HUMAN T-CELL LYMPHOTROPIC VIRUS TYPE I AND II
(HTLV-I/II) INFECTION AMONG BLOOD DONORS AT THE
NATIONAL BLOOD SERVICE (NBSG); IMPLICATION FOR
BLOOD DONOR EXCLUSION

BEINYA DOMINIC KWEKU
(10551312)

THIS THESIS IS SUBMITTED TO THE UNIVERSITY OF GHANA,
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JULY, 2017
DECLARATION

DECLARATION BY THE CANDIDATE

I declare that, I have wholly undertaken the study reported herein under supervision and that except portions where references have been duly cited, this work is the outcome of my research.

Signature………………………..                                          Date ……/……/………….

Beinya Dominic Kweku (10551312)

DECLARATION BY SUPERVISORS

We hereby declare that the principal work and presentation of the thesis were supervised by us in accordance with guidelines on supervision of thesis laid down by the University of Ghana.

Principal supervisor

Signature………………………..                                          Date……/…../………………

(Dr. M.M Addae)

Co-supervisor

Signature………………………..                                          Date……/…../………………

(Mr E.G Narter-Olaga)
ABSTRACT

Background: The human T-cell lymphotropic/leukemia virus (HTLV) was button down in 1980 by Poiesz et al. HTLV are a group of human retroviruses that are known to cause a type of cancer called adult T-cell leukemia/lymphoma (ATL) and also a demyelinating disease called HTLV-1 associated myelopathy/tropical spastic paraparesis (HAM/TSP). There are four main types of HTLV, thus, HTLV-I, HTLV-II, HTLV-III and HTLV-IV. About 10-20 million people are infected with HTLV I and II across the globe. The main modes of transmission of the virus is by mother to child (through breastfeeding), sexual intercourse, sharing of infected needles among drug users and lastly by blood transfusion. HTLV-I and II mostly infect CD4+ and CD8+ T-cells respectively. Reported seroprevalence of HTLV among Ghanaian blood donors over the past ten years is between the ranges of 0.5%-4.2%. Blood donors in Ghana and Africa as a whole are still not screened for HTLV-I and II since the report of the discovery of the virus. This therefore serves as a risk factor for transmitting the virus to blood recipients through infected blood.

Aim: To determine the prevalence of HTLV-I and II antibodies among blood donors at the national blood service, Ghana.

Methods: The research design was a descriptive prospective cross sectional study involving 300 consecutive voluntary blood donors from the southern area blood centre of the National Blood Service, Ghana (NBSG). The study used stored (-20°C) sera separated from 3ml of blood samples obtained from voluntary blood donors at the National Blood Service, Ghana (NBSG) who had consented to join the study orally by writing and who had been proven to be in good health to donate. HTLV-I and II antibodies were duplicately screened for using an enzyme linked immunosorbent assay as a screening test. A well structured questionnaire was also administered
to the voluntary blood donors to obtain some basic information such as age, gender, marital status, occupation and their frequency of donating blood. The statistical software SPSS version 20 was used to generate the descriptive analysis of the results obtained.

**Results:** With the total 300 voluntary blood donors sera samples tested, 10(3.3%) were positive for anti-HTLV-I/II. The ages of the donors ranged between 18-59 years. The majority 104(34.7%) of the donors were in the age range of 25-31 years, and the age range with the least number of donors was 53-59 years 5(1.7%). Three females (3.8%) were infected out of 78(26%) while 7(3.2%) out of 222(74.0%) males were infected. Majority of the donors were single 200(66.7%) and 6(3.0%) out of the total unmarried donors 200(66.7%) tested positive while 96(32.0%) were married and 4(4.2%) of them were infected. Two hundred (66.7%) of the total participants of the study were first time donors and none (0.0%) of those were infected while 34(11.3%) were frequent donors with more than 3 blood donations had 7(20.6%) of them testing positive.

**Conclusion:** This study therefore reports an HTLV-I/II seroprevalence of 3.3%. The findings of this study implies that, HTLV-I/II occurs among apparently healthy voluntary blood donors at the southern area blood centre of the national blood service Ghana. Thus, there’s the need to conduct a nationwide large population based research on the same HTLV-I/II infection among voluntary blood donors so as to determine or ascertain the exact prevalence and then determine whether to screen for the virus or not among blood donors.
DEDICATIONS

This work is dedicated to the Almighty God, Mr I.C.K. Beinya, my father, Mrs Justina Asem Beinya, my mother, siblings and the Department of Medical Laboratory Sciences of the School of Biomedical and Allied Health Sciences, College of Health Sciences, University of Ghana, especially, the entire 2015-2017 Professional MSc Medical Laboratory Sciences Class.
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</tr>
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<tr>
<td>AIDS</td>
<td>Acquired Immunodeficiency Syndrome</td>
</tr>
<tr>
<td>ATL</td>
<td>Adult T-cell Lymphoma</td>
</tr>
<tr>
<td>CD</td>
<td>Cluster of Differentiation</td>
</tr>
<tr>
<td>DNA</td>
<td>DeoxyriboNucleic Acid</td>
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<tr>
<td>ELISA</td>
<td>Enzyme Linked Immunosorbent Assay</td>
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<td>HAM/TSP</td>
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</tr>
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<td>National Blood Service Ghana</td>
</tr>
<tr>
<td>pol</td>
<td>polymerase</td>
</tr>
<tr>
<td>RNA</td>
<td>Ribonucleic Acid</td>
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CHAPTER ONE

BACKGROUND

1.1 INTRODUCTION

Human T-cell lymphoma/leukemia virus also known as Human T-cell lymphotropic virus or Human T-cell lymphocyte virus (HTLV) was button down in 1980 as the agent that causes adult T-cell leukemia/lymphoma (ATL) (Okoye et al, 2014). This virus in was the first human retrovirus that was described by Poiesz et al (Poiesz et al, 1980; Matsuoka, 2005). HTLV is from the Retroviridae family. It also belongs to the subfamily called Orthoretroviridae, the genus Deltaretrovirus and the species simian T-lymphotropic virus (Matsuoka, 2005). Other Deltaretroviruses include Simian T-leukemia virus and bovine leukemia virus (Matsuoka, 2005).

HTLV is composed of four types; which are HTLV-I, HTLV-II, HTLV-III and HTLV-IV. HTLV-I and II were the initial types to be discovered in human population however HTLV-III and IV were discovered in Central Africa in 2005 (Goncalves, 2015). HTLV-I and II have been associated with several health conditions. HTLV III and IV on the other hand have not been associated with any disease (Goncalves, 2015).

HTLV-I is capable of infecting T-lymphocytes, B-lymphocytes, monocytes and fibroblasts (Okoye et al, 2014). Nonetheless, the provirus of HTLV-I is chiefly detected in CD4 positive lymphocytes. However 10% detection is possible in CD8-positive T-lymphocytes (Matsuoka, 2005; Yasunaga, 2001). The hefty clinical consequence that results from HTLV-I is neoplasia (Okoye et al, 2014). Research currently has proved HTLV-I to be the etiological cause of Adult T-cell Lymphoma/Leukemia (ATLL). This proof is as a result of the fact that almost all cases of
ATL are seropositive for HTLV-I (Okoye et al., 2014). Secondly too the provirus of HTLV-I has also been found to be in leukemic cells and not located in other cells of patients with such health conditions (Forbi & Odetunde, 2007).

Moreover, HTLV-I again causes other health conditions which includes HTLV-I associated myelopathies, infective dermatitis, uveitis, arthropathy, interstitial pneumonitis, immune deficiency with opportunistic infection, cutaneous lymphomas such as mycosis fungoides, Sézary’s syndrome, Sjögren’s syndrome, renal failure, B-cell leukemia and small-cell lung cancer (Okoye et al., 2014). HTLV-I infects as many as 10-20 million individuals across the globe (Gessain & Olivier, 2012). Irrespective of this the dissemination of the virus is not uniform but is marked by clusters of high endemicity (Plancouline, Gessain & Tortevoye, 2006).

Seroprevalence rate of HTLV-I antibodies has been found to be high reaching more than 2% in adults. This high seroprevalence has been determined through research work in southwestern Japan, the Caribbean Basin, South America, parts of the Middle East, Melanesia, the West Indies, Jamaica and Sub-Saharan Africa. The prevalence rate of HTLV-I in the African adult population is approximately 0.5% - 33% (Okoye et al., 2014). Reported endemic areas include Gabon, Cameroon, Guinea, the Democratic Republic of Congo and Ivory Coast (Gessain & Olivier, 2012).

A few of sociodemographic factors linked with high prevalence of HTLV-I infection include geographical location, lower socioeconomic status, female sex, higher age, marital status, promiscuity and recurrent sexually transmitted diseases (STD’s) (Gessain & Olivier, 2012). Age related increase often is more characterized in females than males. Women are considerably most often likely to be infected than men at the age of 40-50 years. As a result, several European
studies have shown that the frequencies of HTLV infection in pre-partum women is 50-100 folds higher than found in blood donors (Machuca et al, 2000). The incubation period of HTLV-I infection is prolonged about 6 months to decades, however, the onset of myelopathy is shorter in individuals who acquired the infection via breastfeeding or by the vertical route. Duration between blood transfusion and then HTLV-I associated myelopathy development is also short in immune compromised individuals (Okoye et al, 2014).

There are four main routes through which HTLV-I is transmitted. This includes; vertical transmission (mother to child), sexual transmission and blood transfusion as well as sharing of infected needles among drug user. Blood transfusion is most efficient, since the infecting viral load in the blood is usually high (Chang et al, 2013). A range of transfusion transmissible agents are spread via transfusion of infected blood donated by seemingly healthy and asymptomatic blood donors (Allain et al, 2009). Among the identified virulent pathogens are viruses, bacteria, fungi, protozoa and helminthes but approximately 175 species are well thought-out to be emerging pathogens (Taylor, Latham, Woolhouse, 2001). The diversity of transfusion transmissible infectious agents range from hepatitis B virus (HBV), hepatitis C virus (HCV), human immuno-deficiency viruses-1/2 (HIV-1/2), human T-cell lymphotropic viruses-I/II (HTLV-I/II), Cytomegalovirus (CMV), Parvovirus B19, West Nile Virus (WNV), Dengue virus, Trypanosomiasis, Malaria parasites to variant Creutzfeldt-Jakob Disease (variant CJD) (Allain et al, 2009).

Intentional and unpaid blood donors are the safest categories to donate blood. In Iran for example, the number of blood donations according to literature exceeds 1.7 million units every year and a hundred fold of such donations are intentional and unpaid for (Karimi et al, 2013). Additionally, in the same country, 40% of all blood donations were made from regular blood
donors during the year 2007 (Maghsudlu et al, 2009). In such countries that have recognized HTLV-I/II prevalence, all donated blood are tested or screened for HIV-1 and 2 antigen-antibody, HTLV I/II, HCV antibody, Syphilis and HBV surface antigen (HBsAg). This is one of the main strategies for protecting against serious transfusion-transmissible infections (TTI’s) in blood recipients (Karimi et al, 2013).

Therefore, in recent years the danger of transfusion-transmitted infections has been greatly lessened by progress made in donor screening and testing so that today the supply is safer. Nevertheless, emerging and re-emerging infections (including also infectious diseases) are thought to be important factors of mortality and morbidity in different populations (WHO, 2004; Taylor, Latham, Woolhouse, 2001).

Emerging infections are defined to be those infectious diseases whose occurrence has elevated within the past two decades or threatens to elevate in the forthcoming years (Karimi et al, 2013). Numerous factors are part of the advent of emerging disease. These infections may result from ecological modification or originate from genetic, biological, social and economic factors (Karimi et al, 2013). The entirety of these factors will be the forerunner to the development of emerging disease. Emerging and re-emerging microorganisms like other microbial agents can endanger blood safety (Karimi et al, 2013).

In HTLV endemic areas, about 44-63% cases of HTLV-I seroconversion has been observed. This observation occurred in people who had been transfused with contaminated blood cells (Oladipo et al, 2015). The HTLV-I contamination of blood prevalence has been reported to be about 0.3% in the United States of America and 0.7% in Brazil (Oladipo et al, 2015).
1.2 PROBLEM STATEMENT

Blood safety continues to be a critical public health concern in Africa as mortality and morbidity due to unsafe blood go on to harmfully impact on populations (Bloch, Vermeulen, & Murphy, 2012). Currently HTLV has been thought to be one of the utmost worries frightening the health of blood recipients in some endemic areas (Karmi et al, 2013). Intravenous contact with infected blood or blood products is the most effective fashion of HTLV transmission with up to 63.4% seroconversion rate reported in Japan (Goncalves et al, 2010; Manga et al, 2016). In addition, blood transfusion linked HTLV associated diseases build up more hastily than with other modes of transmission (Manga et al, 2016).

Human T-cell lymphotropic virus is prevalent mainly in Japan, Central and West Africa as well as the Caribbean Basin (Gessain & Olivier, 2012). Seroprevalence among healthy Ghanaian blood donors that has been reported over the past 15-20 years is between the range of 0.5% - 4.2% (Sarkodie et al, 2001; Ampofo et al, 2002). These findings point to the fact that HTLV infection occurs in Ghana.

HTLV is part of the transfusion transmissible infections (TTI’s) but is not part of the TTI’s that are screened for before blood donation in Ghana which is a common challenge in most parts of Africa and the world at large. Since HTLV has not been routinely screened for before, during and after the establishment of the National Blood Service, Ghana (NBSG), there is the need to investigate the seroprevalence of HTLV that occurs among blood donors at the NBSG. As the mission of the NBSG is to save lives of patients by providing safe and adequate blood products and other related blood services through professionalism of their staff and generosity of their
voluntary non-remunerated blood donors, there is the need to investigate the seroprevalence of HTLV I and II antibodies that occurs among screened donated blood at the NBSG using less expensive assays for screening, confirmation and characterization of all positive cases.

1.3 JUSTIFICATION

At the moment in Ghana, blood donors are not screened routinely for HTLV-I/II antibodies at all blood transfusion units which includes the NBSG which helps supply blood and blood components to most hospitals in the southern part of the country. There is the need to determine the prevalence of HTLV infection among blood that is cleared as safe for transfusion by the NBSG after the routine screening of the donated blood available. The outcome of this research will inform health policy makers as well as the scientific community in the country, the continent and beyond with the necessary data with respect to the prevalence of HTLV that occurs among blood donated at the NBSG. National Blood Service Ghana was chosen as the study site because it serves the southern part of the country with large amounts of blood that is devoid of HTLV screening and thus serves as source of spreading and maintenance of HTLV infection in the population. This is due to the fact that the virus is not screened prior to the donor donating his or her blood. Thus knowledge of the current prevalence of HTLV in the country will help to establish prophylactic measures geared at them and have a strategic impact on its spread.
1.4 AIM

To determine the prevalence of HTLV I and II antibodies among voluntary blood donors at the southern blood center of the National Blood Service, Ghana.

1.5 SPECIFIC OBJECTIVES

1. To determine the presence of HTLV I and II antibodies in serum or plasma of blood samples using the enzyme linked immunosorbent assay as a screening test.

2. To determine the most HTLV-I/II affected age group, gender and occupation of the voluntary blood donors
CHAPTER TWO

LITERATURE REVIEW

2.1 TRANSFUSION TRANSMISSIBLE INFECTIONS

Although the past 30 years has seen the emergence of de novo and remerging infections as well as the strict donor screening and initiation of sophisticated technology institution in most transfusion centers, transfusion-transmissible infections remain a challenge to transfusion experts. Many factors have been identified to decrease the danger of spreading such “infectious agents by donor exclusion for clinical history of risk factors, screening for the serological markers of infections and nucleic acid testing (NAT) through viral gene amplification for direct and sensitive detection of the recognized infectious agents” (Allain et al, 2009).

In the industrialised countries, especially in the western part of the world, there has been a considerable reduction in the occurrence of transfusion-transmitted infections as a result of enhancement in donor screening, testing and inactivation of viral particles in blood products (Allain et al, 2009). Nonetheless, the persistence of markers of infectious agents amid blood donors coupled with the challenges of inadequate resources such as lack of sophisticated, sensitive and expensive technologies for screening of blood product are the factors for the declining standards in blood safety in developing countries (Allain et al, 2009) like Ghana.

