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UNIVERSITY OF GHANA

COLLEGE OF HEALTH SCIENCES

DEPARTMENT OF HAEMATOLOGY

THE ROLE OF HUMAN T-CELL LYMPHOTROPIC VIRUS TYPE 1 (HTLV-1) INFECTIONS IN HAEMATOLOGICAL MALIGNANCIES

BY

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DECLARATION

I, FRANK AWUKU do hereby declare that this project was carried out by me at the Departments of Haematology and Medical Microbiology of the University of Ghana, College of Health Sciences under the supervision of DR. AMMA BENNEH-AKWASI KUMA and PROF. KWAMENA SAGOE.

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DEDICATION

I dedicate this project to GOD ALMIGHTY, His Son JESUS CHRIST my Lord and personal saviour and THE HOLY SPIRIT my enabler, to my family, my wife and my daughter **ELDORA KLENAM AWUKU**.

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LIST OF ABBREVIATIONS

AA	Amino Acids
ALL	Acute Lymphoblastic Leukaemia
AML	Acute Myeloid Leukaemia
ATF	Activating Transcription Factor
ATLL	Adult T-Cell Leukaemia/Lymphoma
CA	HTLV-1 capsid
CLL	Chronic Lymphocytic Leukaemia
CML	Chronic Myeloid Leukaemia
CNS	Central Nervous System
CREB	cyclic AMP-Response Component Binding Proteins
CREM	cyclic AMP-Responsive Component Modulator
CTLs	Cytotoxic T Lymphocytes
DNA	Deoxyribonucleic Acid
EBV	Epstein–Barr virus
EIA	Enzyme Immunoassay
ELISA	Enzyme-Linked Immunosorbent Assay
Env	Envelope Glycoprotein

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Gag	Group Specific Antigen
GLUT1	Glucose Transporter 1
gRNA	Genomic RNA
HAM/TSP	HTLV-1 Associated Myelopathy/ Tropical Spastic Paraparesis
HB	Haemoglobin
HBC	Hepatitis C virus
HBZ	HTLV-1 Basic Leucine Zipper
HIV	Human Immunodeficiency Virus
HL	Hodgkin Lymphoma
HSPG	Heparin Sulfate Proteoglycan
hTERT	Human Telomerase Reverse Transcriptase
HTLV-1	Human T-cell Lymphotropic virus type I
HTLV-2	Human T-cell Lymphotropic virus type 2
HTLV-3	Human T-cell Lymphotropic virus type 3
HTLV-4	Human T-cell Lymphotropic virus type 4
ICAM-1	Inter Cellular Adhesion Molecule 1
IFA	Indirect Immunofluorescence Assays
IN	Integrase

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LDH	Lactate dehydrogenase
LFT	Liver Function Test
LTR	Long Terminal Repeat
LYM	Lymphocytes
MDS	Myelodysplastic Syndrome
MHC	Major Histocompatibility Complex
MM	Multiple Myeloma
MPS	Myeloproliferative Neoplasm
MTCT	Mother-To-Child-Transmission
MTOC	Microtubule Organizing Center
NF-κB	Nuclear Factor-Kappa B
NHL	Non-Hodgkin Lymphoma
NRP-1	Neuropilin-1
ORF	Open Reading Frame
PBMC	Peripheral Blood Mononuclear Cells
PCR	Polymerase Chain Reaction
Pol	Polymerase
PLT	Platelets

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pVL	Proviral Load
Rex	Regulator of Viral Expression
RNA	Ribonucleic Acid
RT	Reverse Transcriptase
RXRE	Rex-Responsive Element
STLV	Simian T-cell Leukaemia Virus
SU	Surface Glycoprotein
Tax	Transcriptional Activator
TM	Transmembrane Protein
TRE	Tax Responsive Elements
TWBC	Total White Blood Cells
VS	Virological Synapse
WBC	White Blood Cells

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ABSTRACT

Background: The Human T-Cell Lymphotropic Virus (HTLV) was first recognized in the 1980s by Poiesz *et al* and was the first retrovirus identified in human. HTLVs are human retroviruses identified as the causative agents of Adult T-Cell Leukemia/Lymphoma (ATLL), a lymphoproliferative malignancy of CD4+ activated cells and HTLV-1 associated Myelopathy/Tropical Spastic Paraparesis (HAM/TSP), a demyelinating disease. There is a significant association of HTLV 1 with lymphoid malignancies. Worldwide, about 10 to 20 million persons are estimated to be living with HTLV-1 infection; a greater part of these infections are not symptomatic. Approximately, 5% of these infections progress to cause diseases, which are exceptionally severe (Confidence et al., 2018; Gessain & Cassar, 2012; Gross & Thoma-kress, 2016; Verdonck et al., 2007). The main transmission routes of the virus are by mother to child (mainly through breastfeeding), sexual intercourse, sharing of infected needles among drug users and by blood transfusion.

In Ghana, reported seroprevalence of HTLV-1 is between 0.5-4.2% among different study groups mainly among blood donors and pregnant women (Ampofo et al., 2002; Armah et al., 2006; Sarkodie et al., 2001) but none of the studies included malignant haematological disorders. Current evidence with regards to the role of HTLV-1 in hematological malignancies in Ghana is scarce.

Aim: To determine the role of Human T-cell Lymphotropic Virus type 1 (HTLV-1) infections in haematological malignancies among patients diagnosed at the Department of Haematology, Korle-Bu Teaching Hospital.

Methods: This was a cross-sectional study involving 200 study participants randomly selected. Data abstraction forms were used to obtain demographic data and clinical history from the study

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participants. A blood sample of 5mls was collected from the antecubital fossa into a sterile, plain tube. The sera was then be separated into sterile cryovials and stored in aliquots at -70°C prior to analysis. Sera from study participants were tested using commercial immunoassays for Anti-HTLV-1 specific for HTLV-1 by ELISA method using anti HTLV (MP Diagnostics HTLV ELISA 4.0). The testing was done in duplicates. Analysis of the data was done using Excel and SPSS software version 23.0.

Results: A total of 200 respondents were tested with median age of 49.50 years. There were more females (54.5%) than males. Out of the 200 samples, 14 of them were seropositive for HTLV-1 infection comprising 3 males and 11 females resulting in an overall seroprevalence of 7.0%. This research found no statistically significant association between haematological malignancies, gender and transfusion history and HTLV-1 infection in the study population (P-value = 0.081, 0.061 and 0.551 respectively). This study also did not show significant association between Hb (P-value = 0.941), TWBC (P-value = 0.924), PLT (P-value = 0.281) and LYM (P-value = 0.496) and HTLV-1 infection.

Conclusion: The findings of this current study show the seroprevalence of HTLV-1 of 7.0% among patients with haematological malignancies. The HTLV-1 seroprevalence obtained in this study is high. Consequently, it is possible to consider HTLV-1 screening during clinical and laboratory workup of patients with haematological malignancies in Ghana. However, there was no significant association between HTLV-1 infection and haematological malignancies, previous history of blood transfusion as well as some haematologic parameters (Hb, lymphocyte count, total WBC and platelet count) studied.

