 RNA and the HIV-1 QS RNA.
- Determines the HIV-1 RNA concentration based upon the Ct values for the HIV-1 RNA and HIV-1 QS RNA and the lot-specific calibration coefficients provided on the cassette barcodes.
- Determines that the calculated cp/mL for HIV-1 L(+)C and HIV-1 H(+)C fall within the assigned ranges.

3.4.5.1 Sample Pre-Extraction Procedure

For the detection of HIV nucleic acids in plasma prepared from human whole blood. 100ul of sample is pipetted into an eppendorf S-tube containing 1000ul of Specimen Pre-Extraction Reagent, SPEX. The S-tubes filled with the samples and controls are incubated in an Eppendorf Thermomixer Comfort at 56 degrees celcius and 1000 rpm continuous shaking for 10 minutes.

3.4.5.2 Sample Preparation

The COBAS Ampliprep instrument utilizes a generic silica-based capture technique. The HIV-1 virus particles are lysed by incubation at elevated temperature with a chaoptropic lysis/binding buffer that releases nucleic acids and protects the released HIV-1 RNA or proviral DNA from nucleases in the sample. Magnetic particles along with the lysis reagent and a known number of HIV-1 internal control (IC) Armored RNA molecules are introduced into each sample. Subsequently, the mixture is incubated and the HIV-1 RNA

or proviral DNA and HIV-1 IC RNA are bound to the surface of the magnetic glass particles. Unbound substances, such as salts, proteins and other cellular impurities, are removed by washing the magnetic glass particles. After separating the magnetic glass particles and completing the washing steps, the adsorbed nucleic acids are eluted at elevated temperature with an aqueous solution. The processed sample, containing the magnetic glass particles as well as released HIV-1 RNA or proviral DNA and HIV-1 IC is added to the amplification mixture and transferred to the COBAS 48 Taqman Analyser.

3.4.5.2 Reverse Transcription and PCR Amplification

3.4.5.2.1 Target Selection

Selection of the target sequence for HIV-1 depends on identification of regions within the HIV-1 genome that show maximum sequence conservation among various group M HIV-1 subtypes. Generic silica based sample preparation is used to capture the HIV-1 target RNA or proviral DNA and HIV-1 IC RNA and defined oligonucleotides are used as primers in amplification of the HIV-1 target RNA or proviral DNA and HIV-1 IC RNA. A target-specific and an IC-specific dual-labeled oligonucleotide probe permit independent identification of HIV-1 target amplicon and HIV-1 IC amplicon.

The COBAS Ampliprep/COBAS TAqman HIV-1 Qual Test uses reverse transcription and PCR amplification primers that define a sequence within the highly conserved region of the HIV-1 gag gene (Kwok *et al.,* 1993).

The gag region encodes the group-specific antigens or core structural proteins of the virion. The nucleotide sequence of the primers has been optimized to yield comparable amplification of group M subtypes of HIV-1.

3.4.5.2.2 Reverse Transcription and PCR Amplification

The reverse transcription and PCR amplification reaction is performed with the thermostable recombinant enzyme *Thermus specie* DNA Polymerase (Z05). In the presence of manganese (Mn^{2+}) and under the appropriate buffer conditions, ZO5 has both reverse transcriptase and DNA polymerase activity (Meng *et al.,* 2001, Smith *et al.*,., 2003)This allows both reverse transcription and PCR amplification to occur together with real-time detection of the amplification.

Processed samples are added to the amplification mixture in amplification tubes (k-tubes) in which both reverse transcription and PCR amplification occur. The reaction mixture is heated to allow downstream primer to anneal specifically to the HIV-1 target RNA and to the HIV-1 target RNA and to HIV 1 IC RNA. In the presence of Mn^{2+} and excess deoxynucleotide triphosphates (dNTPs), including deoxyadenosine, deoxyguanosine, deoxycytidine, deoxyuridine and deoxythymidine triphosphates, Z05 polymerase extends the annealed primers forming a DNA strand complementary to the RNA target.

3.4.5.2.3 Target Amplification

Following reverse transcription of HIV-1 target RNA and HIV-1 IC RNA, the Thermal Cycler in the COBAS Taqman 48 Ananlyzer heats the reaction mixture to denature the RNA:cDNA hybrid or proviral DNA and to expose the specific primer target sequences.

As the mixture cools, the primers anneal to the target DNA. The Thermostable Thermus specie Z05 DNA Polymerase(Z05) in the presence of Mn^{2+} and excess deoxynucleotide triphosphates (dNTPs), including deoxyadenosine, deoxyguanosine, deoxycytidine, deoxyuridine and deoxythymidine triophosphates, extends the annealed primers along the target template to produce a double-stranded DNA molecule termed an amplicon. The COBAS Taqman 48 Analyzer automatically repeats this process for a designated number of cycles, with each cycle intended to double the amount of amplicon DNA. The required number of samples is preprogrammed into the COBAS Taqman 48 Analyzer. Amplification occurs only in the region of the HIV-1 genome between the primers; the entire HIV-1 genome is not amplified.

3.4.5.2.4 Internal Control Amplification

In enzyme- based amplification such as PCR, efficiency can be reduced byinhibitors that may be present in the sample. The HIV-1 internal control (HIV-1 IC) has been added to the COBAS AmpliPrep/COBAS Taqman HIV-1 Qual Test to permit the identification of the processed sample containing substances that may interfere with PCR amplification. The HIV-1 IC is a non-infectious Armored RNA construct that contains HIV sequences with identical primer binding sites as the HIV-1 target RNA, a randomized internal sequence of similar length and base composition as the HIV-1 target sequence and a unique probe binding region that allows HIV-1 IC amplicon to be distinguished form HIV-1 target amplicon. These features were selected to ensure equivalent amplification of the HIV-1 IC and the HIV-1 target. The HIV-1 IC is added to each sample and is carried through the

sample preparation, reverse transcription, PCR amplification and detection steps and serves as an extraction control for each independently processed sample.