Blood transfusion persists to perform a crucial role in modern health care. In order to assure the necessity for a secure and effective blood provision, it is critical to not only keep hold of active donors but as well to endlessly enroll de novo donors to supplant those who withdraw from
donation (Burgdorf et al, 2017). The considered most effective course through which viral transmission occurs is blood transfusion and the seroconversion of the recipients is approximately two months whilst for the extra means of transmission, the seroconversion is just about six months or more (Goncalves et al, 2010). The usual blood screening to detect immunoglobulin to HTLV-I and HTLV-II has been established among blood banks in many countries including Australia, USA, Canada, Japan, Brazil, France and the Caribbean (Gessian et al, 2011). Presently, in almost all African countries, Ghana being part, there is no routine screening planned for HTLV among blood donors (Terry et al, 2011). Prevalence studies on HTLV from some regions of Ghana as well as some African states do exist. However, there is only a small amount of data on the load of this virus over the last ten years (Burgdorf et al, 2017).

HTLV are known cancerous agents threatening the health of blood recipients. In Iran for instance, HTLV in about four years back was of immense concern (Karimi et al, 2013). Nonetheless, the usual screening tests are being conducted in a few provinces of Iran with a high prevalence of HTLV (Karimi et al, 2013).

The transmission frequency of HTLV-I or HTLV-II in a recipient of an infective blood unit is between 20-60% (Kaur and Basu, 2005). The threat of transmission of HTLV from a screened blood unit is low, that is 1 in 640,000 (Kaur and Basu, 2005). Contact with infected viable lymphocytes can cause infection as both the viruses are cell associated (Kaur and Basu, 2005).

Implicated blood components for HTLV transmission are whole blood, packed red blood cells and platelets (Kaur and Basu, 2005). As refrigeration of blood product over 10 days results in degradation of lymphocytes and a reduction in load of infectious viruses, plasma and plasma
derivatives do not transmit the virus (Kaur and Basu, 2005). The connection between infectivity and fresh cellular components increases the possibility that transmission of HTLV by transfusion requires viable T-lymphocytes and that their elimination from blood donations may clear the probably infectious cells (Kaur and Basu, 2005). The security of blood donation is a multifactorial process. Donor screening and testing are two main stays in blood processing centers (Karimi et al, 2013).

Currently, blood transfusion has become a substantial part of medical practice (Burgdorf et al, 2017). Across the globe, during every second of the day, someone needs blood for surgery, trauma, severe anemia or complications of pregnancy (Burgdorf et al, 2017). In other words, without blood transfusion, life saving medical treatments such as surgical procedures, pregnancy-related complications, the treatment of anaemic and other multi-transfused patients, cancer treatment, organ transplants and bone marrow transplants would not be a reality (Karimi et al, 2013). It is as a result necessary that adequate blood supplies are obtainable within a very short possible time without overlooking the safety of the blood (Burgdorf et al, 2017).

2.2 HUMAN T-CELL LYMPHOTROPIC VIRUS

Human T-cell lymphotropic virus type-I (HTLV-I) and Human T-cell lymphotropic virus type-II (HTLV-II) are fit in the Retroviridae family and the genus Deltaretrovirus. The first retroviruses to be identified in humans are namely HTLV-I and HTLV-II (Poiesz et al, 1980). HTLV-III and HTLV-IV are the two novel retroviruses that have been secluded recently in Central Africa in 2005 (Wolfe et al, 2005; Ewa, 2015). In spite of this new discovery, these two new viruses have not been linked with any human disease and it spread among humans has not been established (Gessain et al, 2012).
Retroviruses are RNA microbes which employ an enzyme called reverse transcriptase to yield deoxyribonucleic acid (DNA) from RNA. DNA produced is then inserted into the host’s genome. HTLV largely infects T-lymphocytes (Ewa, 2015).

Previous to 1989, seclusion of retroviruses was possible only in non human primates (NHP). The actual believe among the scientific community then were that retroviruses did not exist. In 2005, in the journal Retrovirology, Gallo mirrored earlier concepts that supported this belief (Ewa, 2015).

Three decades after the first identification, four HTLVs are well-known. Both HTLV-I and HTLV-II are implicated within the keen dissemination of epidemics thus influencing 15-20 million persons across the globe (Proietti et al, 2005). The total prevalence in the United States is 22 per 100,000 population with HTLV-I being extra widespread than HTLV-II however current data has revealed a broad-spectrum reduction since the 1990s (Cook & Taylor, 2014). HTLV-I is the most clinically important of the two, as it has been shown to be the causal agent of multiple abnormalities. As a minimum, 500,000 of the people infected with HTLV-I finally build up a fast lethal leukemia whilst development of an incapacitating myelopathy occurs in others. Also some develop inflammatory disorders such as uveitis, infectious dermatitis etc. HTLV-II is attributed to milder neurologic disorders and chronic pulmonary infections (Ewa, 2015). While HTLV has the competence toward infecting several cell varieties such as T cells, B cells, endothelial cells, glial cells and monocytes of both human and non-human origin, the only cells susceptible to HTLV transformation are primarily T-lymphocytes.
2.3.0 GENETIC STRUCTURE

Human retroviruses employ the use of their genomic constituents very effectively due to their restricted genetic make-up size. Regulating the replication of genome copy is a sole responsibility of their accessory gene (Gallo, 2002). Most importantly the HIV robustly duplicates to yield progeny virus while HTLV amplifies the amount “of infected cells by the action of accessory genes” (Yorifumi et al, 2005). HTLV-I and II are genetically associated and multifaceted retroviruses that are capable of immortalizing human T-cells \textit{in-vitro} and set-up lifelong and lasting infections \textit{in-vivo}. Despite such seeming connection, HTLV-I and II demonstrate an appreciably dissimilar pathogenic capability (Rende et al, 2012). However, HTLV-I and II has 70% or more genetic structure similarity, as a result much light will be thrown on the genetic structure of HTLV-I since almost all literature reviewed emphasized on HTLV-I due to the similarity that occurs between the two viruses.

2.3.1 GENETIC STRUCTURE OF HTLV-I

HTLV is an exogenous human retrovirus which varies a bit in sequence in contrast to HIV-I. Picking information from the latest classification, HTLV-I is categorized as an intricate retrovirus (Houshang et al, 2006; Koralnik, 1996; Brady, 1996). The genetic material of retroviruses is the ribonucleic acid (RNA) while the deoxyribonucleic acid (DNA) makes up their genetic constituent in the host cell it attacks (Houshang \textit{et al}, 2006). The stretch of the HTLV-I genome is 9.032 base pair (bp) (Seiki \textit{et al}, 1983; Houshang \textit{et al}, 2006).

The cluster of antigens are solely like the other retroviruses, that is, gag, polymerase (pol) and envelope (env) genes are lined by long terminal repeats (LTR) (Houshang \textit{et al}, 2006). Components of the LTR include, U3, R and U5 regions. Function of U3 is to control the
transcription of virus. The U3 region is composed of essential elements for example, the TATA box. The TATA box is important for viral transcription. The TATA box is simply a sequence that ensures the polyadenylation and termination of the RNA messenger. Another constituent of the U3 region is the Tax Responsive elements (TRE) involved in TAX protein transcription. TRE is responsible for regulating the transcription of the HTLV-I provirus (Houshang et al, 2006). There is an overlap between the R region and the 3’ of the U3 region. Majority of the Rex-response elements are found in the Rex region (Houshang et al, 2006). Core protein of the virus is encoded by the gag gene. The initial synthesized molecular weight of the viral core protein is approximately 53kD (Houshang et al, 2006). “However, there is cleaving of the initial synthesized viral core protein in the course of viral maturation into matured matrix P19 (MA), the capsid P24 (CA) and the nucleocapsid P15 (NC)” (Houshang et al, 2006).

“The protease of HTLV-I extends the 5’end of the pol region and also the end of the gag region. This gene contains information for the synthesis of a P234 amino acid protein with a molecular weight of 14kD”. The pol gene’s 5’end overlies the 3’end of the protease. Numerous enzymes are encoded by the pol gene, these include: RNase H, reverse transcriptase (RT) and integrase. RT is important for production of viral DNA. Responsibility of RNase H is RNA template and tRNA primer degradation. Integrase is crucial for the incorporation of the viral DNA into the cell of interest (Houshang et al, 2006).

The connection between the virion and the host cell hence it’s entry into the host cell is made possible by the envelope proteins. The envelope proteins are produced as precursors of 62kD molecular weight. “This precursor is cleaved into a gp45 surface protein (SU) and also a gp20 transmembrane protein (TM)”’. The surface protein has a molecular weight of 45kD and is also a hydrophobic glycoprotein. The surface protein is important for ensuring binding of the virus to
its receptor on the host cell (Houshang et al., 2006). The transmembrane proteins also have a molecular weight of 20kD and a glycoprotein as well. Essence of the transmembrane protein is for ensuring the anchorage of the surface protein and also the transmembrane complex at the surface of the infected cells or virion (Houshang et al., 2006).

PX region is found between the env gene and the 3’LTR of HTLV-I genome (Houshang et al., 2006). Contained in the PX are four open reading frames (ORFs), X-I, X-II, X-III and X-IV (Houshang et al., 2006). Two significant proteins encoded by the PX region of the HTLV-I include Rex and Tax.

The Tax gene is encoded by the IV open reading frame. Tax is a protein that has a 40kD molecular weight and composed of 353 amino acids (Houshang et al., 2006; Jeang et al., 2004). Tax is confined to the nuclei of HTLV-I infected cells. Tax is again a trans-activating nuclear phosphoprotein which controls transcription of HTLV-I by acting together with TRE1 and TRE2 found within the U3 of the proviral LTR (Houshang et al., 2006). It moves into the cytoplasm by means of a nuclear export indicator (Burton et al., 2000). How this movement occurs is vague, nonetheless, bits of information points to the fact that Tax attaches to tristetrapolin (Twizere et al., 2003) also, tristetrapolin relates with nucleoporin (Carman & Nadler, 2004) and this increases the likelihood that tristetrapolin may aid as a probable nucleocytoplasmic transporter for Tax (Jeang et al., 2004). Attachment of Tax to these DNA elements occurs indirectly, however, it triggers extra transcription factors which binds to TRE1 and TRE2 (Houshang et al., 2006; Ng et al., 2001).

There has been an identification of three key pathways of Tax transactivation (Houshang et al., 2006). This includes:
a. “c-AMP responsive element binding protein and activating transcription factor pathway (CRE/ATF)”.

b. “The nuclear factor kappa binding pathway (NF_{\kappa}B/Rel)”


Adding up to the HTLV-LTR, the promoter regions of several genes which posses NF_{\kappa}B binding sites are transactivated by Tax (Houshang et al, 2006). “All these includes: interleukin 1(IL-1), IL-2, IL-3, granulocyte macrophage colony stimulation factor (GM-CSF), c-fos, C-sis, c-myc, vimentin, transforming growth factor B1 (TGF-B1), tumor necrosis factor β (TNF β), IL-6, IL-10, and IL-5” (Houshang et al, 2006; Chung et al, 2003). Moreover, extra genes including various transcription factors (Fos/Jun), nerve growth factor (NGF), interferon gamma (INF-γ), parathyroid hormone-related protein and major histocompatibility complex (MHC) class I are transactivated by Tax (Houshang et al, 2006). The DNA repair enzyme named DNA polymerase β is the solitary enzyme recognized to be repressed by Tax.

Rex which is also a 27kD phosphoprotein, performs crucial tasks as a controller of viral structural gene expression. However, this represses the transcription of the genome of the virus (Houshang et al, 2006). Encoding of this protein is performed by ORF III. There is a link between Rex protein and intracellular transcription of unspliced as well as singly spliced mRNA of the virus.
2.4 STRATEGY USED BY HTLV-I DURING REPLICATION

As soon as a retrovirus penetrates into several host cells, there occurs a reverse transcription of the genomic RNA into a double stranded form of DNA (Satou & Matsouka, 2012). Expression of viral genes after DNA integration results in synthesis of infectious virions. Retroviral replication involves two models. This includes new infection from old infected cells to cells uninfected as well as clonal expansion of infected host cells (Matsouka & Jeang, 2010). The expression of viral structural proteins and the assemblage of the viral particle is a requirement for both cell to cell mediated de novo infection and free viral particle (Satou & Matsouka, 2012). Tax expression boosts viral structural genes transcription from the HTLV-I plus strand in this circumstance. Scientific reports available on this subject under discussion have it that, free viral particles of HTLV-I are not means of HTLV-I infection, rather, through cell to cell transmission
due to the virological synapse (Igakura et al., 2003). A study has also shown that, the extracellular biofilm like structure located on host cells has a task in cell to cell transmission of HTLV-I (Thoulouze & Alcover, 2011). The cell to cell transmission of HTLV-I is known to aid the early development of a population of cells infected (Satou & Matsouka, 2012). Cells with Tax expressed can be removed by the host cytotoxic T cell lymphocytes (CTL) response due to development of host immunity against HTLV-I (Satou & Matsouka, 2012). Following the development of anti-HTLV-I, immunity of HTLV-I occurs largely only by clonal expansion of cells infected (Etoh et al., 1997; Cavrois et al., 1998; Satou & Matsouka, 2012). The replication of HTLV-I as a provirus and thus increasing the number of host cells infected enables the virus to avoid immune attack by the host cells. Replication of viral cells through the clonal expansion of infected cells aids endurance of the HBZ-expressed cells due to low immunogenicity of HBZ cells (Suemori et al., 2009). This could be justified as a result of the weak attaching activity of HBZ peptide to MHC molecules (MacNamara et al., 2010).

In the course of Chronic stage of HTLV-I infection, there is a steady proviral load in the majority of infected persons. Nonetheless, with individuals infected is a wide array of variation of proviral load. Host genomic factors such as MHC class I molecules are known to be essential determinants of proviral load as the variation of HTLV-I sequence among infected persons is vastly restricted (Satou & Matsouka, 2012).

Tax was formerly regarded as the most significant antigen for the host immune response which regulates proviral load (Bangham & Osame, 2005). However topical proof regarding not long discovered viral protein HBZ has changed the focus of research. This discovery implies that persons with MHC alleles that can effectively attach and present proteins form HBZ have essentially lower proviral load and are unlikely to establish HAM/TSP (Macnamara et al., 2010).
Thus, expression of HBZ is an essential determining factor of viral persistence in the chronic phase of HTLV-I infection (Satou & Matsuoka, 2012). Worthy to note is the fact that expression of Tax is often repressed or diminished in ATL cells while there is a constitutive detection of HBZ expression (Satou & Matsouka, 2012; Gillet et al, 2011).

A sign that proliferation of cells infected and viral replication is regulated by regulatory and accessory genes together is based on the fact that, although HBZ can play an essential task in viral persistence, in vivo persistence of HTLV-I is reduced by mutation of other accessory genes such as p30, p13, p12 (Collins et al, 1998; Bartoe et al, 2000; Franchini et al, 2003; Hiraragi et al, 2006; Valeri et al, 2010; Andersen et al, 2011; Satou & Matsouka, 2012).

2.5 CELL-TO-CELL SPREAD: THE HTLV-I VIROLOGICAL SYNAPSE

Mode of spreading of HTLV-I and HTLV-II are similar to that of HIV. Nevertheless, unbound infectious HTLV units are commonly located in plasma (Paiva & Casseb, 2014). Straight cell to cell linkin is important to ensure effective spread of HTLV-I among cells infected and non-infected cells through dedicated as well as greatly ordered apparatus called virological synapse. Through the virological synapse the virus weakens the conventional T-cell physiology. Straight cell connection swiftly stimulates cytoskeleton polarization of cells infected (Paiva & Casseb, 2014). HTLV-I core (gag protein) complexes as well as HTLV-I genetic material accrue before the cell to cell junction hence quickly transformed into the non-infected cell and this needs about 120 minutes to complete (Igakura et al, 2003). A further likely, mode of in vivo spread of HTLV-I among cells includes engulfment of uninhibited HTLV-I cells by dendritic cells and their ensuing transfer to T-cells via cell-to-cell contact (Jones et al, 2008). Additionally, viral particles can be maintained on the exterior cellular layer of the cell in a biofilm-like makeup before it is
tangentially relocated unto beneficiary cells that are external to the cell-to-cell contact regions (Pais-Correia et al, 2010). Whereas HTLV-I infects movable cells (lymphocytes), the virological synapse amplifies transformation effectiveness and hinders virus contact to host defence mechanisms (Sattentau, 2008). Glut-1 human glucose transporter has been discovered as a receptor for infection by cell-free virus (Manel et al, 2003).