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CHAPTER ONE

1.0 INTRODUCTION

Haematological malignancies are cancers of the blood and blood forming tissues. They result from genetic alterations of cells in the bone marrow or lymphoid tissue thereby giving rise to clonal diseases (Hoffbrand & Moss, 2016). Types of haematological malignancies include Non - Hodgkin Lymphomas, Leukaemias, Plasma Cell Myelomas and Myeloproliferative Neoplasms. The aetiology of these malignancies are thought to be varied; from inherited factors such as Down's syndrome, environmental factors including exposure to industrial chemicals, drugs especially alkylating drugs, radiations especially to the marrow. Infections due to bacteria and viruses are most often implicated. Approximately 18% of all malignancies including haematologic ones are attributed to infections (Hoffbrand & Moss, 2016). Some microorganisms etiologically have been linked to the development of lymphomas with some studies implicating viruses especially as having links to malignant lymphomas. Infections of viral origin are indicated in many haematologic malignancies especially in different subtypes of lymphomas. Notable examples include the Epstein–Barr virus (EBV) which is linked to most Burkitt lymphomas and some subtypes of Hodgkin lymphomas especially the mixed cellularity subtypes (Shannon-lowe, Rickinson, & Bell, 2017). The Human Immunodeficiency Virus (HIV) is also linked to lymphomas at unusual sites especially the central nervous system (Grogg, Miller, & Dogan, 2007). Human T- Cell Lymphotropic virus type 1 (HTLV-1) has been linked with cases of Adult T- Cell Lymphoma/Leukaemia (ATLL) and Hepatitis C virus (HCV) is linked with lymphatic tissue marginal as well as different lymphomas (Carbone & Gloghini, 2013; Confidence et al., 2018). Other microorganisms such as Helicobacter pylori is associated with

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gastric MALT malignant neoplastic disease. Beside the main malignancies, that these viruses are associated with, ample evidence suggest that they may cause or are associated with other haematological malignancies. There is limited data on the extent of impart of HTLV-1 and its role in haematologic malignancies in Africa, West Africa and for that matter Ghana.

HTLVs are enveloped RNA (Ribonucleic Acid) viruses that belong to the group of viruses known as Retroviridae family and subdivision Deltaretrovirus (Switzer, Heneine, & Owen, 2015). The discovery and identification of HTLVs begun as far back as the 1980s when a group of scientists in Japan who isolated the viruses from patients suffering from Adult T-Cell Leukaemia and Hairy T-Cell Leukaemia (Besson & Kazanji, 2009; Carneiro-proietti, Catalan-soares, Proietti, Ba, & Murphy, 2005). These viruses were later named HTLV-1 and HTLV-2. Even though the two viral agents can infect and transform T-cells in vitro and cause long-lasting disorders in vivo, they show considerably dissimilar infective properties. HTLV 1 is documented as the causative agent of ATLL and HTLV-1 associated Myelopathy/Tropical Spastic Paraparesis (HAM/TSP). By distinction, HTLV-2 is not as of yet linked to any human malignancy. Nonetheless, extensive cohort researches discovered that HTLV-2 infection is also linked with increased lymphocytes counts, elevated chance of developing inflammatory disorders, infective maladies and with elevated all-cause death (Rende et al., 2012). Currently there are four known types of HTLVs; HTLV type 1 and type 2 and very lately the discovery of type 3 and type 4 in parts of Central Africa. There is scarcity of information on the virulence and pathological processes of HTLV types 3 and 4 (Switzer et al., 2015; Wolfe et al., 2005).

HTLV-1 is the most pathogenic of them all. Globally, HTLV-1 infections remains projected at about 20 million people with the highest being in Southern Japan (Ciminelli, Melo, Copin, Carneiro-proietti, & Meireles, 2016; Hewagama, Krishnaswamy, King, Davis, & Baird, 2014).

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HTLV-1 was first described and identified over 35 years ago and since then various epidemiological reviews conducted to better understand this viral pathogen (Verdonck et al., 2007). HTLV-1 is the foremost retrovirus linked to malignant disease in humans. This finding opened the way for greater comprehension of retroviruses, especially HIV, and their special effects on humans. Even though HIV-1 and HTLV-1 are closely related with regards to genomic structure, cellular tropism, and viral replication, they are not utterly compatible with regards to treatment, particularly due to the contrasting outcome of their effect on cells: death against immortalization respectively. Currently, the antiretroviral therapies being used for the treatment of HIV-1 infections and to control its escalation globally have only little effect on HTLV-1 infections, and hence, cannot avert the occurrence of HTLV-associated maladies, that require extremely efficient therapies (Futsch, Mahieux, & Dutartre, 2018). Sadly, HTLV-1-infected individuals are yet to benefit from extremely efficient therapies only persons with symptoms are given treatment with unreliable results.

Epidemiologically it has been proven that HTLV-1 causes major diseases such as ATLL, HTLV associated uveitis, infective dermatitis and HAM/TSP (Mahieux & Gessain, 2003). Three main routes are involved in its transmission; the most prominent among them is vertical transmission mostly through prolonged breastfeeding after six months of age. The next most common route is sexual transmission. Women are more at risk by this route of transmission than men are. Next is blood transfusion, especially blood transfusion involving cellular products (Carneiro-proietti et al., 2005; Gessain & Cassar, 2012; Masao Matsuoka, 2005).

In a study by Monavari *et al*., in Tehran, Iran HTLV-1 was detected in some few cases of haematologic disorders like Acute Myeloid Leukaemia (AML), Chronic Myeloid Leukaemia (CML), and Acute Lymphoblastic Leukaemia (ALL). A significant association was also

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established between seropositive cases and previous history of blood transfusion. In another study in Lagos, Nigeria, amongst persons with lymphoid disorders, 5.1% of the cases were seropositive for HTLV-1 infection. Malignancies such as Myeloproliferative disorder, Non-Hodgkin Lymphoma, Chronic Lymphocytic Lymphoma (CLL) and Hodgkin Lymphoma were among the seropositive cases (Akinbami et al., 2014).

Currently in Ghana, HTLV-1/2 screening is not available yet for blood donors thus blood and or blood products recipients are in danger of being infected with HTLV-1/2 thereby increasing their risk of developing malignant haematologic lymphomas. Research conducted by Einsiedel *et al*., in Australia in 2016, revealed that conditions like haematological malignancies among other conditions provide the basis for ordering HTLV-1 serological tests during the usual medical and laboratory diagnosis of patients (Einsiedel et al., 2016). However, during the routine medical and laboratory diagnosis of patients with haematological malignancies at Korle-Bu Teaching Hospital, patients are not tested for HTLV-1/2 infection as part of the management of their malignancies. This may be owing to the presumed low prevalence of HTLV-1/2 here in Ghana.