3.4.5.2.5 SelectiveAmplification

Selective amplification of target nucleic acid from the sample is achieved in the COBAS AmpliPrep/COBAS Taqman HIV-1 Qual Test by the use of AmpErase (uracil-N-glycosylase) enzyme and deoxyuridine triphosphate (dUTP). The AmpErase enzyme recognizes and catalyzes the destruction of DNA strands containing deoxyuridine but not dNA containing deoxythymidine. Deoxyuridine is not present in naturally occurring DNA, but is always present in Amplicon due to the use of deoxyuridine Triphosphate as one of the contaminating amplicon susceptible for destruction by the AmpErase enzyme prior to amplification of the target DNA. Also, any nonspecific product formed after initial activation of the Master Mix by manganese is destroyed by the AmpErase enzyme. The AmpErase enzyme which included in the Master Mix reagent catalyzes the cleavage of deoxyuridine-containing DNA at the deoxyuridine residue by opening the deoxyribose chain at the C1-position. When heated in the first thermal cycling step, the amplicon Dna chain breaks at the position of the deoxyuridine, thereby rendering the DNA non-amplifiable. The AmpErase enzyme remains inactive for a prolonged period of time once exposed to temperatures above 55^0C, i.e. throughout the thermal cycling steps, and therefore does not destroy target amplicon formed after PCR.

3.4.6 Detection of PCR Products in a COBAS TaqMan Test

The cOBAS AmpliPrep/COBAS Taqman HIV-1 Qual Test utilizes real-time PCR technology. The use of dual-lAbeled fluorescent probes allows for real-time detection of PCR product accumulation by monitoring of the emission intensity of fluorescent reporter dye realeased during the amplification process. The probes consist of HIV-1 and HIV-1 IC specific oligonucleotide probes with a reporter dye and a quencher dye. In the COBAS AmpliPrep/COBAS Taqman HIV-1 Qual Test, the HIV-1 and HIV-1 IC probes are labelled with different fluorescent reporte dyes. When these probes are intact, the fluorescence of the reporter dye is suppressed by the proximity of the quencher dye due to Foster type Energy transfer effects. During PCR, the Probe hybridizes to a target sequence and is cleaved by 5'→3' nuclease activity of thermostable Z05 DNA polymerase. Once the reporter and the quencher dyes are released and separated, quenching no longer occurs, and the fluorescent activity of the reporter dye is increased. The amplification of HIV-1 target and HIV-1 IC are measured independently at different wavelengths. The process is repeated for a designated number of cycles, each cycle effectively increasing the emission intensity of the individual reporter dyes, permitting independent identification of HIV-1 target and HIV-1 IC.

3.5 DATA ANALYSIS

Data was entered into a database and analysed statistically using SPSS v 20 (SPSS Inc.). The data was analyzed for significant differences and/or associations between categorical data with the chi-square tests. P-values of < 0.05 was considered significant. The prevalence of HIV was compared between male and female elderly patients at the study sites. For each generally accepted risk factor for the disease under investigation, relative

was determined between those with the disease (cases) and those without the disease (controls). Most analyses were conducted by comparing infected cases with non-infected cases. Variables or risk factors used in this comparison included alcohol and drug use, number of sexual partners, participation in homosexual activities, use of condoms, participation in paid sex or the offering of sexual favours, history of STIs, relationships (living with regular partners). Other analyses conducted by comparing disease and risk factor prevalence between males and females. Logistical progression and other multivariant analysis techniques were used to analyze for possible interactions between the different risk factors for HIV, and to correct for possible confounding effects of age and other variables.

3.6 ETHICAL ISSUES

The Proposal was submitted to the ethical and review committee of the University of Ghana Medical School which approved the study. All data was handled anonymously to ensure high confidentiality. Voluntary written informed consent and permission for drawing of blood samples were sought from the patients before inclusion in the study.

If the patient expresses willingness to participate in the study, an in-depth explanation was given. All explanations and consenting procedures were done in the language that the patient found most comfortable.

CHAPTER FOUR

4.0 RESULTS

A total of 1,100 elderly persons on admission at the Korle-bu teaching hospital participated in the study. Of the consenting patients, 440 (40%) were males (median age: 63 years, range 50-100 years), and 660 (60%) were females (median age: 62 years, range 50-97 years). The participation for the study at the 2 sites ranged from 39.5% (SME) to 60.5% (Medical Block). All the 1,100 consenting patients on admission at both wards completed interviews and blood testing, and the results herein presented are from the 1,100 patients.

Of the 1,100 patients, 75.19% (827 out of 1,100) had been on admission at the hospital for less than 4 weeks while24.81% (273 out of 1,100) had been in hospital for more than 4 weeks. The most common cause for their admission was hypertension (blood pressure ≥ 140/90 mm Hg) in 51%% (561 out of 1,100), diabetes in 37.55% (413 out of 1,100)followed by cancer 5.64% (62 out of 1,100). Other diagnosis accounted for 5.18% of the study population. About 80% of the consenting elderly patients had never been tested for HIV in their lifetime.

4.1 Prevalence of HIV among study patients.

The overall prevalence of HIV-1 was 4.18% (46 out of 1,100) with none testing positive for HIV-2.

Table 1 shows the ORs and corresponding 95% CIs for age, gender and HIV sero-positivity among the study patients. Among the study patients, HIV sero-prevalence was highest in patients aged >75 (7.69%; 11 out of 143) years compared to patients aged 50-60 years (4.82%; 24 out of 498) and 61-75 years (2.40%; 11 out of 459).There were no significant differences between them. Of the patients who tested positive for HIV-1, 58.66% (27 out of 46) were females and 41.34% (19 out of 46) were males. There was no significant difference ($p>0.05$) between males and females.

Table 1: ORs and the corresponding 95% CIs of age and gender for HIV sero-positivity among the study patients.

		N=(1,100)	HIV Status		OR	95% CI	p-value
			Pos (46)	Neg (1054)			
Age	50-60	498	24	474	*		
	61- 75	459	11	448	0.49	0.24-1.00	0.03
	>75	143	11	132	1.65	0.79-3.45	0.13
Gender	Male	440	19	421	1.06	0.58-1.93	0.48
	Female	660	27	633	*		

*: reference for calculations of OR

Table 2 shows ORs and the corresponding 95% CIs for demographic factors and HIV sero-positivity among the study patients. Married patients accounting for 86.91% of the study patients had a 2.21-fold (95% CI: 0.68-7.23) higher risk of HIV infection as compared with those who are not married. Traders (OR 2.28; 95% CI 0.85-6.12), dressmakers (OR 2.73; 95% CI 0.81-9.19) and hairdressers (OR 2.59; 95% CI 0.28-23.28) were at increased risk of HIV infection.