2.6 STRUCTURE OF HTLV-I BASIC LEUCINE ZIPPER (HBZ)

HTLV-I basic leucine zipper (HBZ) is an antisense mRNA transcribed from the 3’LTR. HBZ expression has been shown currently to occur constantly within cells of ATL, however, Tax gene and other viral genes are often not identified in cells of ATL (Satou et al, 2006; Takeda et al, 2004). This implies that, HBZ could have an active role in cellular transformation and leukemogenesis (Matsuoka & Green, 2009).

HTLV-I anti-sense transcription was initially reported in 1989 (Larocca et al, 1989; Matsuoka & Green, 2009). Ten years on after 1989, the viral protein was identified in cell lines transformed by HTLV-I and again detected as an attaching protein to CREB2 by the yeast two hybrid method (Satou et al, 2006). This viral protein attaches to CREB2 via its bZIP domain (Gaudray et al, 2002) hence named the HTLV-I bZIP factor (HBZ).

The quick amplification of the 5’ ends of complementary DNA (cDNA) led to the detection of two dissimilar HBZ transcripts, that is, spliced (sHBZ) and unspliced (usHBZ) forms (Cavanagh et al, 2006; Murata et al, 2006; Satou et al, 2006). There has been the detection of the promoter regions of both HBZ transcript forms; however both promoters are devoid of a TATA. Sp1 also has been detected as a crucial transcription factor for the occurrence of the sHBZ gene (Yoshida et al, 2008). Three domains constitute the HBZ protein, thus, activation domain, central
domain and bZIP domain (Gaudray et al., 2002; Hivin et al., 2005). HBZ attaches to host factors using bZIP domain, including c-Jun, JunB, JunD, CREB2 and CREB (Basbous et al., 2003; Thebault et al., 2004). Just a little amino acid located at the N-terminus differentiates sHBZ from the usHBZ (Murata et al., 2006). Nonetheless, there are markedly definite features. There’s much difference between their half-life too, thus, sHBZ has a longer half-life unlike usHBZ (Yoshida et al., 2008; Landry et al., 2009). The mRNA of sHBZ is highly prevalent than usHBZ mRNA (Usui et al., 2008).

2.7 HTLV MODES OF TRANSMISSION

Mode of spreading HTLV among the population follows the pattern of several other transfusion transmissible infections, including Hepatitis viruses B and C as well as HIV (Martin, Taylor, & Jacobson, 2014). Likelihood of spread is dependent on the effectiveness of the mode of spreading, infectivity of the donor, vulnerability of the recipient as well as the frequency of contact. The risk of spread of HTLV is at such a maximum when transfused blood is not depleted of its leucocytes (8.6-64%) (Hewitt et al., 2013). However this is perceived to be elevated via organ transplantation, sharing needles and syringes founded on subjective details, less so with transplacental exposure (3-5% of all new born of infectd mother), breast feeding (about 22% if breast-feed to 540 days) as well as unsafe sexual intercourse (approximately 1% per annum among steady, harsh partners) (Kaur & Basu, 2005; Paiva & Casseb, 2014). Rarity of transplacental HTLV-I infection is due to the restricted migration of infected lymphocytes through the placenta (Fujino & Nagata, 2000; Hino, 2011). Nonetheless, HTLV-I infection by this route needs extra investigation for evaluation of its result on the fetus and this can have repercussion for counseling serologically differing couples who want to procreate.
The spread of HTLV-I happens by way of using infected cellular blood components which culminates in seroconversion in over 40% of recipients (Paiva & Casseb, 2014). However, the transfusion of plasma and plasma derivatives does not lead to seroconversion (Manns et al., 1992). In comparison with other routes of transmission, contact to blood contaminated with the virus intravenously is the most effective means of HTLV transmission (Xie et al., 2015).

The elevated prevalence of HTLV-II about two decades ago was proven in injection drug users (Fiegal et al., 1999; Khabbaz et al., 1999; Dourado, Andrade & Galvao-Castro, 1998; Paiva & Casseb, 2014).

The year 2001 marked the period during which the initial cases of HTLV-I spread by way of organs that were received by transplantation from infected donors that were asymptomatic thus were reported in Europe (Toro et al., 2005). Among the recipients of the donor organs, three developed subacute myelopathy shortly after transplantation (Toro et al., 2005).

HTLV exists in the genital secretions of people, thus can be spread by way of sexual intercourse hence the most widespread mode of HTLV transmission (WHO, 2012). Endemic areas maintain infection by horizontal transmission by way of unsafe sexual intercourse then vertical transmission during breastfeeding, thus from mother to offspring (Paiva & Casseb, 2014). Human behaviours of high risks such as unsafe sex, numerous partners, coitus with injection drug users, sexual practices and an account of additional sexually transmitted diseases have been recognised as threats HTLV transmission (Paiva & Casseb, 2014).

2.8.0 HTLV-I HOST CELL

HTLV-I can cause alteration and clonal transformation solely in CD4 T cells; nonetheless, it has the capability to infect numerous kinds of cells like dendritic cells (DCs), macrophages, B cells
and T cells (Matsuoka & Jeang, 2007). It’s important to uncover the means by which HTLV-I increases and changes the CD4 T-cell population (Satou & Matsuoka, 2012). There are two broad compartments of the CD4 T-cells, which is, the regulatory T-cells and the Effector T-cells. Homeostasis of the immune system is known to be the function of the regulatory T-cells through suppression of undue immune responses. An essential role performed by the effector T-cells in immune response is to exude cytokines that encourage and stimulate immune systems (Satou & Matsuoka, 2012). The homeostasis, differentiation and purpose are a bit dissimilar among these two T-cell groups (Satou & Matsuoka, 2012). There is therefore an essential need to regard the characteristics of each T-cell group so as to comprehend how HTLV-I operate and upset such CD4 T-cells.

2.8.1 EFFECTOR CD4 T CELLS INFECTION BY HTLV-I

An encounter between naïve T-cells and antigens is a requisite and then their further activation and then conversion into effector or memory T-cells prior to the exercise of their function as effector T-cells. HTLV-I infection is often identified in effector/memory CD4 T-cells unlike naïve CD4 T-cells as documented by earlier reports (Richardson et al, 1990; Yasunaga et al, 2001). The following will help to explain this tendency.

2.8.2 ELEVATED VULNERABILITLY TO NEW INFECTION

Cell to cell infection helps to accomplish de novo infection. This new infection is instigated by the lymphocyte function-associated antigen-1-Intercellular Adhesion Molecule-1 complex (LFA-1-ICAM-1) and their interaction among cells infected and those uninfected (Nejmeddine et al, 2009). Effector/memory T-cells have more LFA-1 and ICAM-1 expressed than naïve T-cells (Shimizu et al, 1990; Satou & Matsuoka, 2012) hence effector/memory T-cells are probably to
be more vulnerable to cell to cell \textit{de novo} infections than naïve CD4 T-cells (Satou & Matsuoka, 2012).

2.8.3 INCREASED PROLIFERATIVE ABILITY

Naïve CD4 T-cells proliferate at a lower rate than the effector/memory CD4 T-cells \textit{in vivo}. This has been proven by the fact that, the doubling period of effector/memory CD4 T-cells, 28 days, thus, by far shorter than the doubling period of naïve CD4 T-cells which is 199 days. This doubling period of the two categories of CD4-T cells was determined by \textit{in vivo} marking of lymphocytes with deuterium labeled glucose (Macallan \textit{et al}, 2004). A property of memory CD4 T-cells, lasting survival can also be a factor to the up keep of HTLV-I \textit{in vivo} (Satou & Matsuoka, 2012). Implying that, of benefit to clonal expansion of infected cells is HTLV-I infection in effector/memory CD4 T-cells. Also, it is described that HTLV-I infected effector/memory CD4 T-cells proliferate appreciably quicker unlike cells uninfected \textit{in vivo} within individuals infected with HTLV-I (Asquith \textit{et al}, 2007).

2.8.4 ENSURES ENHANCEMENT OF THE DIFFERENTIATION FROM NAÏVE TO EFFECCTOR/MEMORY CD4 T-CELLS

Just a little information is available about HTLV-I infection outcome on CD4 T-cell differentiation due to the fact that a handful studies has been centered on the impact of viral gene expression on T-cell differentiation. However, a study has documented that the relative amount of effector/memory CD4 T-cells was high in HBZ expression can compel the differentiation from naïve T-cells to effector T-cells to or improve cell differentiation more intensely in effector/memory T-cells than naïve T-cells (Satou & Matsuoka, 2012). HTLV-I employs this effector CD4 T-cell population as a host cell. This ensures that this may cause the dysregulation of helper and effector function, playing a role in the viral persistence (Satou & Matsuoka, 2012).
2.8.5 REGULATORY CD4 T-CELLS INFECTION BY HTLV-I
CD4+CD25+FOXP3+ regulatory T-cells (T-regs) are known to be part of the key immune regulatory apparatuses which avert autoimmune disease (Sakaguchi et al., 2008). Down regulation of certain exact immune responses in the course of infectious diseases involves the actions of T-regs. Reports prove it that; the rate of occurrence of T-regs is high in chronic viral infections like hepatitis C virus (HCV) (Belkaid, 2007). T-regs in HTLV-I infection have a distinctive task because of the direct infection of CD4 T-cells by the virus involving both progenitor T-reg cells and T-reg cells (Toulza et al., 2008; Satou & Matsuoka, 2012). The occurrence of HTLV-I infection among CD4+FOXP3+ cells is elevated than other T-cell population (Toulza et al., 2008). This could be due to the following:

2.8.6 INCREASED VULNERABILITY TO NEW INFECTION
Contact between T-regs and dendritic cells occurs often (Toulza, 2008) and this could elevate the likelihood of new infection between the same cells. There is vulnerability of dendritic cells to HTLV-I infection; also, DCs infected with HTLV-I activate proliferation of T-cells (Macatonia et al., 1992; Makino et al., 1999; Satou et al., 2006). A study has also illustrated that cell-free HTLV-I cells ably infects DCs hence infected DCs enhance new infection of CD4 T-cells (Jones et al., 2008).

2.8.7 EXCESSIVE PROLIFERATIVE CAPACITY
Tagging of lymphocytes in vivo with deuterium labeled glucose has exhibited the fact that FoxP3+ T-regs are exceedingly proliferative in vivo with a doubling time of 8 days (Vukmanovic-Stejic et al., 2006). HTLV-I infection may well extra promote proliferative action of T-regs through HBZ expression proven in T-regs of HBZ-Tg mice (Satou et al., 2011).
2.8.8 HTLV-I INFECTION AUGMENTS DIFFERENTIATION OF T-regs

Reports have illustrated the fact that HBZ helps improve the production of CD4+Fox+P3+ T-cells in transgenic mice. Therefore, indicating that HBZ has an improving effect on production and/or increase of FoxP3+ T-reg cells (Satou & Matsuoka, 2012). The means by which HBZ supports the production of FoxP3+ T-reg is through augmenting the TGF-β signaling pathways (Zhao et al., 2011) which is a critical pathway for production of induced T-regs.

2.8.9 MERIT OF AVOIDING THE HOST IMMUNE SYSTEMS

Main function of T-regs is the effect of immune suppression. This is made possible through both cell-contact-dependent and independent means (Sakaguchi et al., 2008). Suggesting the fact that, HTLV-I definite CTL killing unlike HTLV-I infected non-T-regs ensuing in better survival of HTLV-I infected T-regs in vivo (Satou & Matsuoka, 2012).

2.9.0 TAX ACTIVITY IN HTLV-I INDUCED ONCOGENESIS

Tax as a transactivator protein, initiates several events such as DNA repair inhibition and impeding with checkpoint regulation, cell cycle control and cell signaling (Kannian & Green, 2010). Expression of tax is from doubly spliced mRNA transcript (Kannian & Green, 2010). While Tax and Rex mRNA are alike, nonetheless, translation of Tax is preferred above Rex as a result of a Kozak sequence. Cytoplasm formed Tax is moved into the nucleus and then attaches to its reaction constituent and stimulates viral LTR-mediated transcription (Kannian & Green, 2010).

2.9.1 FUNCTION OF TAX IN HTLV PATHOGENESIS

It is still uncertain with regards to the reasons for which HTLV-I infection is very pathogenic when contrasted to HTLV-II infection. However scientists have assumed that this disparity in
pathogenic potential may be linked to contrasts that exist in the various Tax activities (Feuer & Green, 2005). HTLV-II Tax (Tax-2) and the HTLV-I encoded transactivator Tax (Tax-1) share approximately 78% homology at the amino acid level as well as exhibiting several features characteristic of viral oncoproteins (Feuer & Green, 2005). Also, a robust proof point to the fact that, Tax leads an essential task in HTLV pathogenesis (Feuer & Green, 2005). HTLV Tax expression happens very early following infection as it aids in transactivation of viral gene expression (Feuer & Green, 2005). The capacity to turn on several cellular genes such as proto-oncogenes transcriptionally is an imperative means for Tax to be considered as a moderator of cellular alteration (Feuer & Green, 2005).

The oncogenic ability of Tax is considered to be dependent on both its capacity to change cellular genes expression implicated in growth of cells as well as proliferation, then, its straight contact with cell cycle (Lemoine et al, 2001; Marriott & Semmes, 2005; 2005; Lewis et al, 2002). Genomic instability which is a known major characteristic of HTLV-I altered cells, is considered to be a double effect of Tax, entailing inhibition of cellular DNA repair pathways as well as liberation of cell cycle checkpoint controls (Neuvent & Jeang, 2000; Gatza et al, 2003; Jeang et al, 2004).

2.9.2 ROLE OF TAX IN CELL CYCLE DYSREGULATION/IMPAIRMENT

Findings made available through scientific research by scientists have demonstrated that, Tax aims at some crucial controllers of G1/S and M progression such as p16INK4a, Cyclin D1, Cyclin D3, cdk and the mitotic spindle checkpoint apparatus (Neuveut et al, 2000). This means that Tax induces the advancement of cells in several of the stages in cell cycle. Tax therefore influences cell cycling through the following
a. Via direct association, Tax can negate the inhibitory function of p16INK4a on the G1-cdk.

b. Tax can again directly manipulate cyclin-D-cdk duties via a protein interaction

c. Tax targets the HsMAD1 mitotic spindle-assembly checkpoint protein.

By these different ways, the HTLV-I oncoprotein dysregulates cellular growth controls and produces a tendency for the loss of DNA-damage surveillance (Neuveut et al., 2000).

Cell division and proliferation of T-lymphocytes is by and large a stringently controlled event (Feuer & Green, 2005). One major and frequent characteristic in the alteration of cells by viral oncoproteins like EIA, HPVE7 and SV40T antigen is the intense impairment of the cell cycle (Feuer & Green, 2005; Jeang et al., 2004, Helt & Galloway, 2003; Lavia et al., 2003, Duensing & Munger, 2003). Among the familiar characteristics of altered cells, including HTLV infected cells are imperfect control of cell cycle advancement and stimulation of DNA damage (Feuer & Green, 2005). The control of Rb phosphorylations by cyclin-cdk complexes as well as cdk inhibitory proteins like p16INK4a, p21CIP1/WAF1, lastly p27Kip1 is an important means of manipulating gatekeeper operations (Helt & Galloway, 2003). HTLV-I infection has been proven to be proficient in manipulating transition of G1/S also (Feuer & Green, 2005; Lemoine & Marriott, 2001; Haoudi & Semmes, 2003).