1.1 PROBLEM STATEMENT

Worldwide, about 10 to 20 million persons are projected to be living with HTLV-1 infection; a greater part of these infections are not symptomatic. Approximately, 5% of these infections progress to cause diseases, which are exceptionally severe (Confidence et al., 2018; Gessain & Cassar, 2012; Gross & Thoma-kress, 2016; Verdonck et al., 2007). In Ghana, various studies between 2001 and 2006 reported HTLV-1 seroprevalence between 0.5-4.2% among different study groups (Ampofo et al., 2002; Armah et al., 2006; Sarkodie et al., 2001) but none of the studies included malignant haematological cases. It is however important to ascertain the role of HTLV-1 infections in haematological malignancies seen at the Department of Haematology,

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Korle-Bu Teaching Hospital and also the seroprevalence of HTLV-1 infections of these cases in order to maximize care and improve treatment outcomes. Epidemiologically, it has been proven that HTLV-1 causes major diseases such as ATLL, HAM/TSP, uveitis and infective dermatitis (Mahieux & Gessain, 2003; Manns et al., 1991). Association of HTLV-1 with more haematologic disorders like Acute Myeloid Leukaemia (AML), Hodgkin Lymphoma (HL), Chronic Myeloid Leukaemia (CML), Acute Lymphoblastic Leukaemia (ALL), non-Hodgkin Lymphoma (NHL), Chronic Lymphocytic Leukaemia (CLL), and myeloproliferative disorder have been reported in other studies (Adedayo & Shehu, 2004; Monavari et al., 2011). Ghana is considered an endemic region for HTLV-1 infection (Carneiro-proietti et al., 2005; Djuicy et al., 2018; Gonçalves et al., 2010) however during clinical and laboratory workup of patients with haematological malignancies at Korle-Bu Teaching Hospital Haematology department, patients are not screened for HTLV-1/2 as the impact of the virus on these diseases is not known. Management of these malignancies can however be enhanced with the information gathered from this study.

1.2 JUSTIFICATION

Ghana's foremost document on the control of cancer (National strategy for cancer control in Ghana, 2012-2016) has noted an increase in haematological malignancies. This research seeks to ascertain the role of HTLV-1 in haematological malignancies as there is currently no data. Results obtained will indicate whether HTLV-1 is a significant predisposing factor that contributes to the rising incidence of these diseases in the country. Treatment protocols and policies for their management will thus be duly updated. This study being the first HTLV-1 studies in patients with various haematologic malignancies in Ghana will also pave the way for further research in this area.

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1.3 AIM

To determine the role of HTLV-1 infections in haematological malignancies among patients diagnosed at the Department of Haematology, Korle-Bu Teaching Hospital.

1.3.1 SPECIFIC OBJECTIVES

1. To determine the seroprevalence of HTLV-1 among patients with haematological malignancies.
2. To determine the association between haematological malignancies and HTLV-1 infection.
3. To determine the association between HTLV-1 infection and haematological parameters (haemoglobin, lymphocyte and platelets) and history of blood transfusion.

1.4 EXPECTED OUTCOME

It is expected that this study will provide information pertaining to the seroprevalence rates of HTLV-1 among patients with haematologic cancers at Korle-Bu Teaching Hospital Haematology department. It would also establish the fundamental data for association of HTLV-1 with haematological malignancies hence the role of HTLV-1 infection in haematological malignancies in Ghana. Similarly, the study will make available the much needed data on changes related with haematological parameters such as haemoglobin levels, lymphocyte and platelets counts in HTLV-1 infected persons.

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1.5 BENEFICIARIES OF THE STUDY

The beneficiaries of the study will primarily be the patients with haematologic malignancies since informed decision would be taken concerning their clinical and laboratory workup and management policies for effective management. Ghana Health Service (GHS) and Ministry of Health (MOH) as well as Korle-Bu Teaching Hospital will benefit from study to help shape policies. Additionally professionals in the pharmacological industry, faced with holistic development of drugs and vaccines to combat malignant lymphomas and people in academia will greatly benefit from this study. The research findings will be disseminated at workshops as appropriate and publications in peer review journals.

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CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 DISCOVERY OF HTLV

HTLVs are RNA viruses that are enveloped with a diameter of about 80-100nm that belong to the family of viruses known as Retroviridae family and of the genus Deltaretrovirus. HTLVs together with Simian T-Cell Lymphotropic Viruses (STLVs) collectively denoted as Primate T-Lymphotropic Viruses (PTLVs) seem to have arisen from a shared predecessor virus which might have been passed to humans via interaction with non-human primates (Johnson, Harrod, & Franchini, 2001). Retroviruses are often classified in keeping with their virion core structure or in accordance with their homology sequences that become obvious subsequent to phylogenetic analyses. Currently there are 4 types of HTLVs that have been discovered; HTLV types 1 and 2 were discovered between 1980 and 1984 and in recent times types 3 and 4 in parts of Central Africa (Switzer et al., 2015). In 1977, a group of researches in Japan (Takatsuki and his co-workers) described Adult T-Cell Leukaemia (ATL) as a different form of leukaemia.

Around 1979 to 1980, another group of researchers (Robert C. Gallo and co-workers) described the first novel pathogenic human oncovirus in ATL individual and termed HTLV which was associated to ATL later by another group of researchers in 1981 (Gallo, 2005; Masao Matsuoka, 2005). This was later designated as HTLV-1 due to the discovery of other HTLVs. HTLV 2 was first recognized in patient with another form of T-Cell malignancy called Hairy T-Cell Leukaemia. In light of serologic cross-reactivity, this infection was demonstrated to be identified with (yet unmistakable from) HTLV-1 and was named HTLV-2 (Taylor & Matsuoka, 2005). Only small number of HTLV-2 infected persons are characterized very well, hence there is scarce information on the role of HTLV-2 in leukaemia. Some recent reports have provided

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evidence that suggests the association of HTLV-2 with lymphocytosis and thrombocytosis. The Internationally recognized committee on virus taxonomy, provided guidelines in 2005 where the naming of HTLV types using Roman numerals was replaced with Arabic numerals (Switzer et al., 2015).

2.2 DESCRIPTION OF THE AGENT

Retroviruses vary from other viruses in their mode of replication. Ordinarily, the flow of genetic information follows this order: DNA (Deoxyribonucleic Acid) codes for an RNA which subsequently can code for a protein. However, a retrovirus, made up of a single RNA encapsulated by a protein, attaches to and penetrates the cell it enters, then copies itself into DNA by going backward, or "retrograde", RNA creating DNA instead of DNA making copies of RNA, hence the name "retrovirus". From there, the virus becomes a part of the host chromosome, known as a provirus, and undergo the standard viral replication cycle to create viral proteins and new viral particles (Rafatpanah, Farid, Golanbar, & Azad, 2006).

The nomenclature of retroviruses depends on juxtapositions of the magnitude of the viral genome and morphological attributes. HTLV was once classified within the genus lentivirus, (*lenti* in Latin means "slow") a category of slow viruses of the Retroviridae family, which comprises HIV. They are distinguished by a lengthy incubation phase and non-oncogenic properties. Comparative to other viruses in the retroviridae family, lentiviruses are capable of delivering a significant amount of genetic data into the DNA of the host cell, so they are among the foremost efficient strategies of gene delivery vector (Switzer et al., 2015).