Table 2: ORs and the corresponding 95% CIs demographic factors and HIV sero-positivity among the study patients.

		N (1,100)	HIV Status		OR	95% CI	p-value
			Pos (46)	Neg (1054)			
Education	Illiterate	466	14	452	0.62	0.20-1.93	0.29
	Basic	230	12	218	1.10	0.35-3.51	0.57
	Secondary	320	16	304	1.05	0.34-3.24	0.60
	Tertiary	84	4	80	*		
Marital Status	Married	956	43	913	2.21	0.68-7.23	0.13
	Unmarried	144	3	141	*		
Religion	Christian	668	24	644	0.46	0.19-1.11	0.08
	Muslim	338	15	323	0.58	0.23-1.46	0.18
	Other	94	7	87	*		
Occupation	Accountant	98	5	93	2.20	0.62-7.80	0.18
	Dressmaker	96	6	90	2.73	0.81-9.19	0.09
	Driver	68	2	66	1.24	0.24-6.56	0.54
	Farmer	61	2	59	1.39	0.26-7.35	0.49
	Hairdresser	17	1	16	2.56	0.28-23.28	0.38
	Teacher	133	3	130	0.95	0.22-4.03	0.62
	Trader	417	22	395	2.28	0.85-6.12	0.07
	Unemployed	210	5	205	*		

*: reference for calculations of OR

Table 3 shows the ORs and corresponding 95%CI according of HIV status according to behavioral characteristics of the elderly hospitalized patients. Elderly patients who had multiple sexual partners, accounting for 36.18% of the study patients had a 1.25 fold higher risk (95% CI: 0.69-2-28) higher risk of HIV infection as compared with elderly patients who did not have multiple sexual partners. Homosexual elderly patients accounting for 0.27% of the study patients were at an increased risk (OR 47.86; 95% CI 4-27-537.91) of HIV infection as compared with heterosexual patients.

A previous history of alcohol use, no condom use, of paying or being paid for sex, of sexually transmitted diseases, tattoos or blood transfusion were all associated with decreased HIV sero-positivity. However, hospitalized elderly patients reporting a previous history of tobacco chewing had a 1.57-fold increased risk (95% CI 0.69-3.64) of HIV infection.

Table 3: ORs and corresponding 95%CI according of HIV status according to behavioral characteristics of the elderly hospitalized patients

		N (1,100)	HIV Status		OR	95% CI	p-value
			Pos (46)	Neg (1054)			
Condom Use	No	1053	43	1010	0.67	0.20-2.23	0.35
	Yes	47	3	44	*		
Concurrent sexual partners	No	702	27	674	*		
	Yes	398	19	379	1.25	0.69-2.28	0.28
Type of sex partner	Homosexual	3	2	1	47.86	4.27-537.91	<0.01
	Heterosexual	1097	44	1053	*		
Drug use	No	1088	46	1042	*		
	Yes	12	0	12		-	0.60
Alcohol use	No	663	28	635	*		
	Yes	437	18	419	0.97	0.53-1.78	0.53
Smoking history	No	979	42	937	*		
	Yes	121	4	117	0.76	0.27-2.17	0.42
Chewing tobacco	No	986	39	947	*		
	Yes	114	7	107	1.59	0.69-3.64	0.19
History of STI	No	1095	46	1049	*		
	Yes	5	0	5	-	-	0.81
Symptoms of STI	No	167	6	161	*		
	Yes	933	40	893	1.20	0.50-2.88	0.44
Payment for sex	No	985	40	945	*		
	Yes	115	6	109	1.30	0.54-3.14	0.35
Tattoos	No	633	28	605	*		
	Yes	467	18	449	0.87	0.47-1.59	0.38
Blood Transfusion	No	568	27	541	*		
	Yes	532	19	513	0.74	0.41-1.35	0.20

*: reference for calculations of OR

A greater percentage of the respondents had heard of HIV and were aware of of STIs. Among 9 items used to measure HIV awareness was the participant's opinion on correct and consistent use of condoms in preventing the transmission of HIV. Only 26.10% of the elderly patients knew that correct and consistent use of condom could protect people from getting HIV, while some 60.9% did not know that a person can protect himself through abstinence. Some 73% thought that healthy looking individuals could not be infected with HIV.

Table 4: HIV awareness among patients.

		Response (%)	
		Yes	No
HIV awareness	Have you heard of HIV/AIDS?	93.7	6.3
	Are you aware of STIs?	87.7	12.3
	Can correct use of condoms prevent HIV?	26.1	73.9
	Can HIV be acquired through food?	25.8	74.2
	Can HIV be acquired through handshakes/touch?	94.3	5.7
	Can HIV be acquired through contaminated needles?	66.8	33.2
	Can HIV be acquired through contaminated blood?	73.5	26.5
	Can a person protect himself from HIV by abstaining?	39.1	60.9
	Can healthy individual be infected with HIV?	37.0	63
	Is there Medication to cure HIV?	42.3	57.7
	Can pregnant woman pass HIV to unborn child?	21.4	78.6

CHAPTER FIVE

5.0 DISCUSSION

Despite the global attention being paid to the epidemic of infection with HIV, the infection rates of HIV among older people in the sub-Saharan Africa has been a neglected area of study. There are several reports on the prevalence rates of HIV infection for individuals aged 15-49 years (UNAIDS, 2011, WHO, 2003, WHO/UNAIDS, 2009), and the indicators or markers used by WHO, UNAIDS and/or other several organizations focus predominantly on the same age range. Interestingly the burden of HIV/AIDS on the elderly (>50 years) has been ignored and this raises a major global public health concern in the response to the epidemic of HIV/AIDS.