One major assumption made by scientist to be the molecular source for cellular transformation is that, Tax stimulates T-cell proliferation in the dearth of correct signals and also dysregulation of expression of cellular genes critical for cell growth and cell cycle advancement (Jeang et al., 2004). Reprogramming of G1 to S advancement by Tax occurs by several means. This includes
the following, transcriptional stimulation/suppression, post-translational alteration like phosphorylations, lastly, direct protein-protein attachment (Jeang et al., 2004). In support of the mechanistic ways, for example, there is a direct attachment of Tax to p16INK4a, CyCcD2, pro-IL-16 and Cdk4 (Wilson et al., 2003). Then again, p18INK4c, CycA, CycC, CycD2, CycE, cdk2, p21CIP1/WAF1 as well as E2F are controlled by Tax through transcriptional stimulation/suppression (Kibler & Jeang, 2001; Ng et al., 2001; Iwanaga et al., 2001; Haller et al., 2002; de La Fuente et al., 2000; Schavinsky-Khraprunsky, 2003; Ohtani et al., 2000). Finally, through an unspecified means, Tax manipulates the phosphorylations of CycD3 (Jeang et al., 2004). In order to deliberate accurately on this complicated manner of interactions, there is the need for one to understand that the background of Tax’s up or down regulation is important (Jeang et al., 2004).

A well informative instance is portrayed by Tax-p21CIP1/WAF1 interaction (Jeang et al., 2004). There is an appreciable increase in levels of p21CIP/WAF1 in cells expressing Tax as proven by several scientific research studies (de La Fuente, 2000; Kawata, Arium, & Shimotohno, 2003; Schavinsky-Khaprunsky, 2003). Nonetheless, relying on whether p21CIP1/WAF1 forms a complex with CycD/cdk2 or CycA/cdk2, it has been observed that the resultant 3 parts complex, whichever, enhances or inhibits G1/S advancement (Kehn et al., 2004). The capability to reduce G1 duration by Tax in order to hasten cells into S represents a constitutive that is, DNA-damage-independent and a damage-induced component (Jeang et al., 2004; Lemoine & Marriott, 2001).

Implying that, direct Tax attachment to Cdk4 as well as its improvement of CycD-Cdk4 actions (Haller et al., 2002) happen constitutively, so, are free of any DNA damage induced incidents
(Jeang et al, 2004). During the same period, Tax can again undermine the DNA damage-triggered G1 arrest inflicted via p53 (Van et al, 2001). The other mechanism by which Tax upsets the other stages of the cell cycle is not fully clear. However, findings that have surfaced from other scientific research studies indicate that, this viral oncoprotein (Tax) is able to disrupt DNA damage-triggered checkpoint in G2/M switch (Haoudi et al, 2003; Liang et al, 2002).

2.9.3 ROLE OF TAX IN APOPTOSIS REGULATION

It is projected that human body cells proliferate $10^6$ periodically when in existence (Jeang et al, 2004). To regulate and avoid mistakes during cell divisions, cells of mammals have developed caretakers and gatekeepers to control the pace of cell growth and the reliability by which cellular genetic information is shared among daughter cells (Jeang et al, 2004). Gatekeepers check the remaining proliferative ability of a cell whilst caretakers operate to eliminate DNA impairments.

Consequently the lone viewpoint of scientist is that cellular transformation happens when both caretaker and gatekeeper roles are negated (Jeang et al, 2004). An ancient cancer enigma is that, oncogene expression does not merely offer proliferative benefit to cells but often also induces cells to go through apoptosis. The conclusion made from this oncogenic transcription factors like myc, E1A and E2F-1 illustrates this twofold facts as a statute instead of the exception (Nilson & Cleveland, 2003).

Certainly, it is now clear and evident that oncogenic upsets stimulate countervailing reactions by the cell; these are evident in cell cycle apprehension as well as apoptosis (Jean et al, 2004). The means which Tax employs to overcome cellular mechanisms for hindering cell advancement has been reviewed in the previous pages. None of the cell cycle or genetic instability expressions of
Tax can provide selective growth advantage if the cells themselves fall shot to bear such phenotypic and genotypic alterations thus chooses apoptotic death. Meaning that destroying the cellular apoptotic reaction is still a need for cellular alteration (Jean et al, 2004). Apoptosis is an effective physiological course that performs a crucial task in the removal of cancerous or viral infected cells (Feuer & Green, 2005). The clinical manifestations of ATL means that, in a subgroup of CD4+ T-cells, HTLV-I infection favors proliferation over apoptosis (Jean et al, 2004). However, the mechanisms for which the oncoprotein HTLV-I Tax makes this possible is uncertain.

Several scientists have scrutinized the involvement of Tax to stress stimulated apoptosis (Jean et al, 2004). All the findings have been controversial and divergent. A section of scientists are of the view according to their findings that, Tax shields cells off stress stimulated cell cycle apprehension and apoptosis (Torgeman et al, 2001) while the other section are also of the view that, Tax induces cells to stress-stimulated apoptosis (Kasai & Jeang, 2004). Possibly the choice amid proliferation and apoptosis is determined by the cellular milieu, type of cell, genetic setting as well as several concomitant signaling events (Jean et al, 2004). Based on the circumstance, the group of genes that Tax transcriptionally turns on and or which bunch of gene products that Tax attaches (Wu et al, 2004) will imply that the usual cellular action versus oncogenic stress will either occur, that is, apoptosis or be redirected, that is, proliferation, by HTLV-I (Jean et al, 2004).

Blemishes which occur in the machineries regulating apoptosis perform a greater task in the development of cancer and neurodegenerative as well as immunological disease (Feuer & Green,
2005). It is therefore not completely revealing that Tax has been found to hinder stimulation of apoptosis or programmed cell death (PCD) (Feuer & Green, 2005).

2.10 PATHOPHYSIOLOGY OF HTLV INFECTION

HTLVs are intracellular proviruses that pass through formation of a virological synapse, allowing the viral genome to be passed from one cell to another. Once infection has occurred, little replication takes place. Infection affects the expression of T-lymphocyte gene expression, leading to increased proliferation of affected T-lymphocytes (Ewa, 2015). HTLV primarily affects T lymphocytes, specifically, HTLV-I predominantly affects CD4+ lymphocytes while HTLV-II also predominantly affects CD8+ lymphocytes (Ewa, 2015). In vitro, HTLV-I is also capable of infecting other cell types, possibly accounting for the diverse pathogenesis of HTLV-I. Recently, GLUT-1, a ubiquitous glucose transporter, has been identified as a receptor for HTLV-I (Manel et al, 2003).

In ATL, the main pathogenic protein, Tax, leads to leukogenesis and immortalization of T-lymphocytes in vitro (Boxus et al, 2008). This is achieved by stimulation of interleukin-15 (IL-15) and interleukin-2 (IL-2), in turn leading to T-cell growth and transformation (Ewa, 2015). Research on this subject is ongoing, and the expression of this gene is not always found in ATL cells. However Tax is inherent to both HTLV-I and HTLV-II although HTLV-I is more pathogenic. Recently, the HTLV-II basic zipper factor gene (HBZ) has been found to be consistently expressed in ATL cells, suggesting a role in cellular transformation and leukemogenesis (Ewa, 2015). This might correlate with the increased pathogenesis of HTLV-I (Matsuoka & Jeang, 2007). The expression of HBZ gene also correlates with the provirus load of HTLV-I (Ewa, 2015).
2.11.0 GLOBAL EPIDEMIOLOGY AND ALLOCATION OF HTLV-I INFECTION

Although HTLV is the first oncogenic retrovirus of man determined about three decades along the line and it is not a ubiquitous virus. HTLV is located all over the globe, with bands of elevated endemicity identified mostly near places the virus is almost nonexistent (Gessain & Olivier, 2012). The exact HTLV-I highly endemic areas are the foci in Middle East and Australo-Melanesia, southwestern part of Japan, sub-Saharan Africa, Caribbean area as well as South America (Gessain & Olivier, 2012). Irrespective of the different cultural and socio-economic environments, the HTLV-I prevalence elevates slowly along side age particularly amongst female in all extremely endemic regions (Gessain & Olivier, 2012). Two decades ago, a group of epidemiologist valuated the overall total HTLV-I carriers ranges between 15-20 million individuals (Gessain & Olivier, 2012). However, per that time of the valuation several location have not been examined, little inhabitant-based investigations were accessible as well as tests used for HTLV-I serology were not adequately definite (Gessain & Olivier, 2012). The foundation of this baffling geographical or somewhat ethnic second distribution is plausibly related to an originator consequence in certain categories with perseverance of an elevated viral spread rate (Gessain & Olivier, 2012).

2.11.1 HTLV-I IN EUROPE

Europe represents over 700 million of people since 2015. Several epidemiological studies have been conducted in Europe frequently in pregnant women and in blood donors (Ades et al, 2000; Machuca et al, 2000; Tseliou et al, 2006; Vrielink & Reesink, 2004; Taylor et al, 2006; Davidson et al, 2006; Laperche et al, 2009; Brant et al, 2011). Also many ATL and HAM/TSP have been described (Martin et al, 2010; Ceesay et al, 2012). The well investigated populations include the metropolitan France, Spain as well as United Kingdome (Gessian & Olivier, 2012).
With regards to all these studies, it is now understandable that most of the individuals with HTLV infection habiting in Europe precisely come from or are descendants of immigrants from a high endemic locality including West Indies and Africa. In UK, majority of HTLV carriers come from former British West Indies, explicitly, Jamaica as well as Barbados, Trinidad and Tobago (Payne et al., 2004; Dougan et al., 2005). More uncommonly, they come from West African countries like Ghana, Sierra Leone (Gessian & Olivier, 2012). In France, most carriers come from West or Central Africa such as Senegal, Mali, Guinea, Cote d’Ivoire, or Cameroon (Duval et al., 2010). In blood donors and part of the wide range inhabitants, a particular fraction of the infected individuals are females of Caucasian decent. Such persons got infected with HTLV-I via sexual contacts with an infected partner coming from an HTLV-I endemic area. This is a representation of about 5-10% in varying European states including France and UK (Dougan et al, 2005).

Majority of the affected persons are descendants of South America, specifically Spain (Toro et al., 2002; Padua et al., 2011; Trevino et al.; 2012). In Portugal, HTLV-I carriers often come from ancient African colonies including Mozambique and Angola (Padua et al, 2011). Additional European countries that have vastly little HTLV-I infection as established by HTLV-I frequency in new donors of below 0.4/10,000 as well as an enormous infrequency of ATL or HAM/TSP reports (Laperche et al, 2009).

2.11.2 HTLV-I IN AFRICA

An assessment of the entire African population is approximately 1 billion as at 2012. The African population has increased vastly after 2012 (Gessian & Olivier, 2012). Nonetheless, irrespective of the several epidemiological research as well as accounts of sporadic instances or
even a few series of ATL and HAM/TSP cases, the circumstance about the degree of HTLV-I infection is not actually acknowledged in many states and regions of the largest continent (Gessian & Olivier, 2012).

2.11.3 HTLV-I IN NORTH AFRICA/MAGHREB
Few studies conducted in this part of the continent have been concerned mainly with blood donors or multi-transfused patients from Egypt, Morocco and Tunisia. However, in these regions HTLV-I seroprevalence is known to be very subtle or negative (Kawashti et al, 2005; Stienlay et al, 2009). HAM/TSP along with ATL cases reported are low and are mostly either diagnosed among local people or immigrants, most often in some.(Gharibi et al, 2011).

2.11.4 HTLV-I IN CENTRAL AFRICA
The region under review consist of nine states, thus Angola, Cameroon, Central Africa Republic (CAR), Chad, Democratic Republic of the Congo (DRC) formerly Republic of Zaire, Equatorial Guinea, Gabon, Republic of the Congo, Sao-Tome-et-Principe. This region of Africa represents about 130 million people (Gessian & Olivier, 2012). Numerous imperative epidemiological researches have been carried out in Cameroon and notably, Gabon. Reports on ATL and HAM/TSP have been documented in Cameroon, Gabon and the DRC (Gessian & Olivier, 2012). Seroprevalence of HTLV-I in Cameroon ranges between 0.5-2% (Mauclere et al, 2011; Fillipone et al, 2012). Gabon is the best studied country for HTLV-I infection (Gessian & Olivier, 2012). HTLV-I infection in Gabon is utmost in the southeastern region of the nation. In a few rural communities of this area, the HTLV-I frequency is over 25% in the elderly, chiefly females (Gessian & Olivier, 2012). Gabon is the country with the utmost hitherto established HTLV-I endemic area on the African continent. The seroprevalence in other countries is low as compared to that in Cameroon and in Gabon (Gessian & Olivier, 2012).
2.11.5 HTLV-I IN WEST AFRICA

This region is composed of 16 states and this denotes over three hundred million populaces. More than a few large studies have been carried out in Senegal, Guinea-Bissau, Cote d’Ivoire and Nigeria (Gessian & Olivier, 2012). ATL and HAM/TSP have been described in majority of these states including Senegal, Mauritania, Mali, Cote d’Ivoire and Nigeria (Gessian & Olivier, 2012). Seroprevalence of HTLV-I amongst blood donors stretched mainly from 0.2-3% (Gessian & Olivier, 2012).

2.11.6 MOLECULAR EPIDEMIOLOGY OF HTLV-I

An analysis from a molecular stance, HTLV-I holds an extraordinary genetic stability which is an atypical characteristic for a retrovirus (Gessain & Olivier, 2012). Viral amplification during clonal growth of cells infected rather than by reverse transcription is really plausible to be the cause for this outstanding genetic firmness (Gessain & Olivier, 2012). The little sequence variations observed in HTLV-I can be employed as a molecular apparatus to trail the movement of populations new infection as well as old ones, as a result acquire recent understanding into the source, evolution and routes of spreading of such retroviruses and of their hosts (Yanagihara 1994).
Figure 2. Geographical allotment of HTLV-I in states where the infection is endemic. The stars highlight regions with elevated frequencies. Individual state borders made known in the diagram are not associated with the regions of endemicity, mirroring the cluster make up of HTLV infection (Goncalves et al, 2010).

The small number of nucleotide substitutions examined amid virus strains is really precise to the geographic source of the patients quite than the pathology (Gessain & Olivier, 2012). There has been so far a report of four key geographic subtypes of HTLV-I. This comprises of the Cosmopolitan subtype A, the Central African subtype B, the Central African/Pygmies subtype D and the Austral-Melanesian subtype C (Gessain & Olivier, 2012). However a small amount of strains established in Central Africa fit in some uncommon subtypes (E, F,G) (Gessain & Olivier, 2012). The cosmopolitan is the most well known as it is prevalent in several geographical subgroups. This subtype is endemic in Japan, Caribbean area, Central and South
America, North and West Africa as well as part of the Middle East (Gessain & Olivier, 2012). The sequence unevenness in subtype A is extremely small. This is extremely possible to echo a somewhat new spreading of this genotype from a shared ancestor (Gessain & Olivier, 2012). The largely divergent is the Australo-Melanesian subtype C. This outcome mirrors an extended time of evolution in remote populations existing in diverse islands of the pacific region (Gessain & Olivier, 2012). The emergence of such HTLV-I subtypes in man was solidly proposed to be coupled to interspecies spread amongst simian T-cell leukaemia virus-I (STLV-I) infected monkeys and human beings followed by uneven cycle of evolution in the human host (Meertens et al, 2001; Nerrienet et al, 2001; Wolfe et al, 2005).

So far there is no firm substantiation that either a precise mutation or a genotype is linked to the advancement of a TSP/HAM or an ATL in an asymptomatic carrier (Gessain & Olivier, 2012).

2.12.0 VIRAL CHARACTERISTICS OF HTLV-I

Just like HIV-1 and HIV-2, HTLV-I also has a non-human primate (NHP) equivalent. This is the simian T-cell lymphotropic virus type-I (STLV-I). HTLV-I and STLV-I both form the primate T-cell lymphotropic virus group (Van, Salemi & Vandamme, 2001). HTLV-I and HIV genomic structure are the same for all retroviruses composed of three basic components: thus, gag, pol and env genes. These genes are flanked by two long terminal repeat (LTR) regions but with the difference of HTLV-I having the pro-proliferative gene tax and the regulatory Rex. Yet another differential fact with HIV is the relatively stable genome of HTLV-I (also HTLV-II) which is a consequence of HTLV-I being a relatively low replicative virus. It has been reported that cell free virions barely exist in vivo. Indeed the virus promotes clonal expansion of the infected cells.
leading to an increase in proviral load without viral replication (Van, Salemi & Vandamme, 2001).