Lentivirus remains the genus name for HIV, and though HTLV is well known for long incubation that qualifies it as a *lenti*, HTLV has the propensity to induce cancer within the host organism. Current researches place HTLV within the taxon Retroviridae and the genus

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Deltaretrovirus and note that it carries oncogenes in its ordering (Mazurov, Ilinskaya, Heidecker, Lloyd, & Derse, 2010). An oncogene usually encodes proteins concerned with cell growth or regulation however might promote malignant processes if mutated or turned on by contact with retroviruses.

HTLVs are retroviruses that are complicated in structure having regulatory genes such as transcriptional activator *(Tax)* and regulator of viral expression (*Rex*) in addition to *Gag* (group specific antigen), *Env* (envelop) and *Pol* (polymerase) bordered by LTRs (long terminal repeats) at either end of the viral genome. On electron micrograph studies, HTLV-1 is 80-100nm in thickness containing an electron-dense, nuclear core located centrally. HTLV types 1 and 2 are similar and shares nearly 70% nucleotide sequence homology with greater preservation within the *Rex, Gag, Pol, Env,* and *Tax* genes. Typically, all classical retroviruses have the*, Pol*, *Env* and *Gag* genes. The similarity is lowered in the LTRs, protease and pX region (Fani et al., 2019; Gonçalves et al., 2010; Rende et al., 2012). HTLV types 3 and 4 have morphologies comparable to that of types 1 and 2. There are seven known subtypes of HTLV-1 (subtypes A to G). All subtypes can cause infections but the cosmopolitan subtype A, causes great majority of infections (Ciminelli et al., 2016). The cosmopolitan subtype A so called because it contains four geographical subgroups; West African subgroup, Transcontinental subgroup, North African subgroup and Japanese subgroup and also it is the most widespread (Ciminelli et al., 2016; Gessain & Cassar, 2012). HTLVs have much more genetic stability compared to other retroviruses (Gessain & Cassar, 2012).

2.3 GENOMIC ORGANIZATION OF HTLV

HTLVs belong to the delta retrovirus family. Beside the genes that are of structural and enzymatic in nature shared by all retroviruses, these complex viruses express regulatory and

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accessory genes. HTLV-1 contains a 9kb RNA genome that is single-stranded. This genome codes for *Gag, Env,* and *Pol* as well as enzymatic and structural proteins (Boxus & Willems, 2009). All deltaretroviruses are complex because they have the "pX region" on their genomic structure in addition to the LTRs on both ends and the other genes existing in all other retroviruses (figure 2.1). LTRs are generated as a consequence of direct repetitions of the reverse transcription process (Martinez, Saleem, & Green, 2019). The LTR comprises regions U3, R and U5. Region U3 main function is to control viral transcription. The U3 region is composed of essential elements like the TATA box. The TATA box is important for viral transcription. The TATA box is mainly a sequence that ensures the polyadenylation and termination of the RNA messenger. Another constituent of the U3 region is the TRE (Tax Responsive elements) responsible for transcribing Tax protein. TRE is responsible for regulating HTLV-I provirus transcription (Barbeau & Mesnard, 2011).

The main viral promoter for transcription is located at the 5' LTR. Integrase and protease functions as well as reverse transcription are encoded in the *Pol* open reading frame. The *Pol* gene encodes essential proteins or enzymes like RNase H, integrase and RT (Reverse Transcriptase). Reverse transcriptase functions as the production house of DNA virion likewise the RNase H is for degrading RNA copy and primers of tRNA. Integrase is also essential for incorporating viral DNA into host cell. The *Env* genes provide viral infectivity functions whereas the *Ga*g genes are responsible for the provision of virion core proteins. The 'pX' region codes for accessory and regulatory proteins such as *Rex, Tax* as well as additional proteins; p12, p13, p30 and p21 (Mesnard, Barbeau, Césaire, & Péloponèse, 2015). HTLV-1 proteins and glycoproteins functions are summarized in Table 2.1 (Verdonck et al., 2007). The *Env* genes and has an open reading frame (ORF) that has sequences for regulatory/accessory viral products.

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Recent studies have found that the complementary strand of the pX encodes an antisense transcript, the HTLV-1 Basic Leucine Zipper (HBZ) (M. Matsuoka & Jeang, 2011). HTLV-1 demonstrate a lot of genetic stability because of limited use of reverse transcriptase.

Out of a number of regulatory proteins utilized by HTLV-1, the *Tax* and HBZ proteins possess exceptionally essential functions in viral persistence and pathologic processes, presumptively via continual stimulation of infected cells growth within the company of a powerful immune systems.