Consequently, there is limited data on the burden of HIV infection in individuals aged ≥ 50 years. The few existing information of HIV infection among the elderly comes from developed countries (Orchi *et al.*, 2008; Bhavan *et al.*, 2008; Schmid *et al.*, 2009). However in developing countries, studies on the prevalence of HIV infection and its impact among the elderly have been ignored, and the few studies available emphasize on the social and economic impact of HIV infection. Recent reports from the department of medicine and therapeutics in 2012 indicate that age-related death was the leading cause of death within the population on admission. HIV/AIDS was not included in the cause of death among the elderly, and this has resulted in the perception that the elderly are at a little or no risk of HIV infection. The purpose of this study is to determine the sero-prevalence of HIV and also the correlates of HIV infection among the patients. This is believed to be the first study to determine the prevalence and risk factors associated with HIV infection among the elderly individuals on admission at KBTH, Accra, Ghana; and demonstrates the high prevalence of and the considerable potential for the transmission of HIV infection among the elderly in Ghana, suggesting the possibility of HIV/AIDS related illness(es) may be one of the causes of death in both wards.

Further studies need to be conducted to determine broad population-based sero-prevalence studies among the elderly as most of the patients on admission were unaware of their HIV status.

The overall sero-prevalence of HIV among the elderly patients on admission at KBTH (4.18%) is higher than the sero-prevalence in the general population (1.37%) and that of similar studies in Cameroon (2.6%; Negin & Cumming (2010)) but lower than the reported sero-prevalence of HIV in elderly individuals in Tanzania (15%; Mtei & Pallangyo (2001)) The increased sero-prevalence of HIV infection among the elderly people admitted at both SME/Medical Wards, KBTH suggests that HIV may be widespread in the elderly populations on admission in other districts and regional clinics and hospitals and therefore reasonable to speculate that HIV infection may circulate among the elderly population within the general population. The diagnosis of HIV at the SME/Medical Wards in the elderly may be difficult. This is because many of the signs and symptoms are non-specific, and that clinicians and/or physicians may not realize that the elderly are at risk. There is therefore the need for further studies to be done in the elderly individuals on admission to heighten awareness of the possibility of HIV infection presenting to the SME and the Department of Medicine and Therapeutics.

The risk of HIV did not correlate with increasing age. However, elderly patients on admission aged >75 years old had a higher risk of HIV infection (Table 1) even though a greater proportion of elderly patients on admission who were sero-positive to HIV infection were in the 50-60 years age group. Similar findings were noted in a study conducted by Mtei and Pallangyo (2001) in Muhimbili Medical Center in Dar es Saalaam, Ethiopia. The reason(s) for this disparity cannot be discerned from this study and hence, there is the need for further studies to be done to define the prevalence, risk factors and impact of HIV on the elderly in the population.

Another finding of interest reported herein in this study is that a greater number of elderly patients who are traders (22 out of 46) tested seropositive for antibodies to HIV. Of the traders who were sero-positive to HIV, majority, (14 out of 22) were women who travelled long distances for several weeks to engage in trading activities. The finding of higher HIV sero-positivity in traders may be attributed to separation from partners and family, peer pressure, alcohol and drug use, low perceived vulnerability to HIV infection and freedom from social norms. The presence of HIV sero-positivity among the traders who travelled long distances and spend weeks away from home to engage in their trading activities is suggestive that the traders may be considered as bridge or core populations in the spread of HIV and other sexually transmitted infections from high risk groups to low risk groups within the elderly population (Brummer *et al.,* 2002; Anderson *et al.,* 2003, Lurie *et al.,* 2003). This is consistent with literature and therefore there is also the need to define the high prevalence of HIV infection and other risk factors associated with the trading profession among elderly females in the country.

Interestingly, homosexual elderly patients on admission has a higher prevalence (67%; 2 out of 3) of HIV infection. Although it is premature and inadvisable to generalize or extrapolate the findings to the larger pool of hospitalized elderly patients, the community and the general population, the results presented herein have a significant implication for polices and strategies to prevent HIV transmission within the elderly homosexual populations. Further studies with a larger cohort elderly group are needed to confirm this finding and determine the true prevalence and gather information and time of HIV infection among elderly individuals.

Of interest, elderly patients on admission at both the SME and Medical wards were less knowledgeable about some HIV preventive measures particularly in relation to condom use. Although the questions asked during the course of the study on HIV-related awareness, behavior and attitudes may differ from other studies (Negin *et al.,* 2012) thus making direct comparisons difficult. Common misconceptions about HIV prevention and sexual activity among elderly patients remain. Of the 46 elderly patients who tested positive for antibodies to HIV, 43 had no previous use of condoms during sexual intercourse. The results presented herein is in agreement with other studies which suggests that non-regular and inconsistent use of condoms may increase the transmission of HIV and STIs (Foss *et al.,* 2004, Holmes *et al.,* 2004). Based on these results, there is therefore the need to intensify and provide general HIV and STI education to hospitalized elderly patients, and all elderly people in the country.

The small sample size, the population studied (hospitalized patients), inability to establish whether the patients were infected with HIV before or after the age of 50 years, under-reporting of sexual activity and other risk behaviors as a result of face-to-face interviews may be the limitations to this study.

CHAPTER SIX

6.0 CONCLUSION

The results suggest that elderly individuals aged 50 years and above at the SME/Medical Wards, Korle Bu Teaching Hospital, Accra, Ghana have a high prevalence (4.18%) rate of HIV infection than the national prevalence rate (1.37%) in the general population.

RECOMMENDATIONS

The following recommendation are suggested:

1. Need to consider strengthening interventions targeting elderly populations against HIV/AIDS and other STIs.
2. Physicians, clinicians and healthcare providers should seek risk behavior information and offer HIV counselling and testing of elderly patients who present at SME/Medical Wards in KBTH and elsewhere.
3. Need to consider expansion of HIV counselling and testing to hospitalized individuals irrespective of the age of the patient.
4. Need for further studies of HIV infection among the elderly in the country.
5. Ghana AIDS Commission, Ministry of Health, National AIDS Control Programme and the Ghana Health Service should direct their efforts and attention to the elderly group in the HIV epidemic.

6.2 LIMITATIONS

The non-association between some of the risk factors and HIV status may be owing to the fact that some eligible elderly patients declined to participate despite re-assurance of confidentiality. In addition, some of the risk factor investigated may have been under reported as a result of face-to-face interview.