Seven HTLV-I subtypes are known. They are labeled a to g and they differ from each other in their geographical distribution. The most ubiquitous subtype, known as the Cosmopolitan subtype is HTLV-Ia. It is present in different areas and the most frequent subtype in Japan (Miura, Fukunaga, & Igarashi, 1994). HTLV-I subtype b and d to g are restricted to Central Africa while HTLV-Ic was isolated in Australo-Melanesia (Wolfe et al., 2005). No differences in outcomes have been reported between different subtypes although most of the information available is focused in the most frequent subtype HTLV-Ia (Nicholas et al., 2015).

2.12.1 DIAGNOSIS OF HTLV-I

Through the regular clinical practice, individuals infected with HTLV-I are diagnosed of being infected via serological testing for anti-HTLV antibodies using peripheral blood (Martin, Taylor, & Jacobson, 2014). Screening for HTLV-I as it has already been stated above is predominantly carried out via ELISA and HTLV-I positive cases obtained from the initial ELISA screen established by means of uncovering antibodies produced in opposition to gag (p11 and p24) as well as envelope (gp21 and gp46) proteins by western blot or immunoblot (Martin, Taylor, & Jacobson, 2014). Differentiation of HTLV-I from HTLV-I is possible due to the add-on of recombinant gp46-1 and gp46-2 to the blots (Martin, Taylor, Jacobson, & 2014). There is a great immunological response to HTLV-I evasion of the human body while antibodies in serum titer which associate with HTLV-I viral load could be as lofty as 1:256,000 (Martin, Taylor, & Jacobson, 2014). Quantification of HTLV proviral DNA is possible by means of PCR using peripheral blood mononuclear cells (PBMCs). This is often stated as HTLV DNA copies per 100 PBMCs or in percentage (Martin, Taylor, & Jacobson, 2014). As every infected cell normally
carries a lone provirus (Cook et al., 2012) hence the proviral load (pVL) is often equated to the percentage of cells infected (Martin, Taylor, & Jacobson, 2014) unlike some cases involving ATL. The viral load is maintained at a low central point of 155 (0.0003-28) copies/100 PBMCs (1.55%) among asymptomatic carriers whilst there is a media viral load of 14 (0.002-112) copies/100 PBMCs (0.14%) among HAM/TSP patients has been reported (Martin et al., 2010). In order to ensure a quick confirmation of HTLV-I associated lymphomas, the qualitative PCR method (qPCR) can be used in uncovering 100% of several pVL in lymphoma cells or other tissue samples (Martin, Taylor, & Jacobson, 2014).

2.12.2 VIRAL CHARACTERISTICS OF HTLV-II

Despite sharing the same viral family and several characteristics, HTLV-I and HTLV-II differ in epidemiology and most importantly, in pathogeny as HTLV-II has not been proven to be real oncogenic virus like HTLV-I (Nicholas et al., 2005). HTLV-II has a significant genetic homology (nearly 70%) with HTLV-I. It shares the same replication patterns and gene structure, including the expression of the regulatory protein Tax (Feuer et al., 1995). Genome sequence analysis has subdivided HTLV-II into four subtypes. The subtypes do not differ in pathogenicity and most likely mirror the geographical genesis as well as movement of ancient populations (Paiva & Casseb, 2014). Each of the subtypes has a characteristic geographic association; subtype a and b are present throughout the western hemisphere and Europe with sporadic distribution in Africa and Asia, subtype c is present in Kayapo indigenous people of the Amazon and urban Brazilian populations and lastly subtype d was discovered in an African pygmy tribe (Ewa, 2015).
2.13.0 HTLV ASSOCIATED DISEASES

HTLV being a retrovirus infects 10-20 million people worldwide as estimated by seroprevalence studies. However HTLV is associated with diseases in only approximately 5% of infected individuals. HTLV infection is linked with different diseases of human. These include adult T-cell leukemia (ATL) and non-neoplastic inflammatory diseases. The latter includes HTLV-I-associated myelopathy/tropical spastic paraparesis (HAM/TSP) and HTLV-I uveitis (HU) and other diseases with unestablished associations such as arthropathy, pneumopathy, dermatitis, exocrinopathy and myositis (Watanabe, 1997). HAM/TSP and HU are depicted by infiltration of HTLV-I infected lymphocytes and dysregulated production of cytokines (Watanabe, 1997).

2.13.1 HTLV-I-ASSOCIATED DISEASES

There has been an established link between HTLV-I infections with ATL, HAM/TSP and HU (Proietti et al, 2005). Opportunistic co-infections have also been linked with HTLV-I infections due to the subclinical immune suppression it causes. Some of these opportunistic co-infections are tuberculosis and strongyloidiasis as well as others (Bangham & Osame, 2005). Former studies have also reported that, urinary tract abnormalities, fibromyalgia, arthritis and major depression are often established in HTLV-I infected individuals (Cruz, Catalan-Soares & Proietti, 2006; Mori et al, 2004; Oliviera, de-Castro & Carvalho, 2007; Stumpf et al, 2008).

2.13.2 ADULT T-CELL LEUKEMIA/LYMPHOMA

ATL is an aggressive lymphoproliferative malignancy of peripheral T-cells. It has a brief existence in its acute kind and an occurrence of five percent in HTLV-I infected individuals. ATL was first portrayed in Japan then in the Carribean region as well as South America (Watanabe, 1997; Goncalves et al, 2010). Occurrence of ATL is high in grown-ups at least 20-30
years following HTLV-I infection. ATL is common in men (Goncalves et al, 2010) but there is no gender prevalence (Matutes, 2007). Documentation of familial ATL in Japan, the USA and England has been reported, nonetheless, it is unspecified if a genetic predisposition stages a part in the buildup of the disease. Also, ATL could coexist or trail other HTLV-I induced non-neoplastic diseases, including TSP (Matutes, 2007). Childhood infections in some people have an elevated threat of ATL development. The fifth decade in the life of HTLV-I infected people in Japan is dominated with the appearance of ATL while the frequency in Brazil and Jamaica is during the fourth decade. Plausibly, factors that occur in the local setting play a role in pathogenesis of disease (Goncalves et al, 2010).

2.13.3 ATL PATHOGENEESIS

The pathogenesis of ATL includes four main phases. These are infection, polyclonal proliferation, clinical latency and tumorigenesis. Stimulated and actively proliferating T-cells are efficiently infected than quiescent T-cells by HTLV-I (Kannian & Green, 2010). Penetration into host cell is made possible by env thus activating T-cells probably by means of CD2/LFA-3, LFA-1/intracellular adhesion molecule (ICAM) and IL-2/IL-2R (Kannian & Green, 2010; Wucherpfennig et al, 1992). Activation of the T-cells results in the formation of a pool of proliferating lymphoblasts, however at this point; the population of polyclonal cells generated is not leukemic (Kannian & Green, 2010). An important task is performed by HBZ and Tax during modification of the cell. This is achieved by initiating alterations in several intracellular signal transduction pathways including up-regulating as well as down regulating cellular and viral gene expression so as to trigger neoplastic transformation (Kannian & Green, 2010; Franchini, 1995; Matsuoka & Green, 2009). The modified T-cells become IL-2 unconnected. It therefore connects with constitutive stimulation of the Jak/Stat pathways and reduced expression of src homology 2
(SH2) containing lynosine phosphatase-1 (SHP-1) protein which controls signaling from numerous hematopoietic surface receptors (Migone et al, 1998). There exist a connection between this changeover and importantly more rapid disease advancement (Kannian & Green, 2010; Ressler, Connor, & Marriott, 1996). A time of clinical latency is noted in carriers of HTLV persisting for 2-4 decades. Expressions of the viral genes at this time have levels that are less noticeable which can evade immune surveillance. Also HTLV-I goes through epigenetic silencing as well as chromosomal aberrations promotion, leading to selection and evolution of monoclonal tumor populations (Kannian & Green, 2010). The extent of cytogenetic abnormality is directly comparative to disease gravity. Leukemogenesis also occurs via transactivation of protooncogenes like c-fos, egr-1 as well as egr-2 by Tax (Kannian & Green, 2010). The tumors describe the conclusion of clinical latency and ATL development among patients (Fujii et al, 1991).

2.14 CLASSIFICATION OF ATL

ATL is a diverse disease alienated into four subtypes clinically (Goncalves et al, 2010). These subtypes are Acute, Chronic, Smouldering and Lymphomatous (Matutes, 2007).

2.15 ACUTE SUBTYPE

This is the most frequent subtype that is presented at health facilities by ATL patients. About 65% ATL patients present this subtype of ATL (Matutes, 2007). It has the following features: presence of systemic symptoms, organomegaly especially lymphadenopathy, as well as leukemic picture. Hypercalcemia that may or may not involve lytic bone lesions occurs in half of the
patients. It may buildup in the course of disease advancement in a further third of the ATL population. Skin lesions are seen in close to a half of these patients (Matutes, 2007).

2.16 CHRONIC SUBTYPE

This subtype presents with lymphocytosis that may be steady for months or yet years. There is a dearth of hypercalcemia, organomegaly but presence of skin manifestations and normal or only a little increased lactate dehydrogenase (LDH) (below two fold the upper limit value) (Matutes, 2007).

2.17 SMOULDERING SUBTYPE

Patients with this subtype are usually asymptomatic. They however present the following characteristics: skin rashes that react to topical steroids, lung infiltrates. Different from the chronic form, the white blood cell count is within range and there is no lymphocytosis and less than 3% atypical circulating lymphocytes (Matutes, 2007).

2.18 LYMPHOMATOUS SUBTYPE

Often, less than a third of patients present with lymphoma with no evidence of blood involvement. There is often organomegaly, circulating leukemic cells often below 1% and LDH is elevated as well as likely hypercalcaemia (Matutes, 2007).

2.19 TREATMENT AND PROGNOSIS OF ATL

The prognosis for ATL is still inadequate irrespective of the progress made in the assistance and development of de novo treatment agents (Goncalves et al, 2010). There has been an
immeasurable testing of several different therapeutic approaches over the past 30 years (Goncalves et al, 2010). It has been shown that patients presenting with aggressive ATL also portray multidrug resistance of malignant cells, a huge tumor burden among multi-organ failure, hypercalcemia and or frequent infectious complications due to weighty T-cell immunodeficiency hence resulting in poor prognosis of their condition (Goncalves et al, 2010; Taylor & Matsuoka, 2005).

Over the past 3 decades, the blend use of arsenic trioxide, zidovudine and alpha interferon has shown an extraordinary remission rate with average toxicity (Kchour et al, 2009). In order to achieve results, the treatment plan should be established on the clinical sub-classification of ATL and prognostic factors, such as, watchful expectant approach, chemotherapy, antiviral therapy as well as allogenic hematopoietic stem cell transplantation (Taylor & Matsuoka, 20005; Tsukasaki et al, 2009). The main aspects driving prognosis include progressed performance status, age of 40 years and beyond, lastly, having three or more lesions. One other aspect which is seen to be an independent poor prognostic factor is the involvement of bone marrow (Goncalves et al, 2010). Earlier, several scientific medical research have proposed that elevated doses of corticosteroids in smoldering ATL patients without concurrent therapy may amplify the threat of acute ATL (Goncalves et al, 1999; Goncalves et al, 2008).

2.20 HTLV-I-ASSOCIATED MYELOPATHY/TROPICAL SPASTIC PARAPARESIS (HAM/TSP)

HTLV-I-associated myelopathy/tropical spastic paraparesis (HAM/TSP) is a chronic meningomyelitis of the gray and white matter in the spinal cord, with perivascular demyelination and axonal degeneration (Cooper, Van der Loeff, & Taylor, 2009).
A gradually progressive spastic paraparesis, without remissions, with increased harm disrupted gait, bladder and bowel autonomic dysfunction as well as intense effects on their capabilities and excellence of life builds up in patients (Goncalves et al, 2010). Those frequently affected are women than men as the disease also sets off in middle age at a mean age of 40 years (Orland et al, 2003). The lesion in the central nervous system triggered by HTLV-I is associated with dense infiltrates of mononuclear cells, largely the CD8+ lymphocyte response (Bangham & Osame, 2005). Thus HAM/TSP is well thought-out as an inflammatory ailment in which cellular impairment leads to demyelination (Goncalves et al, 2008; Bangham & Osame, 2005).

Early in the disease, leptomeninges, blood vessels and parenchyma are infiltrated by CD4+ and CD8+ lymphocytes, B lymphocytes and foamy macrophages. In the chronic phase, the cord infiltrate consists mostly of CD8+ lymphocytes. The whole spinal cord can be affected, although the lower thoracic level is predominantly affected (De Castro-Costa et al, 2006). The first symptoms are weakness of the lower limbs and lumbar pain, although the initial complaint can be sensory, such as tingling, burning or pins and needles. In many patients, urinary and sexual problems can be the first symptoms (De Castro-Costa et al, 2006). Dizziness is common in the early phase, preceding abnormalities upon a neurological exam and would be related to a functional disturbance in the vestibule-spinal and motor tracts (Goncalves et al, 2010). With disease progression, the weakness and the spasticity elevates while the gait deteriorates (Nagai & Osame, 2003).

2.21 CLINICAL CHARACTERISTICS AND DIAGNOSIS OF HAM/TSP

Among the first signs and symptoms presented include the following lower limb weakness, pain in the lumber, urinary and sexual problems, lastly, dizziness (De Castro-Costa et al, 2006;
Orland et al, 2003; Oliveira et al, 2007). Some of these early phase symptoms of HAM/TSP could be associated with a functional disruption in the vestibule-spinal and motor tracts (Felipe et al, 2008; Chen and Nussbaum, 2000). Also, the initial symptom could be sensory like burning, tingling, pins or needles (Goncalves et al, 2010; Lima et al, 2007). There is a link between the weakness in the limbs and medium to severe spasticity, hyperreflexia and Babinski’s sign (Mori et al, 2004; Samson & Cardenas, 2007). However, the sensory injury is weak; the vibratory sense is often injured as well as the proprioception is less impinged upon (Goncalves et al, 2010; Kira et al, 1991). There occurs also hyperreflexia however without muscle weakness in the upper limbs. As the advancement of the disease occurs, the spasticity and weakness also elevates as well as the worsening of gait (Nagai & Osame, 2003; Oliveira et al, 2007). Spasticity happens as a result of velocity reliant elevation in the muscle tone in reaction to unresting motion as well as performing a great task in the advancement of disability (Rhan, Bertorini, & Levin, 2001; Cooper et al, 2009). The motor neuron action is expressed as muscular spasms when the spinal cord is denied of supraspinal control (Goncalves et al, 2010; Khan, Bertorini, & Levin, 2001). When the muscle tone increases, it can result in disrupted function, pain as well as joint contractures (Andrade, 2005; Felipe et al, 2008; Morgan et al, 2007).

2.22 TREATMENT AND PROGNOSIS OF HAM/TSP

As a result of the lack of any curative treatment available, the person’s immune reaction and proviral load describes the prognosis (Goncalves et al, 2010). The use of IFN-β1a, IFN-α and corticosteroids have not shown successful results (Oh et al, 2005). A research work that assessed the blending use of two nucleoside analogue drugs, zidovudine and lamivudine in a randomized, double blind placebo-controlled study found out that there was no clinical improvement (Taylor
et al, 2006). Meanwhile patients included in the study had developed myelopathy to the advanced stage, thus, they thought it to be a limitation of the treatment reaction (Goncalves et al, 2010). An observation was made among corticosteroid using HAM/TSP patients that their proviral load had elevated (Takenouchi et al, 2003). On the other hand, use of retroviral drugs reduced the proviral load (Taylor et al, 2006). These two annotations point out that the proviral load determined in peripheral blood could be of significance in following disease action in therapeutic tests (Taylor et al, 2006). One possible biomarker that can be used to determine accurate progression of HAM/TSP is the proviral load in CSF than the load in the blood (Goncalves et al, 2010).

2.23 HTLV-I ASSOCIATED UVEITIS

Ophthalmological disturbances related to HTLV-I include vasculitis, exudation or degeneration of the peripheral retina and keratoconjunctivitis sicca (Goncalves et al, 2008) and HU is an accepted entity associated with this viral infection (Goncalves et al, 2010).