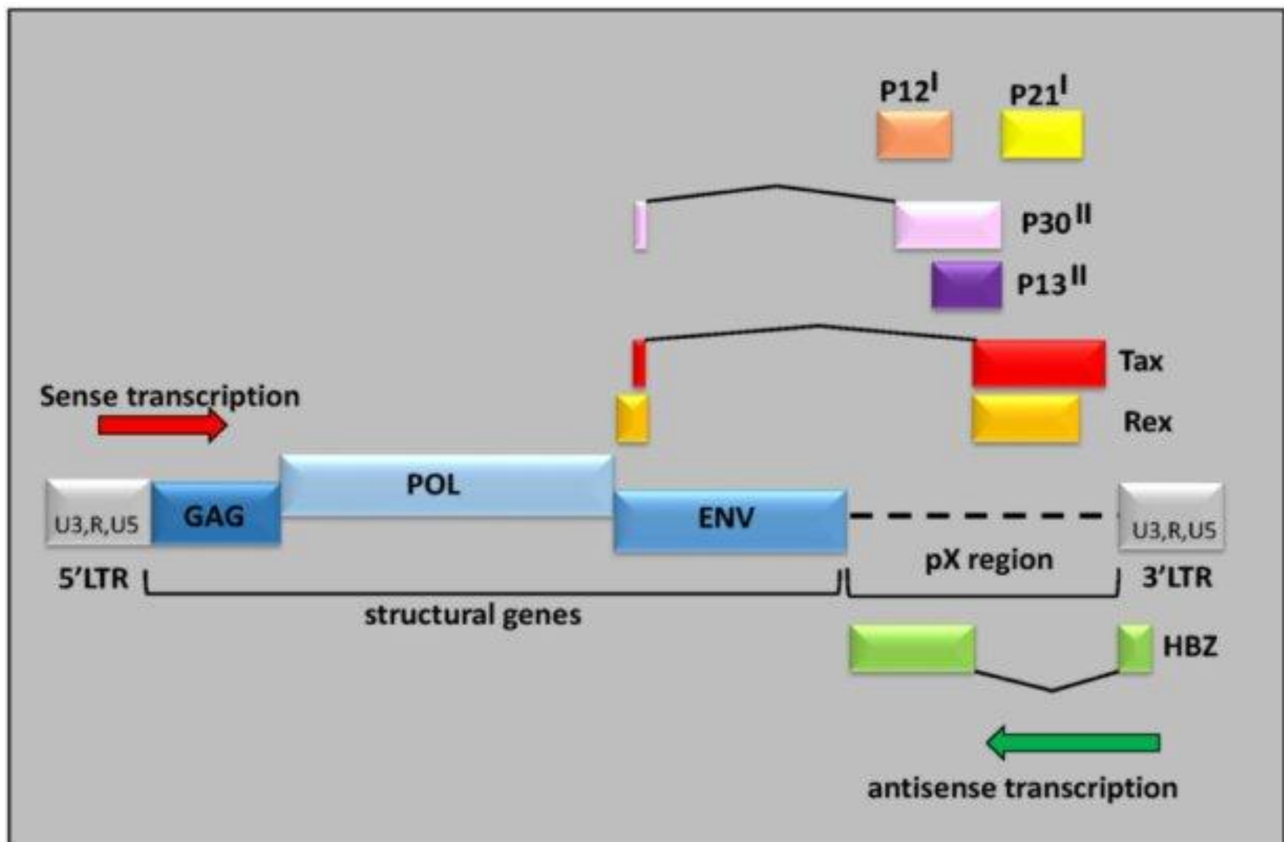

Figure 2. 1: Structure of the HTLV-1 genome

Adapted from (Mesnard et al., 2015)

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Table 2. 1: Proteins and glycoproteins of HTLV 1 and their functions

Proteins and glycoproteins of HTLV 1	**Functions**
***Env* proteins**	
Surface glycoprotein (gp46) Transmembrane protein (gp21)	Aids in Binding to target cell receptor Anchoring of surface glycoproteins to virus
***Gag* proteins**	
Matrix layer (p19) Capsid (p24) Nucleocapsid (p15)	Organizes viral components at the inner cell membrane Protection of viral RNA and proteins Interaction with genomic RNA
Functional proteins	
Protease (p14) Reverse transcriptase (p95) Integrase	Cleaving polyproteins into functional units Converting ssRNA to dsDNA Makes it easier to insert provirus into host cell DNA
Regulatory proteins	
Tax Rex $p12^{I}$ $p13^{II}$ $p27^{III}$ $p30^{II}$ $p40^{IV}$ HBZ	Enables provirus transcription and host genes Modulates Viral RNA Transport Functions in viral replication and activation of T-cells May interfere with mitochondrial function regulates viral gene expression post-transcription Modulate the transcription of cellular genes Transcriptional and post-translational regulator Down regulates viral transcription

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2.3.1 GENE PRODUCTS

The *Tax* gene

HTLV 1 *Tax* is a forty (40) kilo Dalton (kDa) protein, comprising of 353 amino acids (AA), extremely preserved in all serotypes of HTLV 1. Viral replication involves Tax, acting as proviral transcription transactivators. By magnifying the expression of cell genes that govern T-cell propagation and interacting with proteins that regulate mitotic checkpoints and deactivate the pathways of tumour suppressor pathways, *Tax* has important functions in immortalizing and transforming infected cells. In addition, *Tax* is a vital component in moderating the growth of viral continual presence and disease onset. *Tax* potential to cause cancer is evidenced by its capability to immortalize in vitro cells and to cause tumours in models of transgenic mouse (Boxus & Willems, 2009). In addition, in the proliferative cycle of HTLV 1, *Tax* has many functions, including important functions in facilitating the copying of HTLV 1 genes and the engaging Microtubule Organizing Center (MTOC) to cell contact sites. Furthermore, through a medley of mechanisms, *Tax* influences the regulation of the host cell-division cycle, the apoptotic pathways and the proliferative pathways. The most widely studied route by which *Tax* increases cell proliferation is through the NF-κB/Rel family of proteins (Martin, Maldonado, Mueller, Zhang, & Mansky, 2016).

Expression of Tax-induced inter cellular adhesion molecule 1 (ICAM-1) promotes HTLV 1 T-cell tropism. Using chemical inhibitors has shown that the function of the small GTPases Cdc42 and Rac1 is vital for MTOC polarization caused by *Tax*. (Gross & Thoma-kress, 2016).

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The G*ag* gene

The HTLV-1 *Gag* is assembled as a single polyprotein precursor. This is then modified posttranslationally, and is inserted into the inner membrane of the host cell membrane. *Gag* is cut into its functional units: nucleocapsid (NC, p15), capsid (CA, p24) and matrix (MA, p19) by viral proteases. Matrix is essential for *Gag* functions such as *Env* integration, membrane binding and *Gag* targeting, whereas the capsid makes the inner core of the viral particle. Nucleocapsid attaches to the gRNA within the virion. Appropriate spatial and temporal control of viral assembly and budding is essential for HTLV 1 transmission to be efficient. In other retroviruses, this has been shown to be crucial and thus likely the case for HTLV (Boxus & Willems, 2009; Gross & Thoma-kress, 2016).

The *Env* gene

The HTLV 1 *Env* gene is a glycoprotein of 61 kDa to 69 kDa. The precursor protein is located in the surface of cells contaminated with HTLV. The *Env* protein is cleaved into functional units: a 46-kDa surface glycoprotein (gp-46) and a 21-kDa transmembrane protein (gp-21). Culture media from HTLV-transformed cells contain significant quantities of free gp -46, released from the cells' surface. *Env* has pivotal functions in the cell-to-cell transmission of HTLV 1, which is important for infectivity of HTLV1. The functional proteins of the *Env* gene are conveyed to the cytomembrane to begin viral assembly and budding. In order to enable the union of the virus and the host cell membranes, the surface glycoprotein adheres to the host cell surface receptors Glut-1, NRP-1, and to HSPGs (Martin et al., 2016). *Env* is additionally vital for virological synapse development for in vitro and in vivo transmission of HTLV 1.

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The *Rex* gene

HTLV-1 *Rex* gene is a twenty-seven (27) kilo Dalton (kDa) protein, made up of 189 amino acids that confines to nucleus, nucleoli and nucleolar speckles. *Rex* post-transcriptionally encodes viral regulatory proteins that vigorously convey mRNAs within the cytoplasm. Even though primarily detected within the nuclear section, *Rex* frequently alternates between the nucleus and the cytoplasm, an attribute that is closely connected to its capability to move partially spliced viral RNA form the nucleus to the cytoplasm. *Rex's* function is mediated by binding to a 254-nucleotide stem-loop cis acting RNA element, called the Rex-responsive element (RXRE) present in the LTR U3 / R region. Although *Rex* is not necessary for in vitro cell immortalization, it is essential for viral survival and in vivo infectivity, as *Rex* relies on the expression of viral RNAs that encode structural protein proteins. The Rex-RXRE interaction was therefore proposed to serve as a molecular switch that regulates the transition between HTLV-1 infection 's active and latent stages (Gross & Thoma-kress, 2016; Rende et al., 2012).