The estimated prevalence in this study may not reflect the actual situation in Ghana, owing to demographic and geographical differences and therefore further studies in different locations as well as in non-hospitalized elderly persons is required.

The immune profiles (CD4/CD8) could not be carried out due to inability to procure reagents on time.

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APPENDICES

APPENDIX A

PARTICIPANT INFORMATION LEAFLET AND CONSENT FORM

Title of Research: Human Immunodeficiency Virus Among Elderly Medical Patients at the Korle Bu Teaching Hospital, Accra, Ghana.

Name(s) and affiliation(s) of researcher(s): This study is being conducted by ; Mr Seth Agyemang, Department of Pathology, University of Ghana Medical School, College of Health Sciences, Korle Bu, Accra.

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You have been invited to take part in a research study on the commonness of HIV and risk factors among hospitalized elderly patients in Ghana. The researcher will first explain the study and will ask you to participate by signing this agreement which states that the study has been explained, that your questions have been answered and that you agree to participate. The researcher will explain the purpose of the study. He or she will explain how the study will be carried out and what you will be expected to do.

The researcher will also explain the possible risks and benefits of participating in the study. You should ask the researcher any questions you have about any of these things before you decide whether you wish to take part in the study. Please read the form and talk to the researcher about any questions you may have. Then, if you decide to participate, please sign and date this form in front of the person who explained the study to you. You will be given a copy of this form to keep.

Background: Human immunodeficiency virus (HIV) has now spread to all parts of the world and the rates of infection are found to be particularly high in young people than older people. As a result, most of the known epidemiological and clinical features of HIV and acquired immune deficiency syndromes (AIDS) were defined and continued to be defined using children and younger people than older people. Interestingly, most of the HIV/AIDS prevention efforts largely target children and the younger people and little is known about the attitudes towards HIV and awareness of prevention, testing and treatment among older people; although older people are also sexually active and remain at risk of HIV infection. Recent reports from Africa and elsewhere have demonstrated that the diagnosis of HIV infection is recently occurring with increasing frequency in older people. However, little is known about the prevalence or the status of HIV among the older people in the country

Purpose(s) of research: The purpose of this research is to find out how common HIV are among hospitalized older people at KBTH; to describe the reasons or causes for the spread of these infections among the elderly; to offer them counseling and testing as well as treatment for these infections; to inform and educate them of the causes of spread of these infections particularly through unprotected sex with commercial sex workers and sharing

of needles and syringes. They shall also be educated on measures which could be taken to prevent the spread of these infections.

Procedure of the research, what shall be required of each participant and approximate total number of patients that would be involved in the research: To find answers to the issues raised above, when the team of researchers come to your ward on a visit, you will be asked a few questions to obtain information. Some of the questions are educational background, marital status, drug/alcohol use, and smoking history, length of stay, place of origin, sexual partners, HIV/STI status and knowledge/attitude towards HIV/AIDs. These are routine questions asked in studies like the one you have been invited to take part.
In addition you will be asked to provide blood sample (about 1 tablespoon). A trained biomedical scientist will insert a needle into your vein in one of your arms and draw some blood. This may cause pain, discomfort and bruising at the site of needle insertion. Your blood sample will be tested for HIV. You will be taken through individual pretest counseling sessions before data and specimen collection and you will be requested to return in four (4) weeks to receive testing results. The researchers will notify and advice you on referrals for treatment or management in cases where any of these viruses are detected. In total we expect to recruit 500 hospitalized older people.

Risk(s): By participating in this research, you are likely to have some uneasiness of questioning, physical examination and a slight pain from collection of blood. The procedure of blood drawing for laboratory test sample can be associated with rare risks including bruising, bleeding or skin infection. Before blood collection, your arm will be cleaned and a new hollow needle/plastic tube will be placed in your arm to take the blood samples. When the needle goes into a vein, it hurts for a short time. The study team will try and decrease the chances of those risks/dangers happening, but if an untoward event happens, you will be immediately managed by a study physician and will be provided with free medical care in hospital.

Benefits(s): There are no direct benefits to the study patients. However, as part of the objectives of the goal, we hope that the data generated will form the firm basis to find appropriate social interventions that will influence behavioral change toward the risk of HIV/STIs infection.

Confidentiality: All of your records from this study will be treated as confidential medical records. The medical results with participant's name and identifying information will only be available to me, the principal investigators and the study supervisors. Information collected on study forms and database will be given code numbers. No name will be recorded on the research forms or in the electronic database. The findings of this study may be reported in publications or reports but your name will not be mentioned. However, as part of my responsibility to conduct this research properly, I may allow officials from the ethics committees or the safety committee to have access to your records.
The blood will be stored in an ice-chest and transported to the laboratory (Department of Pathology, UGMS). The remaining blood samples will be stored three (3) years after all

study analyses have been completed. All the blood samples will be labeled with a code so that your identity is not revealed to the people who do the tests.

Voluntariness: You do not have to take part in this research if you do not wish to do so, and this will not affect your management, treatment or health care or mobility status. Taking part in this study should be out of your own free will. You are not under obligation to do so. Research is entirely voluntary.
Alternatives to participation: This study does not involve the administration of investigational drugs or use of new curative procedures.

Withdrawal from the research: You may choose to stop participating in this research at any time that you wish to, without having to explain yourself. You may also choose not to answer any question you find uncomfortable or private.

Consequence of Withdrawal: There will be no consequence, loss of benefit or care to you if you choose to withdraw from the study. Please note however, that some of the information that may have been obtained from you before you choose to withdraw may be modified or used in analysis reports and publications without your name being mentioned. These cannot be removed anymore. I do promise to make effort to comply with your wishes as much as practicable.

Contact(s): If you have any questions you may ask those now or later. If you wish to ask questions later, you may contact:

Prof Andrew A. Adjei
Department of Pathology,
University of Ghana Medical School,
Korle Bu, Accra, Ghana
Tel: 020-813-5979; 0274-430-256

Prof. Richard K Gyasi,
Department of Pathology,
University of Ghana Medical School,
Korle Bu, Accra, Ghana.

Dr Francis Offei,
Department of Medicine and Therapeutics,
University of Ghana Medical School,
Korle Bu, Accra, Ghana.