Middle-aged HTLV-I infected adults of both genders are the target population. HU is characterized by a granulomatous or non-granulomatous reaction accompanied by vitreous opacities and retinal vasculities with rare exudative retinochoroidal alterations in one or both eyes (Goncalves et al, 2010). It can occur as the only HTLV-I manifestation or can be associated with HAM/TSP. Uveitis usually occurs as an isolated ophthalmological alteration (Goncalves et al, 2010).
2.24 SCREENING TESTS AND RESIDUAL RISK OF TRANSFUSION TRANSMITTED HTLV

Screening tests for HTLV most often are enzyme-linked immunoassay (EIA) or particle agglutination (PA) assays (Goncalves et al., 2010). HTLV-I and HTLV-II testing is usually merged in EIAs whilst HTLV-I is the sole virus tested for in PA assays. This implies that the technique of choice should be taken into consideration, the kind of retrovirus located in the geographic region (Goncalves et al., 2010). For instance, the assay of choice in Japan is PA because HTLV-II is usually not found (Goncalves et al., 2010). Brazil too has both viral types, thus HTLV-I and II hence EIA is preferred over the other (Namen-Lopes et al., 2009). Some antibodies that recognize HTLV-I antigens can either recognize those from HTLV-II and the serological screening tests are not accurate to distinguish the two infections. Thus, confirmatory test must differentiate between HTLV-I and HTLV-II. Subsequent to the screening tests, a confirmation is performed by blood testing using a different procedure from the initial screening tests which differentiates HTLV-I from HTLV-II (Goncalves et al., 2010). Most regularly used assays are the radioimmunoprecipitation (RIPA), Western blotting (WB) or immunofluorescence techniques (Goncalves et al., 2010). Test results that show to be indeterminate after the confirmatory test are due to the window period, the incidence of a viral variant as well as unspecified reaction of the patient’s serum to viral antigens (Namen-Lopes et al., 2009). Detection of the DNA of HTLV provirus by the use of polymerase chain reaction (PCR) has been used to make clear indeterminate results as well as a confirmatory test too (Goncalves et al., 2010). However, PCR could also be used for the subtyping of the virus when merged with proviral DNA sequencing or restriction fragment length polymorphism (RFLP) analysis (Proietti et al., 2005).
The threats associated with HTLV-I transmission via transfusion differs with the prevalence of this virus in the entire population and among blood donors (Goncalves et al, 2010). Another interfering factor with respect to the residual risk of transmission calculation is the time interval prior to seroconversion (Goncalves et al, 2010). The window period for transfusion transmitted HTLV-I often differs between 41 and 65 days, however could be more (Goncalves et al, 2010). Also the product of blood being used for treatment is one more variable that affects transmission. However, industrial blood products such as albumin, immunoglobulin not forgetting antihemophilic factors as well as plasma have been established not to spread HTLV (Goncalves et al, 2010; Donegan et al, 1990).

Duration of storage varies with regards to spread of HTLV associated with packed red cells and platelet, thus, the highest in the first 14 days (Goncalves et al, 2010; Sullivan et al, 1991). There is a nominal residual threat of transfusion transmitted HTLV in countries with low prevalence (Goncalves et al, 2010). Retrospective studies of HTLV-I/II in all such countries above will show a vastly low yield hence their public health benefit is uncertain (Goncalves et al, 2010). On the other hand, in HTLV endemic regions, the residual risk is higher hence the need of retrospective studies is crucial whilst sustained checking via hemovigilance also ought to be considered because of the higher transmission rates due to the seroconversion in repeat blood donors (Namen-Lopes et al, 2009; Carneiro-Proietti et al, 2006).

2.25 PREVENTION AND COUNSELING

Adult T-cell lymphoma/leukemia and HAM/TSP as stated above has weak prognosis while there are also no available vaccine to help prevent such diseases from developing in individuals infected with HTLV (Goncalves et al, 2010). Individuals with HAM/TSP tend to experience
huge financial loss since it’s a long lasting advancing disease. The financial loss affects the family and the health system as well (Goncalves et al, 2010). With these consequences in mind, there is the need to mount serious counseling and educative programs for high risk persons as well as in populations.

Ever since Japan instituted an agenda to help prevent transfusion-transmission of HTLV-I through screening for HTLV-I during blood donation exercises in 1986, several other countries in endemic regions of the country have followed suit (Carneiro-Proietti et al, 2006). This has proven to be an efficient tactic in the prevention of HTLV transmission via blood transfusion (Goncalves et al, 2010).

There have been several commendations for the execution of policies for selective donor enrolment in regions where the disease is not endemic (Goncalves et al, 2010). As a result of the costly nature of test kits imported for HTLV screening, it is important for developing countries to design and assess more cost effective tactics for screening donated blood (Goncalves et al, 2010).

Blood transfusion remains a threat for HTLV infection for recipients in several African nations including other less established areas that lack the requisite public policies and transfusion services infrastructure (Proietti et al, 2005). Individuals tested to be HTLV-I seropositive ought to be advised not to partake in blood donation exercises, donate semen, organs or milk (where milk banks are in place) (Goncalves et al, 2010; Carneiro-Proietti et al, 2002). All other ways of impacting on the frequency of HTLV aside via blood transfusion should be put in place such as to prevent, mother to child transmission, cross-feeding, suggested cesarean section (CS) for the women that are seropositive so as to reduce the risk of perinatal transmission while establishing prenatal screening for HTLV (Goncalves et al, 2010).
In order to prevent sexually transmitted infections, recommendations for condom use and avoidance of multiple as well as anonymous sexual partners and commercial sex worker. When a partner is seronegative, the use of condom should be stressed (Goncalves et al, 2010). Among the intravenous drug users (IDU) population, they must be counseled and educated so as to minimize practices that harm them and this may be efficient in preventing HTLV-infection (Goncalves et al, 2010).

Several HTLV-I infected persons are challenged with psychological and social problems including fear or guilt about pregnancy, difficulty in creating and sustaining relationships, depression and elevated anxiety have to be dealt with appropriately (Goncalves et al, 2010). Obtaining the right information about HTLV is very crucial as HIV is often contradicted with HTLV even in health facilities, thus, resulting in needles stress on the patient which is often linked to self-destructive intentions (Goncalves et al, 2010), stressing the fact that majority of persons infected with HTLV will not acquire the disease, staying asymptomatic lifelong.

The counseling provided to HTLV-I/II seropositive carriers seeks to explain facets of infection and diseases related with the virus, to offer guidance about the mode of spread of the virus and treatment, lastly assess the need for emotional support. It is also important to explain the essence of the serological results, principally if the person has not been tested by a confirmatory assay or if the result proved to be seroindeterminate. This is very crucial when counseling blood donor candidates in endemic areas since they are often young, asymptomatic and of a reproductive age. HTLV-I/II seroindeterminate persons are not easy to counsel since their status as infected or not are not classified. Such persons should be notified that the result is not clear, since false reactive
results may happen, demanding additional tests (such as molecular tests) or serological monitoring to classify possible seroconversion. Often seropositive women counseled about pregnancy and avoiding breastfeeding tend to comply hence spread of the virus may be hindered (Goncalves et al, 2010). Also, it is suggested that HTLV carriers test for other pathogens that share similar transmission modes.

Lastly, counseling of persons infected is a laborious charge that should require a multidisciplinary team, able to rightly educate the carrier, take his/her questions and avoid hopeless attitude.

2.26 STRATEGIES FOR RISK REDUCTION IN BLOOD BANKS

The initiation of improved technologies of donor screening coupled with viral inactivation methods have contributed to a reduction of the transmission of infections via blood and blood products. The safest source of blood to the various transfusion services across the globe is voluntary non-remunerated blood donation (Kaur & Basu, 2005). Instituting a well grounded voluntary donor base coupled with ensuring awareness to slowly eradicate the replacement donations ought to be one of the ways to guarantee safety of blood and its products (Kaur & Basu, 2005). Research studies conducted across the globe including India have proven that there is an increased seroprevalence of transfusion transmitted disease in replacement donors unlike voluntary blood donors. As a result, emphasis should be on enrolling voluntary non-remunerated repeat donors (Kaur & Basu, 2005). One of the methods used to detect the presence of infected agents in blood is nucleic acid testing (NAT) and is very expensive (Kaur & Basu, 2005).
CHAPTER THREE

METHODOLOGY

3.1 STUDY DESIGN

The study is a prospective cross-sectional study among blood donors donating blood at the Southern National Blood Service (NBSG) in Accra, Ghana.

3.2 STUDY SITE

The study site was the Southern Centre National Blood Service (NBSG) in Accra, Ghana. The study site is located off the Guggisberg Avenue at Korle Bu, Awotse Kojo, adjacent the Korle Bu branch of GCB Bank and behind the Korle Bu district office of the ECG. The NBSG is one of the Agencies under the Ministry of Health that contributes significantly to the implementation of Ghana’s Health sector programme of work. The mandate of the NBSG is to ensure an effective and coordinated national approach to the provision of safe, adequate and efficacious blood and blood products, making it timely, accessible and affordable to all patients requiring transfusion therapy in both public and private health care institutions in the southern part of Ghana. The NBSG currently selects blood donors on the basis of a health check questionnaire and screening for hepatitis B and C, syphilis and HIV.

3.3 STUDY POPULATION

The study population included all consented non-remunerated voluntary blood donors at the NBSG from March to April, 2017. The blood donors were served with a consent form and questionnaire to answer before taking the blood samples. The sampling technique used to select
the participants to be included in the study was random sampling. Those who did not fall in the eligibility criteria were exempted from joining the study.

3.4 SAMPLE SIZE

With a confidence interval of 95%, precision of 0.0105 and a prevalence of 4.2%, the sample size was determined to be 300 after inputting the above values into the formula used for calculating the adequate sample size in prevalence studies, that is, \( n = \frac{Z^2 \times P(1-P)}{d^2} \). Where \( n \) is the sample size, \( Z \) is the statistic corresponding to the level of confidence, \( P \) is the expected prevalence and \( d \) is the precision (corresponding to effect size).

3.5 SAMPLE COLLECTION

Blood samples (3ml) were drawn into 5ml plain tubes and the sera was separated and kept at -20°C until utilized. A well structured questionnaire was provided for data collection on demographic and risk factors after an informed oral and written consent was taken.

3.6.0 VARIABLES

3.6.1 DEPENDENT VARIABLES

The dependent variable for this study was blood donors.

3.6.2 INDEPENDENT VARIABLES

The independent variables for this study are; age, sex, marital status and HTLV infection.
3.7. DATA COLLECTION TECHNIQUES/METHODS AND TOOLS

Questionnaire method was used for demographics data collection among the blood donors. A structured survey questionnaire on the research topic was designed and administered to the blood donors. The questionnaire was used to collect specific and general responses from participants. Investigator administered questionnaire method was employed in filling questionnaire to ensure consistency in asking the questions.

The questionnaire was created and revised based on reference materials and recommendations from supervisors. All questionnaires were checked for mistakes and completeness. Any questionnaire with unclear responses or missing information that could not be clarified was excluded. Double entry of data was done to reduce data entry errors and validate authenticity.

3.8 DATA PROCESSING AND ANALYSIS

Descriptive statistics involving percentages, frequencies and mean were used to analyze demographic characteristics and the other information provided by the respondents. Computer assisted generation was used to clean data to manage outliers and missing data. The results were presented in frequencies and percentages shown in tables. The tests were analyzed using Statistical Package for Social Scientist (IBM SPSS) version 21.

3.9.0 SAMPLE ANALYSIS

Enzyme linked immunosorbent assay (ELISA) was performed in duplicate to determine the HTLV positive samples with strict adherence to the manufacturer’s guidelines.
3.9.1 HTLV I AND II ANTIBODIES SEROLOGICAL ASSAY

Sera from consented participants was screened for the presence of HTLV-I and II antibodies with a commercially available HTLV-I and II enzyme linked immunosorbent assay (ELISA) test kit. The EIAgen HTLV-I/II Ab Kit (ADALTIS, Italy) was used. The EIAgen HTLV-I/II Ab Kit is solid phase enzyme-linked immunosorbent assay intended for the detection of antibodies to Human T-cell Lymphotropic Virus type I and type II in human serum or plasma. It utilizes a mixture of antigens for the *in vitro* diagnostic screening in human serum or plasma (EDTA, Heparin, and Sodium Citrate) as a screening test, requiring repeat testing of initially reactive specimens. The assay is intended to screen individual human donors, including volunteer donors of whole blood and blood components, and other living donors for the presence of anti-HTLV-I/II.

It is also intended for use in testing blood and plasma specimens to screen organ donors when specimens are obtained while the donor's heart is still beating and in testing blood specimens from cadaveric (non-heart-beating) donors.

3.9.2 CHEMICAL & BIOLOGICAL PRINCIPLES OF THE PROCEDURE

The EIAgen HTLV-I/II Ab kit is based on the sandwich principle, a solid-phase enzyme-linked immunoassay technique, to measure antibodies to HTLV-I and HTLV-II levels in serum or plasma. Mixture of recombinant antigen and peptides representing immunodominant epitopes of HTLV-I p19, gp21, gp46 and HTLV-II gp21 and gp46 are coated onto wells of a microplate. Serum or plasma specimens and assay diluent are added to these wells, followed by a solution of biotinylated antigens. If antibodies specific to the antigens (IgG, IgM or IgA) are present in the specimen, they form stable complexes with the HTLV antigens attached to the well and the biotinylated antigens. Antigen-antibody complexes are then identified through the addition of
HRP-Streptavidin conjugate. The catalytic activity of HRP allows for the quantification of these antibody-antigen complexes.

HRP substrate solution is next added, and the catalytic activity of the bound HRP conjugate allows for the proportional revelation of these antibody-antigen complexes, thus establishing their presence or absence in the specimen. Wells containing specimens negative for anti-HTLV antibodies remain relatively colourless. A stop solution is added to each well and the resulting yellow colour is read on a microplate reader at 450 nm and if available at 405 nm and 600-650 nm.

3.9.3 ASSAY PROCEDURE

The assay was carried out according to the manufacturer’s instructions.

Three hundred microcrowsells were placed in the microwell holders. Twenty-five microliters (25µl) of the assay diluents was dispensed into the microwells. Twenty-five microliters (25µl) of negative control was dispensed in triplicate. A volume of 25µl of the positive control was also dispensed in triplicate. Twenty-five microliters (25µl) of each test specimen was added into the individual wells using a separate pipette tip for each specimen. The specimen was added gently avoiding overflowing and contaminating adjacent wells. Twenty-five microliters (25µl) of the first conjugate was dispensed into the wells with the controls and specimens to be tested. The microplates were then covered with adhesive microplate covers and incubated for 60 minutes at +37°C. After this preincubation period, the covers were removed and replaced with new adhesive microplate covers. Fifty microliters of (50µl) the second conjugate solution was dispensed into the individual wells containing the mixture of the first conjugate solution and the specimen. The microplates are covered with new adhesive microplate covers and then incubated at +37°C for 30
minutes. After incubation the microplate covers were removed and also the solution removed from the wells by inverting the microplate and tapping dry on paper towel. The microtiter plate was washed 5 cycles. After the last wash, the microplates were sharply turned upside down on absorbent paper to remove the last remaining liquid, taking care not to have dislodged strips from holder to ensure that all of the solution is removed from each well. A volume of 100µl of substrate solution was dispensed into each well after the washing and the microplates were covered. The substrate is light sensitive so extended exposure to light was avoided. The substrate-filled plate was incubated in the dark for 30 minutes at room temperature (24°C). The timing was started within 3 minutes from the addition of the reagent to the first well. The microplate covers were removed after incubating in the dark. The reaction was halted by adding 100µl of stop solution into each well, in the same order used for the addition of the substrate. The colour intensity of the solution in each well was measured at 450nm and 405nm (for OD higher than 2.0 at 450nm) and 620nm (optical reference wavelength). The readings were carried out just after the addition of the stop solution and not longer than 30 minutes after its addition. Some self oxidation of the chromogen can occur leading to high background. See Appendix III, IV and V for calculation of cut of point and interpretation of results.