The HBZ gene

The gene HBZ is a very important gene of HTLV 1 which is continuously related to ATLL. HBZ mRNA transcripts are present in just about every ATLL cell, and HBZ plays an essential role in T-cell proliferation. The more recently discovered HBZ acts as a negative regulator of the viral transactivation mediated by *Tax*. Compared to tax, HBZ interacts with proteasome subunits and may promote the transmission of cellular factors to the proteasome even without omnipresence. HBZ RNA expression results in the upregulation of target E2F1 genes and activates the proliferation of T-lymphocytes. Inverse, the silencing of HBZ with shRNA decreases the proliferation of T-cells infected with HTLV-1 and their ability to cause solid tumors in mice

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(Boxus & Willems, 2009). HBZ was found to interact directly or indirectly with the following cyclic AMP responsive element binding protein (CREB) cell proliferation-dependent proteins: CREB, CBP, activating transcription factor 1 (ATF-1), and ATF-3. Additionally, HBZ interacts with the Jun, JunB, JunD, and c-Jun family transcription factors. Tax and HBZ 's joint roles are essential for cellular spread (Martin et al., 2016).

2.4 HTLV EPIDEMIOLOGY AND TRANSMISSION

The true estimate of the prevalence of HTLV-1 globally is difficult to establish based on published studies owing to the fact that only a few population-based studies has been carried out. Most of the publications are based on cohort studies of special population groups such as pregnant women, blood donors among others. Since the discovery of HTLV-1, about 20 million persons worldwide are thought to be infected (Besson & Kazanji, 2009; Gessain & Cassar, 2012; Johnson et al., 2001; Taylor & Matsuoka, 2005). However, testing for the virus in areas thought to be endemic for the virus is not very encouraging only about a third of persons are screened in those areas.

The geographical areas involved are diverse and include portions of Japan, the Middle East, the Caribbean, Central and South America, large parts of sub-Saharan Africa and some rare areas in Melanesia, and Romania in Europe (Besson & Kazanji, 2009; Carneiro-proietti et al., 2005; Paiva et al., 2018; Tarokhian et al., 2018; Tienen et al., 2012). Analyses of phylogenetic data revealed HTLV-1c initially separated from STLV about 50,000 or so years past, whereas PTLV-1 transmission within Africa happened a minimum of 30,000 years past. Later, HTLV-1a, separated from the African strain 17,000 years past. These viruses have been with the human race once interspecies transmission occurred a very long time ago (Carneiro-proietti et al., 2005; Masao Matsuoka, 2005).

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The prevalence of HTLV-1 globally is projected to be 2–17%. Prevalence increases with increasing age in endemic regions (Armah et al., 2006; Carneiro-proietti et al., 2005; Caskey et al., 2007; Gessain & Cassar, 2012; Hewagama et al., 2014; Johnson et al., 2001; Martinez et al., 2019; Masao Matsuoka, 2005). A significant epidemiologic factor of HTLV-1 prevalence is age. In endemic areas, seroprevalence significantly increases with age. In a study conducted in 2018 among a cohort of native Australians, seropositive rates increases significantly with age reaching as high as 48.5% in men 50–64 years of age (Einsiedel et al., 2016). Other risk factors for HTLV-1 are economic standing and gender (Martinez et al., 2019).

Monkeys are thought to be reservoir hosts for HTLV-1 infection. Cross-species transmission is likely still actively contributing to the endemic (Tienen et al., 2012). In Africa, it is believed that HTLV-1 transmission from animals to humans still occurs through the bites of non-human primates or slaughtering of these primates. Thus Simian T-cell Leukaemia Virus type 1 (STLV 1) is transmitted to humans via these activities which then mutates to the human form as HTLV 1. These forms of transmission may be contributing to fresh infections on the continent. A current survey in central Africa established that 8% of respondents in the study who were gnawed by monkeys became seropositive for HTLV 1. The types of HTLV 1 seen in these infections are similar to STLV 1 subtypes frequently detected in the simian species from which they were gnawed. The discovery of HTLV 3 and HTLV 4 are likely as a result of zoonotic transmission of STLV since STLV 4 is endemic in gorillas in Africa. Also phylogenetic analysis has revealed that HTLV 4 was only discovered in recent times in the human race. (Martin et al., 2016).

Transmission of HTLV-1 is by infected lymphocytes and not acellular virus. Three main routes have been discovered to be involved; the most prominent among them is vertical transmission

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mostly through prolonged breastfeeding after six months of age. The next most common route is sexual transmission. Women are more at risk by this route of transmission than men are. Next is blood transfusion, especially blood transfusion involving cellular products (Carneiro-proietti et al., 2005; Masao Matsuoka, 2005). Transmission by blood products has decreased significantly in Japan and subsequently in United Kingdom (UK), United States of America (USA) and many others where persons involved with blood donation are screened for HTLV-1 (Armah et al., 2006).

HTLV-1 transmission happens through one of three unique modes. In the first place, infected mothers can transmit the infection to the embryo or new born. The method of transmission here is either through trans placental entry of contaminated maternal lymphocytes or via breastfeeding with infected lymphocytes. With regards to mother-to-child-transmission (MTCT), recent studies in Brazil concluded that MTCT occurs in infants breastfed, at rates ranging from 7.4% to 32%, compared with a rate of less than 5% among bottle-fed children of seropositive mothers (Paiva et al., 2018).

The predominance of HTLV-I among infants breastfed for more than 3 months was fundamentally higher (27%) than that of those breastfed for less 3 months. Of 78 bottle-fed infants, 13% of children born to infected mothers are contaminated with HTLV-1 by courses other than breast milk (Futsch et al., 2018). Polymerase Chain Reaction (PCR) enhancement had recognized HTLV proviral DNA in the peripheral blood and milk of all carrier neonates, demonstrating that trans placental disease with HTLV-I is uncommon and that post-partum contamination by means of breast milk is major perinatal transmission course. These perceptions have delivered suggestions that carrier mothers should cease from breast feeding so as to lessen the occurrence of HTLV-I transmission to their posterity. Great success was achieved in

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efficiently reducing HTLV-1 MTCT in Japan when pregnant women were tested for HTLV-1 antibody and seropositive mothers refrained from breastfeeding their infants (Futsch et al., 2018; Paiva et al., 2018).

Furthermore, HTLV-I is transmittable from man to woman during sex by means of HTLV-I contaminated cells in semen. It is conceivable that female to male sexual transmission likewise happens although at a very low rate.

The third course of transmission is through infected blood and blood products. This is however dissimilar to HIV, in that only blood products that include section of entire lymphocytes from donor to beneficiary can transmit the infection(Carneiro-proietti et al., 2005; Murphy, 2016). A review investigation of HTLV transmission by means of contaminated blood transfusion indicated an evident effective transmission of 12%. This investigation presumed that 700 beneficiaries of blood transfusion in the USA annually were infected with HTLV-1 before routine donor testing started in 1988. In another study, antibodies were detected in 19 (0.3%) of 6,286 plasma contributors from five cities of the USA yet no HTLV-1/2 antibodies were identified in haemophiliacs who were transfused routinely with non-inactivated plasma or its derivate emphasising the fact that HTLV-seropositive plasma transfusion does not transmit the viral infection (Carneiro-proietti et al., 2005). It has been established that for HTLV 1 infection to occur via blood transfusion, it requires a minimum of 80,000 HTLV-1 infected cells to cause infection in the contaminated blood recipient. Hence, leukocyte depletion below that infected cells threshold would significantly reduce HTLV-1 infection by blood transfusion (Futsch et al., 2018).