I have read the foregoing information, or it has been read to me. I have had the opportunity to ask questions about it and any questions that I have asked have been answered to my satisfaction. I consent voluntarily to participate as a subject in this study and understand

that I have the right to withdraw from the study at any time without in any way affecting my stay in the hospital..

Signed by……..
Date……..
Place…….

If illiterate
Signed by the investigator…….
In the presence of an independent literate witness……
(where possible this person should be selected by the participant)
Date……..
Place…….

APPENDIX B

SURVEY QUESTIONNAIRE – DATA SHEET

1. Age:

2. Sex: (a) M (b) F

3. **Educational background:** (a) None (b) Basic (c) Secondary (d) Tertiary

4. **Religion**
   (i) Christian (ii) Muslim (iii) Other (iv) None

5. **Marital status**: (a) Married (b) Single (c) Widowed (d) Divorced
   (e) Other

   If married, please state the type of marriage
   (a) Monogamous (b) Polygamous (c) Other
   (i) Do you have any sexual partner besides your wife (ves)? (a) Yes (b) No
   (ii) If yes state reasons……
   (iii) If not married, do you have any sexual partner(s)?
   (a) Yes (b) No
   (iv) If yes, how many?
   (a) One (b) Two (c) Three (d) Four

6. Do you have children? (a) Yes (b) No
   (i) If Yes, how many children?
   (a) One (b) Two (c) Three (d) Four (e) > Four

7. **Place of Origin:**
   (IF GHANAIAN) Where did you live?
   .....
   (IF NON-GHANAIAN) Where did you live prior to migrating to this city/town in Ghana?
   ……

8. **Occupation:** (i) Teacher (ii) Administrative Assistant (iii) Artisan
   (iv) Accounting Officer (v) Trader (vi) Seamstress, (vii) Tailor
   (viii) Hairdresser (ix) Driver (x) Housewife (xi) Pensioner
   (xii) Farmer (xiv) Unemployed

9. **Race:**
   (i) African (ii) Asian (iii) American (iv) Hispanic
   (v) European

10. How long have you been hospitalized?

(i) Day(s) (ii) Week(s) (iii) < 1 Month (iii) > 1 Month

11. **Alcohol use:**
(i) Do you drink alcohol? (a) Yes (b) No
If Yes, are you (i) Regular/Active drinker (ii) Binge drinker

(ii) How much do you drink?
(a) Per day……...
(b) Per week……

12. If regular/active, For how long have you been drinking alcohol?
(a) < 6 months (b) 6 months-1 year (c) 2-5 years (d) 6-10years
(e) > 10 years

13. **Smoking History:** Do you smoke cigarettes? (a) Yes (b) No
If Yes, are you (i) Regular/Active smoker (ii) Occasional smoker

14. If regular/active, For how long have you been smoking cigarettes?
(a) < 6 months (b) 6 months-1 year (c) 2-5 years (d) 6-10years
(e) > 10 years

15. How many pack(s) of cigarette do you smoke weekly?
(a) one (b) two (c) three (d) four (e) > five

16. **Tobacco Chewing History:** Do you chew tobacco? (a) Yes (b) No
If Yes, are you (i) Regular/Active (ii) Occasional

17. If regular/active, For how long have you been chewing tobacco?
(a) < 6 months (b) 6 months-1 year (c) 2-5 years (d) 6-10years
(e) > 10 years

18. How many pack(s) of tobacco do you chew weekly?
(a) one (b) two (c) three (d) four (e) > five

19. **Drug use:** Do you use drugs? (a) Yes (b) No
If Yes, are you (i) Regular/Active drug user (ii) Occasional drug user

20. If regular/active, For how long have you been using drugs?
(a) < 6 months (b) 6 months-1 year (c) 2-5 years (d) 6-10years
(e) > 10 years

21. What type of drug do you use? (a) Marijuana (b) Heroin (c) Cocaine
(e) Crack (f) Diazepam/Valium (h)Others (specify)……

22. Mode of intake of drugs; (a) Sniff (b) Smoke (c) IV (d) Oral

23. **Sexual behavior, knowledge and attitudes of HIV/STI**
Do you have a sexual partner besides your wife (yes)? (a) Yes (b) No

24. If Yes, how many sexual partners do you have? (a) 1 (b) 2 (c) 3 (d) 4 (5) >5

25. Is/Are your sexual partner(s) a Male or Female? (a) Male (b) Female

26. Have you ever visited a commercial sex worker?
(i) Yes (ii) No

27. Have you visited a commercial sex worker in the past 6 months or before hospitalization? (i) Yes (ii) No

28. If Yes, did you had any sexual contact/intercourse with her? (a) Yes (b) No

29. How may commercial sex workers have you visited in the past 12 months?
(a) 1 (b) 2 (c) 3 (d) 4 (5) >5

30. Do you often request for sex with a commercial sex worker? (a) Yes (b) No

31. Do you use condoms during sex? (a) Always (b) Sometimes (c) No

32. Have you ever heard of HIV or AIDS? (a) Yes (b) No

33. Are you aware there are some diseases that can be passed through sexual contact/intercourse (a) Yes (b) No

34. Can the correct use of a condom every time a person is engaged in sexual contact or intercourse help protect him/her from HIV infection? (a) Yes (b) No

35. Can a person contract HIV infection through the sharing of food with an HIV infected person? (a) Yes (b) No

36. Can a person contract HIV infection by shaking the hand of an HIV infected person? (a) Yes (b) No

37. Can a person contract HIV infection through injections with a needle previously used by someone else? (a) Yes (b) No

38. Can a person contract HIV infection through receiving blood (blood transfusion) from HIV infected individual? (a) Yes (b) No

39. Have you received a blood transfusion before? (a) Yes (b) No

40. Can a person protect himself/herself from HIV by abstaining from sexual contact or intercourse especially with multiple partners or commercial sex workers? (a) Yes (b) No