3.10 ETHICAL CONSIDERATIONS

Before the research commenced, consent was obtained from the Ethics and Protocol Review Committee of the School of Biomedical and Allied Health Sciences as well as the research department of the National Blood Service, Accra, Ghana where the blood samples were obtained. Individual consent was also taken from the voluntary blood donors before questionnaires were administered and blood samples also taken. The participant’s rights were safeguarded by
enlightening them about the essence of the study and not compelling them to participate in the study. Participants were notified that their participation in the study is voluntary hence they reserve the right to withdraw at any time. In addition, participants were presented with phone contacts of the researcher for clarification as well as actions established to guarantee confidentiality of the research data. The general and specific objectives of the research plus the merits of the study were explained to participants. Every consenting participant was made to endorse the informed consent form (see Appendix I) with their signatures/thumbprints.
CHAPTER FOUR

RESULTS

4.1 PREVALENCE OF HTLV-I/II AMONG VOLUNTARY BLOOD DONORS

Three hundred (300) blood samples were tested for HTLV-I/II (Table 4.1). Among the 300 voluntary blood donor samples used for the ELSIA testing, only 10 were repeatedly considered to be reactive and the prevalence of the reactive samples was therefore 3.3%. Two hundred and ninety (290) out of the total 300 samples tested were detected to be non-reactive, thus representing 96.7% (Table 4.1). There were no seroindeterminate samples (0.0%) detected in this study (Table 1).

Table 4.1: PREVALENCE OF HTLV-I/II AMONG VOLUNTARY BLOOD DONORS

<table>
<thead>
<tr>
<th>ELISA TEST</th>
<th>Number of Donors Tested</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reactive</td>
<td>10</td>
<td>3.3</td>
</tr>
<tr>
<td>Non-Reactive</td>
<td>290</td>
<td>96.7</td>
</tr>
<tr>
<td>Seroindeterminate</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Total</td>
<td>300</td>
<td>100</td>
</tr>
</tbody>
</table>

4.2. AGE DISTRIBUTION AMONG HTLV-I/II INFECTED VOLUNTARY BLOOD DONORS

A total of 300 voluntary blood donors (table 4.2), consented orally and by writing to participate in this current study and were enrolled. The ages of the voluntary blood donors ranged from 18 years to 58 years. The mean age of the voluntary blood donors was 30.1±8.7 (table 4.2). To facilitate statistical analysis, voluntary blood donors were categorized into age ranges of equal intervals (table 4.2). Majority of voluntary blood donors were within the age range of 25-31 years and the least number of voluntary blood donors were also within the range of 53-59 years (table 4.2). The age prevalence of HTLV-I/II among the voluntary blood donors was 4(1.3%) for 18-24 years age group, 2(0.7%) for 25-31 years age group, 2(0.7%) for 32-38 years age group, 1(0.3%) for 39-45 years age group, 1(0.3%) for 46-52 years age group and 0(0.0%) for 53-59 years age group (table 4.2).
Table 4.2: AGE DISTRIBUTION AMONG HTLV-I/II INFECTED VOLUNTARY BLOOD DONORS

<table>
<thead>
<tr>
<th>Age Groups</th>
<th>Number of Donors Tested (%)</th>
<th>Number of Donors with HTLV-I/II Antibodies (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>18-24</td>
<td>91(30.3)</td>
<td>5(4.4)</td>
</tr>
<tr>
<td>25-31</td>
<td>104(34.7)</td>
<td>2(1.9)</td>
</tr>
<tr>
<td>32-38</td>
<td>51(17.0)</td>
<td>2(3.9)</td>
</tr>
<tr>
<td>39-45</td>
<td>34(11.3)</td>
<td>1(2.9)</td>
</tr>
<tr>
<td>46-52</td>
<td>15(5.0)</td>
<td>0(0.0)</td>
</tr>
<tr>
<td>53-59</td>
<td>5(0.0)</td>
<td>0(0.0)</td>
</tr>
<tr>
<td>Total</td>
<td>300(100)</td>
<td>10(3.3)</td>
</tr>
</tbody>
</table>

4.3 GENDER OF HTLV-I/II INFECTED VOLUNTARY BLOOD DONORS

With a total of 300 voluntary blood donors who donated blood, there were more male donors (74.0%) than female donors (26.0%). Out of the overall 10 seroreactive HTLV-I/II antibodies detected among the 300 donors enrolled in this study, 7 males were infected with HTLV-I/II representing a prevalence of 3.2% among the males while 3 females were infected, thus representing a prevalence of 3.8% among females. Thus more males were infected than the females considering the number; 7 males compared to 3 females.
Table 4.3: GENDER OF HTLV-I/II INFECTED VOLUNTARY BLOOD DONORS

<table>
<thead>
<tr>
<th>Gender</th>
<th>Number of Donors Tested (%)</th>
<th>Number of Donors with HTLV-I/II Antibodies (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male</td>
<td>222(74.0)</td>
<td>7(3.2)</td>
</tr>
<tr>
<td>Female</td>
<td>78(26.0)</td>
<td>3(3.8)</td>
</tr>
<tr>
<td>Total</td>
<td>300(100.0)</td>
<td>10(3.3)</td>
</tr>
</tbody>
</table>

4.4. MARITAL STATUS OF HTLV-I/II INFECTED VOLUNTARY BLOOD DONORS

The prevalence of HTLV antibodies among single, married, divorced and widowed voluntary blood donor subjects is illustrated in table 4.4. For a total of 96 (32%) married voluntary blood donors, 4 tested positive for HTLV-I/II antibodies, thus representing a prevalence of 4.2%. Also for a total of 200 (66.7%) single blood donors, 6 were infected and this represents prevalence of 3.0%. There were no widows 0(0.0%) and none were divorced 0(0.0%) too.
### Table 4.4: Marital Status of HTLV-I/II Infected Voluntary Blood Donors

<table>
<thead>
<tr>
<th>Marital Status</th>
<th>Number of Donors Tested (%)</th>
<th>Number of Donors with HTLV-I/II Antibodies (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Single</td>
<td>200(66.7)</td>
<td>6(3.0)</td>
</tr>
<tr>
<td>Married</td>
<td>96(32.0)</td>
<td>4(4.2)</td>
</tr>
<tr>
<td>Divorced</td>
<td>4(1.3)</td>
<td>0(0.0)</td>
</tr>
<tr>
<td>Widowed</td>
<td>0(0.0)</td>
<td>0(0.0)</td>
</tr>
<tr>
<td>Total</td>
<td>300(100.0)</td>
<td>10(3.3)</td>
</tr>
</tbody>
</table>

#### 4.5. Occupation of HTLV-I/II Infected Voluntary Blood Donors

For the total 300 voluntary blood donors tested, traders 90(30%) were the majority with an HTLV-I/II prevalence of 2(2.2%) and the least represented occupation were the security personnel 15(5.0%) with an HTLV-I/II prevalence of 0(0%) (Table 4.5). Other occupations of the voluntary blood donors enrolled in this study include; civil servants 66(22%) with an HTLV-I/II prevalence of 0(0.0%), students 20(6.7%) with an HTLV-I/II prevalence of 0(0.0%), artisan 30(10) with an HTLV-I/II prevalence of 1(0.3%), drivers 55(18.3%) with an HTLV-I/II prevalence of 6(2.0%) as well as other occupations 24(8.0%) with an HTLV-I/II prevalence of 0(0.0%).
Table 4.5: OCCUPATION OF HTLV-I/II INFECTED VOLUNTARY BLOOD DONORS

<table>
<thead>
<tr>
<th>Occupation</th>
<th>Number of Donors Tested (%)</th>
<th>Number of Donors with HTLV-I/II Antibodies (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Civil Servant</td>
<td>66(22.0)</td>
<td>1(1.5)</td>
</tr>
<tr>
<td>Student</td>
<td>20(6.7)</td>
<td>1(5.0)</td>
</tr>
<tr>
<td>Artisan</td>
<td>30(10)</td>
<td>1(3.3)</td>
</tr>
<tr>
<td>Trader</td>
<td>90(30)</td>
<td>2(2.2)</td>
</tr>
<tr>
<td>Driver</td>
<td>55(18.3)</td>
<td>4(7.3)</td>
</tr>
<tr>
<td>Security</td>
<td>15(5.0)</td>
<td>1(6.7)</td>
</tr>
<tr>
<td>Others</td>
<td>24(8.0)</td>
<td>0(0.0)</td>
</tr>
<tr>
<td>Total</td>
<td>300(100)</td>
<td>10(3.3)</td>
</tr>
</tbody>
</table>

4.6. COMPARISON BETWEEN NUMBER OF BLOOD DONATION AND HTLV-I/II POSITIVE BLOOD DONORS

For the total 300 voluntary blood donors, 200(66.7%) were first time donors while 34(11.3%) had donated more than three times before enrolled into this study. Also, 26(8.7%) had donated twice while 40(13.3%) had donated thrice. The HTLV-I/II prevalence among the voluntary blood donors donating for the first time, twice, thrice and more than three times is 0(0.0%), 2(0.7%), 1(0.3%) and 7(2.3%) respectively.
<table>
<thead>
<tr>
<th>Number of Donations</th>
<th>Number of donors tested (%)</th>
<th>Number of donors with HTLV-I/II Antibodies (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Once</td>
<td>200(66.7)</td>
<td>0(0.0)</td>
</tr>
<tr>
<td>Twice</td>
<td>26(8.7)</td>
<td>2(7.7)</td>
</tr>
<tr>
<td>Three times</td>
<td>40(13.3)</td>
<td>1(2.5)</td>
</tr>
<tr>
<td>More than three times</td>
<td>34(11.3)</td>
<td>7(20.6)</td>
</tr>
<tr>
<td>Total</td>
<td>300(100)</td>
<td>10(3.3)</td>
</tr>
</tbody>
</table>
CHAPTER FIVE

5.0 DISCUSSION

The findings obtained from this serological study showed that 10 voluntary blood donors out of the 300 enrolled in this study had been infected with HTLV-I/II thus producing antibodies against the virus. Therefore this study comes up with a prevalence of 3.3%. Prevalence of HTLV-I/II has been reported all over the world from various states. Several HTLV-I/II serosurveys in the United States of America (USA) have reported a prevalence of about 3-5%; Japan which is one of the regions where HTLV-I/II is endemic have reports of about 27% whilst a prevalence range of about 3-33% is reported in Central Africa (Oladipo et al, 2015). A research conducted among pre-partum women and commercial sex workers in Nigeria reported a prevalence of 16.7% and 22.9% (Forbi & Odetunde, 2007) respectively. A current study conducted in Dakar, Senegal by Durojaiye et al reported a seroprevalence of 0.16% (Durojaiye et al, 2014) as opposed to a study conducted in 1986 in Nigeria with findings of 2.0% as the seroprevalence among blood donors (Terry et al, 2011).

This current study was undertaken employing the use of EIAgen HTLV-I/II antibodies kit produced by Adaltis, Italy (Lot number: 17093; Reference number: 081321; Expiry date: 2018-10) which the manufacturer claimed to have 100% sensitivity and specificity for HTLV-I/II antibodies detection. In Africa and specifically Ghana where poverty has significant effect on health issues, the ELISA test kit can be used for detecting HTLV-I/II antibodies in blood donors and positive cases must be prohibited from donating blood.

As stated above, this research finding reports a prevalence of HTLV-I/II to be 3.3% among blood donors at the Southern Area Centre of the National Blood Service Ghana. This finding is higher
than the finding obtained in a comparable study conducted among blood donors in sub-Saharan African state of Senegal; where HTLV-I/II prevalence was reported as 0.16% (Diop et al, 2006). This study again reports a prevalence greater than others reported in some regions of the West African state of Nigeria such as 0.0% in Jos (Ma’an et al, 2016), 0.0% in Enugu (Okoye et al, 2015) and 1.4% in Port Harcourt (Iyalla et al, 2015). All these studies were conducted among blood donors. In Ghana the prevalence of HTLV-I/II is reported to be 0.7% among blood donors (Ampofo et al, 2002), and this is lower than the prevalence of 3.3% reported among voluntary blood donors by this study. The differences in the reported prevalence and that of the current study could be attributed to the different sample size and also the different ELISA kits hence the different specificity and sensitivity of the kits used. Also, the difference could be as a result of the stringent criteria for selecting prospective donors presently established ever since HIV/AIDS was reported. However the seroprevalence reported is lower than the reported seroprevalence of 25.8% in Ogbomoso, Oyo state, Nigeria (Oladipo et al, 2015). In the study conducted by Oladipo et al, their sample size was 93 while the sample size for this study was 300. The sample size of these two studies had an effect on the prevalences reported among blood donors.

The study population had an age range of 18-59 years. This age range is the advised and accepted age for prospective blood donors in Ghana. This justifies why no participant in this study was below age 18 and a few above 50 years.

The prevalence of HTLV-I/II among the elderly group, that is 53-59 years age group was 0.0% whilst the younger voluntary blood donor age groups of this study, that is, 18-24, 25-31, 32-38 and 39-45 had different prevalence of 4.4%, 1.9%, 3.9% and 2.9% respectively which may be an indication supporting the fact that the HTLV-I/II infection was not present in the past but is present in the community due to the life style of the young age groups. This particular research
could not ascertain the risk factors that influence this young age group, however, a study conducted in Western Nigeria, determined the prevalence among persons who are sexually active as being 16% and 23% (Forbi & Odetunde, 2007) not excluding commercial sex workers hence it can be inferred that active sexuality is a risk factor since these age group individuals are sexually active.

The participants in the age group 18-24 years recorded the highest HTLV-I/II infection of 5(1.3%). This age range represents the most active age group of this study participant. This could indicate that this is the age range that HTLV-1 infection mostly occurs. This finding correlates with Terry et al and Oladipo et al who had the same findings as this study (Terry et al, 2011; Oladipo et al, 2015). The findings of this study does not correlate with the fact portrayed by Ma’an et al who reported a prevalence of 0.0% and thus explained their findings to be so because of the high population of voluntary blood donors which is characteristic of the Nigerian national blood transfusion services. Nonetheless this current study in Ghana enrolled only voluntary blood donors but identified anti-HTLV-I/II immunoglobulins among such donors hence this may be a wake-up call for transfusion scientists. The reactivity detected among voluntary blood donors could be due to the sexual behavioural life style of the participants.

Also this finding is not in agreement with finding of other studies showing the occurrence of HTLV-I/II spread to be far elevated in females than in males unlike in males greatly than females. This observation can be explained to be due to the greater number of males than females recorded in this research study. This has been observed in several other studies (Graca et al, 2013).
The sex and age pattern of blood donors in this study corresponds to another similar study conducted in Oshogbo, Nigeria with a prevalence of 3.6% (Terry et al, 2011) and another study in Jos, Nigeria with a prevalence of 0.0% (Ma’an et al, 2016). In this study, the 222 (74%) males enrolled were more than the 78 (26%) females. This therefore reflects the reason why more males (7) with a prevalence of 3.2% infected were identified as compared to 3 females who were infected representing a prevalence of 3.8% (table 4.3). This finding corresponds to the reports made by Oladipo et al in a similar study in Ogbomoso, Nigeria. The high prevalence among both the married and the singles can also be attributed to the kind of life the infected persons are living such as unprotected sex with multi-partners who are infected thus being a risk factor.

Although this study did not determine risk factors associated with HTLV-I/II infection, again, inferring from the study conducted by Forbi & Odetunde (2007) among commercial sex workers in South Western Nigeria, individuals who engage such infected individuals are at risk of being infected via unprotected sex. This may be possible to relate both HTLV-I/II infected voluntary blood donors married and single to their sexual life style.

This study reports 90 (30%) traders as the highest occupation to have been enrolled (table 4.5) although the study seems to have various occupations (table 4.5) represented in this study. This somehow fair representation with seemingly different educational background could be attributed to the awareness programmes deployed via media publicity, visit to places of worship schools, market places and also the mass blood donation exercises by the NBSG.