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Nevertheless, HTLV-1 has lately been transmitted widely among intra venous drug addicts (IVDA) apparently through blood lymphocytes in shared needles. Hence the general method of HTLV-I transmission is like that of HIV infection with the exemption that the infection is evidently not promptly transmitted by cell - free body liquids. In Africa and therefore Ghana, the transmission of infection would build due to the utilization of whole blood, which has not been leuco-depleted. Leuco-depletion decreases lymphocytes in whole blood; consequently, HTLV-1 contamination would be decreased.

There is increasing evidence that significant number of persons who are transfused cellular blood products (for example whole blood, packed RBCs or platelets) seroconvert while recipients of cell-free blood products rarely do seroconvert. Major determinant factors for this kind of transmission includes the duration of storage (blood products stored for less than seven days) and immunosuppression in patients who receive these transfusions (Murphy, 2016). Transmission is enhanced by HTLV-1 inducing proliferation of infected cells in vivo. This is achieved principally by two ways: new infections and mitotic division of infected cells. During the chronic stage of the infection, it is believed that the mitotic division is the prominent type (Furuta et al., 2017).

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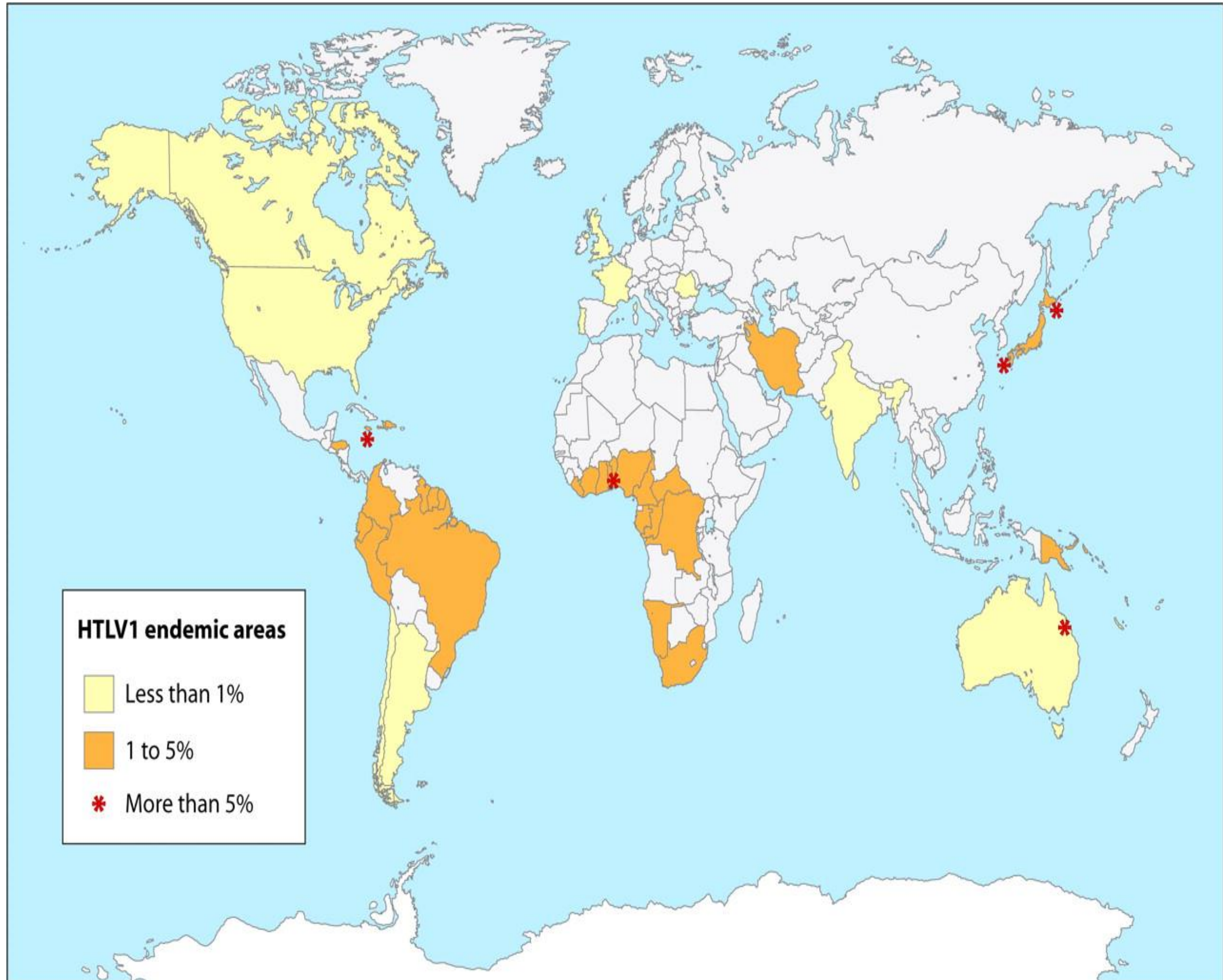

Figure 2. 2: Map showing worldwide HTLV-I distribution.

Adapted from Gonçalves et al., 2010

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2.5 HTLV INFECTIVITY

Direct cell-to-cell contact is typically needed for effective HTLV infection, although infection with cell-free virus preparation can occur. By comparison with other retrovirus, infection by HTLV of susceptible cells is inefficient with a slow course. Usually only a minute number of the cells express viral antigens, and this number may gradually increase over a course of weeks. The reason for this slow kinetics may relate to positive and negative regulation by *Tax/Rex* genes or other unknown processes of infection. Only T cells are known to be transformed by HTLV. Cord and peripheral blood T cells of humans are efficiently transformed. Productive infection with HTLV has only been demonstrated in a few cells of lymphoid origin.

Conversely, HTLV-1 appears to infect but not transform several different cell types in vivo.

On several occasions B-cell lines productively infected with HTLV 1 have been established from patients. B cells can also be infected in culture. There is also evidence that HTLV is capable of infecting immature cells from human bone marrow, which, do not have a T-cell phenotype. Infection of macrophages and cells of neural origin has been documented in vitro but not in vivo. Although cases of ATL with central nervous system (CNS) involvement are rare, direct infection of the CNS may account for such symptoms in a few patients. Ninety to 99% of HTLV 1 DNA in peripheral blood from infected patients is found in CD4+, CD8+ cells.