41. Can a healthy apparently healthy looking individual be infected with HIV? (a) Yes (b) No

42. Based on your current understanding of HIV, is there medication that can cure HIV? (a) Yes (b) No

43. Can the HIV infection in a pregnant woman be passed on to her unborn child? (a) Yes (b) No

44. Have you ever undergone HIV testing in the past 6 months or since you were admitted in this hospital?

45. If No, Why? Give reason(s)

46. Have you ever heard of STIs? (a) Yes (b) No

47. Have you experienced any of the following symptoms in the past 12 months? (a) Genital ulcers (b) Swelling in groin (c) Itching in genital area (d) frequent painful urination

48. Have you ever been treated for STIs diseases? (a) Yes (b) No

49. Which of following STIs diseases did you receive treatment? (a) Gonorrhoea (b) Syphilis (c) *Chlamydia trachomatis* infection

50. Have you had a tattoo or tribal mark done on your body? (a) Yes (b) No

51. Shaving behavior
Do you shave? (a) Yes (b) No

52. If yes, how often do you shave? (a) daily (b) weekly (c) monthly

53. Where do you shave? (a) At home (b) Barbering shop (c) Salon

54. Nail cutting behavior
Do you cut your nails? (a) Yes (b) No

55. If yes, how often do you cut your nails? (a) daily (b) weekly (c)monthly

56. Where do you cut your nails? (a) At home (b) Commercial nail cutters

APPENDIX C

REAGENTS USED

1. ECLIA: Reagents and working solution

The reagent rackpack (M, RO, R1, R2) is labeled as HIVCOMPT where;

M streptavidin-coated micropaticles (transparent cap), 1 bottle, 6.5mL:
Streptavidin-coated microparticles 0.72 mg/mL; preservative.

R0 MES buffer 50 mmol/L, pH 5.5; 1.5% Nonidet P40; preservative(white cap), 1 bottle, 4 mL.

R1 Ant-p24, HIV1/2 – specific recombinant antigens (E. coli)-, HIV1/2-specific peptides-biotin (gray cap), 1 bottle, 7 mL:
Biotinylated monoclonal anti-p24 antibodies (mouse), biotinylated HIV-1/2-specific recombinant antigens (E. coli), biotinylated HIV-1/2-specific peptides >1.3mg/L; TRIS buffer 50 mmol/L, pH 7.5; preservative.

R2 Anti-p24-, HIV-1/2-specific recombinant antigens (E. coli)-, HIV-1/2-specific peptides- $(Ru(bpy)_3^{2+})$ (black cap), 1 bottle, 7 mL:
Monoclonal anti-p24 antibodies (mouse), HIV-1/2 specific recombinant antigens, HIV-1/2 specific peptides labeled with ruthenium complex >1.5mg/mL; TRIS buffer 50 mmol/L, pH 7.5; preservative.

HIVCOMPT Cal1 Negative calibrator (white cap), 2 bottles (lyophilized) for 1.0 mL each: Human serum, non reactive for anti-HIV-1 and anti-HIV-2.

HIVCOMPT Cal2 Positive calibrator (black cap), 2 bottles (lyophilized) for 1.0 mL each: Anti-HIV-1 positive human serum (inactivated) in human serum negative for anti-HIV-1 and anti-HIV-2.

The reagents in the kit are ready for use (except for HIVCOMPT Cal1 and HIVCOMPT Cal2). HIVCOMPT Cal1 and HIVCOMPT Cal2: the contents are carefully dissolved by adding exactly 1.0 mL of distilled or deionized water and allowed to stand, closed in the bottle supplied which is compatible with the system, for 15 minutes to reconstitute.

2. PCR

REAGENTS COBAS AmpliPrep/COBAS TaqMan HIV-1 Test
(P/N: 03542998 190)

HIV-1 CS1 (HIV-1 Magnetic Glass Particles Reagent Cassette)
Magnetic glass particles plus 93%(w/w) Isopropanol

HIV-1 CS2 (HIV-1 Lysis Reagent Cassette)

Sodium citrate dehydrate,

42.5%(w/w) Guanidine thiocyanate

< 14% Polydocanol

0.9% Dithiothreitol

HIV-1 CS3 (HIV-1 Multi-Reagent Cassette) containing:

EB (Elution Buffer)
Tris-base buffer
0.2% Methylparaben

HIV-1 CS4 (HIV-1 Test-Specific Reagent Cassette) containing:
HIV-1 QS ((HIV-1 Quantitation Standard)

Tris-HCl buffer

EDTA
< 0.005% Poly rA RNA (synthetic)
< 0.001% Armored HIV-1 RNA construct containing HIV-1 primer binding sequences and a unique probe binding region (non-infectious RNA in MS2 bacteriophage)
0.05% Sodium azide

HIV-1 MMX (HIV-1 Master Mix)

Tricine buffer,
Potassium acetate
Potassium hydroxide,
20% Dimethylsulfoxide,
Glycerol,
< 0.04% dATP, dCTP, dGTP, dUTP, Dttp,
< 0.003% Upstream and downstream primers to the GAG region of HIV-1,
< 0.003% Oligonucleotide aptamer
< 0.003% Fluorescent-labeled oligonucleotide probes specific for HIV-1 and the HIV-1 QS
< 0.05% Z05 DNA Polymerase (microbial)
< 0.1% AmpErase (uracil-N-glycosylase) enzyme (microbial)0.09% Sodium azide

CAP/CTM Mn^{2+} (CAP/CTM Manganese Solution)

< 0.5% Manganese acetate

Glacial acetic acid

0.09% Sodium azide

HIV-1 L(+)C (HIV-1 Low Positive Control)

< 0.001% Armored HIV-1 RNA construct containing HIV-1 sequences (non-infectious RNA in MS2 bacteriophage)
Negative Human Plasma, non-reactive by FDA licensed tests for antibody to HCV, antibody to HIV-1/2, HIV p24 antigen and HBsAg; HIV-1 RNA, HCV RNA and HBV DNA not detectable by PCR methods
0.1% ProClin 300 preservative

CTM (–) C [COBAS TaqMan Negative Control (Human Plasma)]

Negative Human Plasma, non-reactive by FDA licensed tests for antibody to HCV, antibody to HIV-1/2, HIV p24 antigen and HBsAg; HIV-1 RNA, HCV RNA and HBV DNA not detectable by PCR methods
0.1% ProClin 300 preservative

HIV-1 L(+)C Clip 1 x 4 Clips (HIV-1 Low Positive Control Barcode Clip)