Drivers had a higher prevalence (7.3%) as compared to the other occupations. The prevalence of 7.3% (table 4.5) among drivers who enrolled in the study, which is higher than the other occupations corresponds to the same occupation (Driver, 18.2%) also found to be higher than other occupations in a similar study findings by Terry et al (2011). Thus, vehicle drivers have
been noted to be linked with this high prevalence of HTLV-I/II infections. The job description of drivers does not expose them to blood or blood products hence that cannot be speculated to be a risk factor. However, the possible reasons that can be provided is that, one major occupational hazard associated with their work are road motor accidents. Some fatal accidents may require transfusion therapy due to loss of large volumes of blood as a result of the nature of injuries associated with such accidents. This can lead to the risk of being infectd with HTLV during the transfusion therapy. Also, the nature of the motor vehicle driving occupation involves travelling long distances away from home hence they tend to satisfy their sexual pleasures through prostitutes (Terry et al, 2011) at their temporary destinations.

HTLV is known and has been proven to be a blood borne infection (Terry et al, 2011). In this study, 200 (66.7%) (table 4.6) first time donors constituted the majority of the voluntary blood donors. Among this group, HTLV-I/II antibodies were not detected in the sera. Two (7.7%) (Table 4.6) donors were identified to have HTLV-I/II antibodies in their sera. These are donors 26 (87.6%) (table 4.6) who are part of the voluntary blood donor group donating blood for the second time in their lives. Among those donating for the third time and frequent donors (more than 3 times) groups, 1 (2.5) and 7(20.6%) donors were infected with the virus respectively. If HTLV-I/II is screened for among blood donors then it implies that such HTLV-I/II positive donors will be excluded. However as it stands now, there is no routine screening of HTLV-I/II in Ghana hence such positive HTLV-I/II donors cannot be excluded from donating blood hence they tend to serve as sources of spreading the virus among transfusion therapy beneficiaries. This simply implies that, although HTLVSs are capable of being spread via transfusion therapy, anti-HTLV-I/II screening while excluding seroreactive donors and their donation units can efficiently interrupt this route of spreading HTLV-I/II. Infections detected after transfusion therapy have
been documented in other regions of the world (Terry et al, 2011). This implies that such individuals can easily infect other recipients of such HTLV-I/II contaminated blood. So, in as much as drastic measures have been put in place to curtail the spread of some TTI’s through routine screening, the same needs to be done for HTLV-I/II in the blood banks located in endemic areas.

Several states across the length and breadth of the globe with least prevalence are controlling infection of HTLV-I/II. Through routine screening of blood donors, this is done to obtain evidence of HTLV-I/II infection hence prohibit such prospective donors from donating. This measure has helped reduce HTLV-I/II spreading in USA. Adjei et al (2003) and Ampofo et al (2003) (4.2% and 0.7%) have both reported HTLV-I/II antibodies a decade and half ago but HTLV-I/II screening is still not a routine test in blood banks in the country.

The prevalence of HTLV-I/II findings of this study implies there is some bit of evidence of recent infection among the young age groups. This seroprevalence furnishes the setting of transfusion scientist with the information about the incidence of this HTLV-I/II infection in the community. This finding together with previous findings should emphasize the urgency for regular epidemiological surveillance to help in the policy decision-making as to blood safety since not a single prospective blood donor is ever screened routinely for HTLV-I/II antibodies.

The spread of blood borne infections through the use of blood, its derivative or components is evident since the inception of transfusion. The last five decades has seen an elevation in the use of blood, its components and derivative. Nonetheless, the progress made with regards to the understanding of blood borne transmission of diseases has brought about a tremendous technological change especially in the area where blood donor screening is involved. The phobia
that goes along with the acquisition of AIDS after blood transfusion therapy, made transfusion transmitted disease an issue of great public interest with major effects on blood transfusion science. Several medico-legal issues have erupted ever since TTI’s became a public concern. This concern has made some affected patients to sue physicians, health facilities and blood banks to the extent of asking for the particulars of the blood donor “who was the source of the infection”. The safe use of blood and blood products with regards to TTI’s is based principally on the ability to detect apparently healthy asymptomatic carriers. TTI’s have been known to cause a huge burden on transfusion therapy, importantly in Africa as a result of an elevated request for transfusion.

HTLV-I/II infection renders about 15-20 million people worldwide as asymptomatic carriers of the virus (Iyalla et al, 2015). The general prevalence of HTLV-I/II is estimated at 15-20% although rates as high as 24%, 27% and even 33% has been reported in Nigeria, Japan and Central Africa respectively (Oladipo et al, 2015). The elucidation of HTLV-I/II has put the virus in the known as a blood borne infectious agent which can be spread via transfusion therapy. The knowledge of this virus together with other TTI’s has necessitated an increased emphasis to be placed on two basic goals thus; safety and protection of life (Oladipo et al, 2015). There is a difference between HTLV-I/II and HIV; however, they possess the same routes for spreading. Among all the routes through which HTLV-I/II spreads, the most effective mode is through transfusion therapy.

5.1 CONCLUSION

Several investigators have determined via their work a number of sociodemographic factors that affect the prevalence of HTLV-I/II infection. Some of these factors include increasing age, sex,
geographical location, marital status, poor socioeconomic status, recurrent sexually transmitted infection and female sex (Okoye et al, 2015). Although this present study did not investigate such risk factors, it may be possible such factors play a role in the spread of HTLV-I/II among the blood donors.

The findings of this study report an HTLV-I/II antibodies prevalence of 3.3% implying the virus is part of a few major transfusion transmissible viral infections among voluntary blood donors at the Southern Area Blood Centre of the National Blood Service Ghana. This study also reports Males 7(3.2%), 18-24 year age group 5(4.4%), Single 6(3.0%), Driver 4(7.3%) and frequent donors (more than three times) 7(20.6%) as the most infected groups.

Although such prevalence has been determined in several countries in Africa and the world as a whole, larger and multi-center prospective studies should be conducted among several groups apart from blood donors such as multi-transfused patients in order to conclusively establish the exact seroprevalence of HTLV-I/II among blood donors in Ghana.

5.2 RECOMMENDATIONS

A large multi-centered prospective study should be conducted to determine the seroprevalence of HTLV-I infection among blood donors and multi-transfused blood donors. Outcome from such studies will help support the need for routine screening of HTLV-I/II infection among blood donors.

1. An accepted and concurrent national epidemiological study must be conducted in all the regions of Ghana to determine the exact seroprevalence of HTLV-I/I infection and its
pattern of dissemination. The national data produced from such a study should be reviewed and updated on timely basis.

2. A national based research body should be put together and mandated with the payments and procurement of materials, kits and reagents needed for such research. Research in this area of transfusion science and others should be facilitated by such a national research body in place and functioning perfectly.

5.3 LIMITATIONS

1. This study could not perform a confirmatory test on the positive cases.

2. This study was unable to determine risk factors associated with HTLV-I/II infection.

3. Questionnaire did not find out voluntary donor ever being transfused.
REFERENCES


Tsukasaki, K., Hermine, O., Bazarbachi, A., Ratner, L., Ramos, J. C., Harrington, W., O’Mahony, J. D., Janik, J. E., Bittencourt, A. L., Taylor, G. P., Yamaguchi, K., Utsunomiya, A.,


APPENDIX I:

CONSENT FORM

TITLE: HUMAN T-CELL LYMPHOTROPIC VIRUS TYPE I AND II (HTLV-I/II) INFECTION AMONG BLOOD DONORS AT THE NATIONAL BLOOD SERVICE (NBSG); IMPLICATION FOR BLOOD DONOR EXCLUSION

PRINCIPAL INVESTIGATOR: BEINYA DOMINIC KWEKU

INSTITUTION: COLLEGE OF HEALTH SCIENCES, SCHOOL OF BIOMEDICAL AND ALLIED HEALTH SCIENCES, DEPARTMENT OF MEDICAL LABORATORY SCIENCES, UNIVERSITY OF GHANA, LEGON.

EMAIL: dominickwekubeinya@yahoo.com

TELEPHONE: 0200627226/0542805626

PROCEDURE: This study anticipates determining the prevalence of HTLV among blood donors at the national blood service. This will be made possible by issuing questionnaires to voluntary blood donors enrolled into the study and also sampling of blood from the same group of voluntary blood donors that consent to join the study.

You are therefore kindly requested to partake in this study embarked by Beinya Dominic Kweku, a student of University of Ghana pursuing a Professional Master in Medical Laboratory Science in the School of Biomedical and Allied Health Sciences. This form is aimed to obtain your concurrence to join this study. This consent form includes details regarding this study and so as to guarantee your understanding about participating, you are encouraged to read and
sign/thumbprint. Prior to commencement of the study, it is essential for you to provide us with some facts about yourself. You are kindly entreated to answer a questionnaire that will take approximately 10 minutes of your time to complete. You may voluntarily turn down to answer any questions and also refuse inclusion at any level of the study. Participants who agree to participate in the study will not be given any monetary reward. There is no direct benefit to participant; nonetheless, information gathered at the end of the study could be used to enhance the screening requirements during blood donation exercises.

CONFIDENTIALITY

Assurance is given to all participants that, whatever details you provide will be handled with stringent confidentiality and will be solely for research reasons. Your answers will not be disclosed to anybody who is not a member of the research team. Data testing will be done on a cumulative basis to ensure obscurity. This study has been reviewed and approved by the ethics and protocol review committee of the school of biomedical and allied health sciences. Information provided will be hoarded in protected places such as secured cabinets. Data will be inputted into SPSS and electronic files accessible by only principal investigator and research supervisors. Participants’ identity such as names will not be written on the questionnaire. However, each questionnaire will be given a unique identification number. If you have any questions and concerns, we will be more than happy to answer and address them now or later at your convenient time. You may contact me on the telephone number and email provided. Telephone: 0200627226/0542805626. Email: dominickwekübeinya@yahoo.com.
PARTICIPANT CONSENT FORM

I……………………………………………………………………. have been informed in details on the whole methodology and significance of this current study which is being conducted by Beinya Dominic Kweku. On my own free will, I therefore agree to partake of the study centered on my comprehension of what the study involves. I am doing this on requirement that under no condition should my details to my actual identity be made available to another person(s) after providing all the facts requested of me for this exact study as assured by the researcher.

Respondent’s signature……………………….. Date :……./……./…….(dd/mm.yy).

Researcher’s signature……………………….. Date :……./……./…….(dd/mm.yy).
APPENDIX II:

QUESTIONNAIRE

HUMAN T-CELL LYMPHOTROPIC VIRUS TYPE I AND II (HTLV-I/II) INFECTION AMONG BLOOD DONORS AT THE NATIONAL BLOOD SERVICE (NBSG); IMPLICATION FOR BLOOD DONOR EXCLUSION

My name is Beinya Dominic Kweku. This research is being undertaken for academic reasons as part of the prerequisites for the award of professional Master of Science (MSc) Degree at the University of Ghana, Legon. The rationale of the research is to determine the occurrence of HTLV among blood donors at the National Blood Service. Involvement in this study is completely voluntary. Please do not hesitate to articulate yourself as truthfully as possible in your reply to the questions that I will be asked. Replies will be confidential and will not be circulated to non members of the research team. Thank for agreeing to partake in the study.

Beinya Dominic Kweku (BSc Biological Sciences). Mobile Phone Contact: +2330200627226/+233542805626. Email: dominickwekubeinya@yahoo.com
QUESTIONNAIRE

1. Age of respondent: ………………… Date of birth of birth: ………………… (Dd/mm/yy)

2. Gender
   Male □
   Female □

3. Occupation of respondent: …………………………………………………………………………………

4. Marital status of respondent: M/S

5. Have you ever donated blood: Yes/No

6. How many times
   Once □
   Twice □
   Three times □
   More than three times □
APPENDIX III:

TABLE SHOWING INFORMATION PROVIDED BY THE TEN VOLUNTARY BLOOD DONORS THAT TESTED POSITIVE

<table>
<thead>
<tr>
<th>ID</th>
<th>Age</th>
<th>Gender</th>
<th>Marital Status</th>
<th>Occupation</th>
<th>Frequency of Donation</th>
<th>Mean of absorbance at 450nm</th>
<th>Htlv ELISA Test</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>H070</td>
<td>20</td>
<td>Male</td>
<td>Single</td>
<td>Student</td>
<td>Twice</td>
<td>0.363 POS</td>
</tr>
<tr>
<td>2</td>
<td>H072</td>
<td>23</td>
<td>Male</td>
<td>Single</td>
<td>Driver</td>
<td>More than 3</td>
<td>0.370 POS</td>
</tr>
<tr>
<td>3</td>
<td>H075</td>
<td>38</td>
<td>Male</td>
<td>Married</td>
<td>Civil Servant</td>
<td>Twice</td>
<td>0.352 POS</td>
</tr>
<tr>
<td>4</td>
<td>H127</td>
<td>22</td>
<td>Female</td>
<td>Single</td>
<td>Security</td>
<td>More than 3</td>
<td>0.381 POS</td>
</tr>
<tr>
<td>5</td>
<td>H140</td>
<td>24</td>
<td>Male</td>
<td>Married</td>
<td>Trader</td>
<td>More than 3</td>
<td>0.351 POS</td>
</tr>
<tr>
<td>6</td>
<td>H167</td>
<td>30</td>
<td>Male</td>
<td>Married</td>
<td>Driver</td>
<td>More than 3</td>
<td>0.351 POS</td>
</tr>
<tr>
<td>7</td>
<td>H222</td>
<td>33</td>
<td>Female</td>
<td>Married</td>
<td>Trader</td>
<td>More than 3</td>
<td>0.360 POS</td>
</tr>
<tr>
<td>8</td>
<td>H223</td>
<td>24</td>
<td>Male</td>
<td>Married</td>
<td>Artisan</td>
<td>More than 3</td>
<td>0.359 POS</td>
</tr>
<tr>
<td>9</td>
<td>H252</td>
<td>31</td>
<td>Male</td>
<td>Single</td>
<td>Driver</td>
<td>Thrice</td>
<td>0.358 POS</td>
</tr>
<tr>
<td>10</td>
<td>H263</td>
<td>22</td>
<td>Female</td>
<td>Single</td>
<td>Driver</td>
<td>More than 3</td>
<td>0.362 POS</td>
</tr>
</tbody>
</table>
APPENDIX IV:

DETERMINATION OF CUT OF POINT

<table>
<thead>
<tr>
<th></th>
<th>NEGATIVE CONTROL (NC)</th>
<th>POSITIVE CONTROL (PC)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.198</td>
<td>2.461</td>
</tr>
<tr>
<td>2</td>
<td>0.214</td>
<td>2.267</td>
</tr>
<tr>
<td>3</td>
<td>0.222</td>
<td>2.313</td>
</tr>
<tr>
<td>TOTAL</td>
<td>0.211</td>
<td>2.347</td>
</tr>
</tbody>
</table>

\[
\text{NC_{MEAN}} = \frac{0.198 + 0.214 + 0.222}{3} = 0.211
\]

\[
\text{PC_{MEAN}} = \frac{2.461 + 2.267 + 2.313}{3} = 2.347
\]

\[
\text{CUT OF POINT (CO)} = \frac{\text{NC_{MEAN}} + \text{PC_{MEAN}}}{8} = \frac{0.211 + 2.347}{8} = 0.320
\]

\[
\text{GRAY ZONE} = 0.85 \times \text{CO} = 0.85 \times 0.320 = 0.271
\]
APPENDIX V:

MANUFACTURER’S GUIDELINES FOR INTERPRETATING ELISA RESULTS

1. If the absorbance value is less than 85% of the calculated cut-off value (CO × 0.85), then the specimen is considered non-reactive (negative) for HTLV-I/II antibodies.

2. If the initial absorbance value is equal to or greater than 85% of the cut off (Gray zone or initially reactive), retest the specimen in duplicate. Position the Gray zone specimens and weakly positive specimens close to the Positive Control (PC).

3. If the absorbance values of both retests are less than 85% of the calculated cut-off value, then the specimen is considered non-reactive (negative) for HTLV antibodies.

4. If the absorbance value of one or both retests is greater than or equal to the cut-off, then the specimen is considered reactive or positive for HTLV antibodies by the criteria of the EIAgen HTLV I-II Ab Kit.

5. If the absorbance value of one or both of the retests is within 85-100% of the calculated cut-off value, it is again fallen into the Gray zone. Test again with a new specimen from a move recent blood drawing.