HIV-1 (–) C Clip 1 x 4 Clips (HIV-1 Negative Control Barcode Clip)

PG WR (COBAS AmpliPrep/COBAS TaqMan Wash Reagent)
(P/N: 03587797 190)

Sodium citrate dehydrate
< 0.1% N-Methylisothiazolone-HCl

SPEX (COBAS Ampliprep/ COBAS TaqMan Specimen Pre-Extraction Reagent)
Sodium Citrate dehydrate
42.5% Guanidine thiocyanate
< 5% Polycocanol
1.8% Dithiothreitol
0.01% Citric Acid

3. Reagent Preparation for Genscreen™ ULTRA HIV Ag-Ab

NOTE: Before use, allow reagents were allowed to reach room temperature (18 – 30°C).
Reagent for use

1) Reagent 1 (R1): Microplate
   Each frame support containing 12 strips was wrapped in a sealed foil bag. The bag was cut using scissors or a scalpel 0.5 to 1 cm above the sealing. The bag was opened and the frame taken out. The unused strips were put back into the bag. The bag was closed carefully and put back into storage at +2–8°C.
2) Reagent 3 (R3): Negative Control

3) Reagent 4 (R4): HIV Ab positive control
   Reagent 5 (R5): HIV Ag positive solution
4) Reagent 6 (R6): Conjugate 1
5) Reagent 10 (R10): Stopping solution

Reagents to reconstitute:
Washing solution (20x concentrate): Reagent 2 (R2) was diluted 1:20 in distilled water to obtain the ready-to-use washing solution. 800ml was prepared for use for one plate of 12 strips.

Conjugate 2 working solution: reagent 7a (R7a) + Reagent 7b (R7b)
The vial of the lyophilised conjugate 2 (R7a) was gently tapped on the workbench to remove any substance from the rubber cap. The cap was carefully removed and the content was poured of the Conjugate Diluent vial (R7b) into the Lyophilised Conjugate vial (R7a). The cap was replaced and allowed to stand for 10 minutes, whilst gently shaking and inverting from time to time to ease dissolution.

Enzyme development solution: Reagent 8 (R8) + Reagent 9 (R9)
The chromogen (R9) was diluted 1:11 in the Substrate Buffer (R8) eg. 1ml reagent R9 + 10ml reagent R8. Stability was for 6 hours in the dark once prepared.

APPENDIX D

Protocol for Genscreen™ ULTRA HIV Ag-Ab

1.0 PROCEDURE & QC

1. Carefully establish the sample distribution and identification plan
2. Prepare the **diluted washing solution**
3. Prepare the **conjugate 2 working solution**
4. Take the carrier tray and the strips (R1) out of the protective pouch
5. Apply directly, without prior washing of the plate and in succession (suggested plate distribution)
   a. 25ul of conjugate 1 (R6) in each well
   b. 75ul of HIV Ag positive control (R5) in well A1
   c. 75ul of HIV Ab control (R4) in well B1
   d. 75ul of negative control (R3) in well C1, D1 and E1
   e. 75ul of specimen 1 in well F1
   f. 75ul of specimen 2 in well G1, etc...

i. Homogenise the mixture by a minimum of 3 aspirations with 75ul pipette or by shaking the microplate after the pipetting step.

ii. Depending on the used system, it is possible to modify the position of controls or the order of distribution.

NB: the sample and conjugate 1 distribution can be visually controlled at this step of the manipulation: after adding the sample, the conjugate 1 turns yellow-green to blue

6. When possible, cover the microplate with adhesive film. Press firmly all over the plate to ensure a tight seal.
7. Incubate the microplate in a thermostat-controlled water-bath or microplate incubator at 37^{o}C ±1^{o}C for 1 hour ±4 minutes
8. Remove the adhesive film. Aspirate the contents of all the wells into a container for biohazardous waste (containing sodium hypochlorite). Add into each well a minimum of 0.370ml of washing solution. Allow a soak time of at least 30 seconds. Aspirate again. Repeat this procedure a minimum of two times (i.e. in total of a minimum of three washes). The residual volume must be lower than 10ul (if necessary dry the plate by

turning it upside down on absorbent paper). If an automatic washer is used, follow the same procedure.

9. Quickly dispense 100ul of conjugate 2 solution (R7a + R7b) into all wells, the conjugate must be shaken before use.
10. When possible, cover the plate with new adhesive film and incubate for 30 minutes ±4 minutes at room temperature (18-30^{o}C)
11. Remove the adhesive film, empty all wells by aspiration and wash a minimum of 5 times as described above. The residual volume must be lower than 10ul (if necessary, dry the strips by turning them upside down on absorbent paper)
12. Quickly dispense into each well 80ul of prepared substrate solution (R8+R9), freshly prepared before use. Allow the reaction to develop in the dark for 30±4 minutes at room temperature (18-30^{o}C). Do not use adhesive film during incubation.
13. Add 100ul stopping solution (R10) by using the same sequence and rate of distribution as for the substrate solution.
14. Carefully wipe the plate bottom. At least 2 minutes after stopping solution addition and within 30minutes of stopping the reaction, read the optical density at 450/620-700nm using a plate reader within 30minutes of stopping the reaction(the stripes must always be kept away from light before reading)
15. Check for agreement between the spectrophotometric and visual reading and against the plate and sample distribution and identification plan.

RESULT RECORDING AND INTERPRETATION

The presence or absence of detectable HIV antigen or antibodies to HIV-1 and/or HIV-2 is determined by comparing the absorbance measured for each sample to the calculated cut-off value.

APPENDIX E

OraQuick® ADVANCE Rapid HIV-1/2 antibody test

Procedure

- Places kits on bench to bring to room temperature.
- Allow samples to thaw at room temperature for 30-60 minutes
- Put 40µl of serum into test well and leave on a flat work bench.
- Results read after 20 minutes

APPENDIX F

First Response HIV Card Test 1-2.0

Procedure

- Places kits on bench to bring to room temperature.
- Allow samples to thaw at room temperature for 30-60 minutes
- Place a drop of serum into test well with the integrated micropiprtte and leave on a flat work bench.
- Results read after 10 minutes