Until recently, the cellular receptors for HTLV were not identified. However, recent studies have demonstrated the existence of receptors HTLV on a wide range of human and animal cells, which includes primate, canine, feline and rodent cells. The presence of a cell-surface receptor for HTLV has also been demonstrated by virus-induced cell fusion, leading to syncytium formation (Johnson et al., 2001; Martinez et al., 2019). The likely cells HTLV1 infects includes T-cells, B-cells, macrophages, fibroblasts and dendritic cells however the virus mainly infects

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the T-cells. This means that the receptor for the virus entry is expressed commonly on these cells. Researchers have found the three important proteins or receptors for HTLV-1 viral entry to be; HSPGs (heparin sulfate proteoglycans), NRP 1 (neuropilin-1) and GLUT-1 (glucose transporter 1) (Bangham & Matsuoka, 2017; M. Matsuoka & Jeang, 2011; Masao Matsuoka, 2005; Mesnard et al., 2015; Taylor & Matsuoka, 2005). When the virus gets to the cell surface it first makes contact with HSPG and form complexes with NRP-1 followed interaction with GLUT1 on the cell surface before the final fusion of the membrane and then enters the cell. This is illustrated in Figure 2.3. Furthermore it is worthy of note that notwithstanding the wide distribution of the cellular receptors for HTLV 1, the HTLV 1 provirus is detected primarily in CD4+ T-cells and to a some extent in CD8+ T-cells (Sachse, Guadagnini, Robbiati, & Lasserre, 2009). This could be because of the effects of on these cells while HTLV 1 initiates proliferative cell cycling in CD4+ T-cells, HTLV 1 however causes delay in CD8+ T-cells death. Consequently, the different outcomes of the effects of the virus on CD8+ and CD4+ cells may be significant in determining the seeming specificity for CD4+ cells than receptor-binding and cell entry variations. There is however proof that cellular receptors has a crucial function in deciding the cellular response of HTLV 1 (M. Matsuoka & Jeang, 2011; Mesnard et al., 2015).

Once inside the host cell, uncoating takes place, the viral genome gets integrated into the host genome and the RNA genome is transcribed to double stranded DNA by reverse transcription (Satou & Matsuoka, 2012). The accessory proteins p30, p12/p8 and p13 are essential for virus entry into cells and maintenance in vivo whereas *Rex* and *Tax* genes are responsible the replication of the virus. Cell-cell contact is necessary for HTLV-1 infection. Unlike HIV, HTLV is mainly transmitted through cell-to-cell contact, not by acellular virions. Once contact is made with uninfected cell, HTLV-infected cells will within a short time produce much higher levels of

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Tax and intercellular adhesion molecule-1 (ICAM-1) which form virological synapse (VS) or a viral biofilm (Gross & Thoma-kress, 2016). Virions can transfer through this synapse and hence spreading the infection.

HTLV-1 appears to infect but not change a number of different cell types in vivo. The *Tax* oncoprotein is adequate to immortalize T-cells hence plays a vital part in HTLV-1-induced cellular transformation. The HBZ gene also plays a significant part in the pathogenic processes of HTLV. HTLV replicates by enhancing de novo infections or by mitotic division and clonal proliferation of infected cells (Gross & Thoma-kress, 2016; Johnson et al., 2001; Satou & Matsuoka, 2012). HTLV 1 and HTLV 2 are thought to utilize the same cell receptor molecules during cellular infection.

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 new CD4+ T-cells as documented by some scientific reports (Gross & Thoma-kress, 2016; Mesnard et al., 2015; Pique & Jones, 2012).

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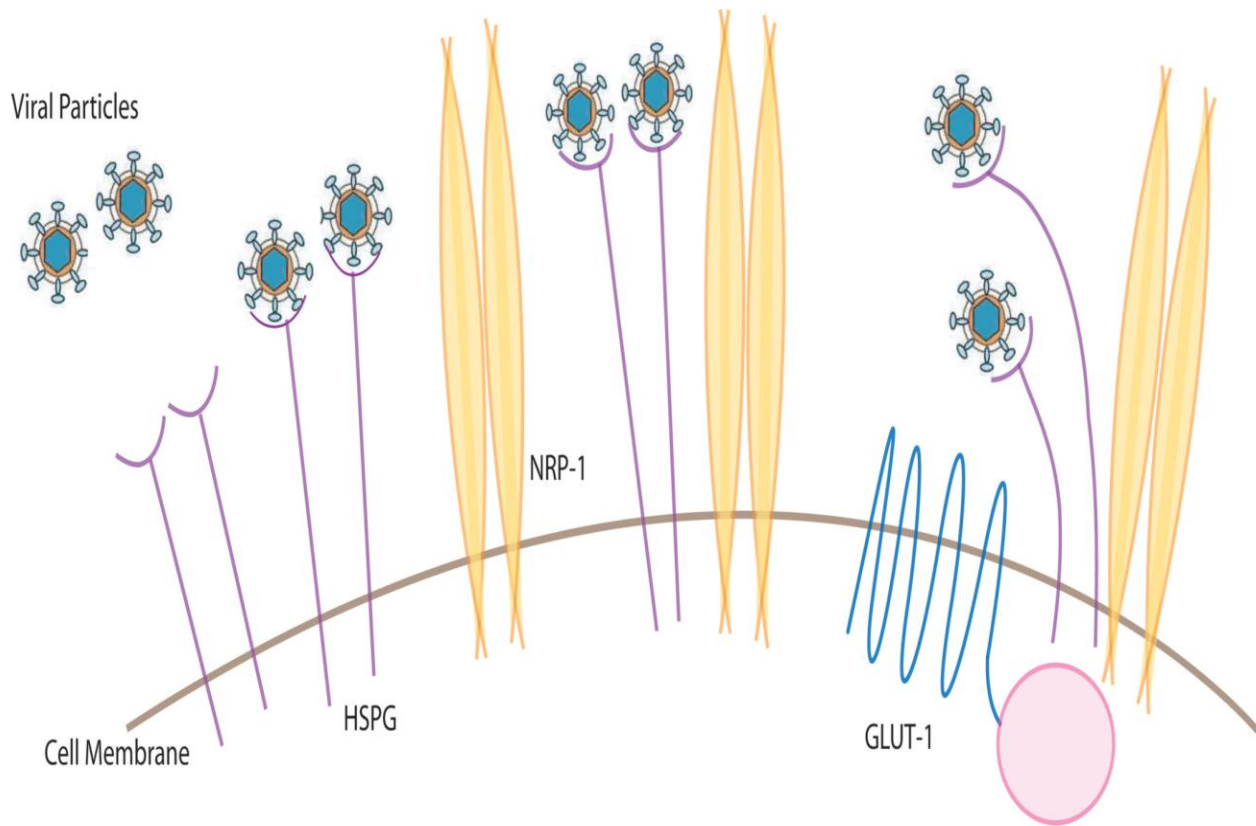

Figure 2. 3: Illustration of HTLV infectivity

HTLV interact with HSPG, NRP-1 and Glut-1 to establish infection. Adapted from (M. Matsuoka & Jeang, 2011)

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2.6 HTLV VIRAL REPLICATION

Basically, all viruses replicate by following these basic steps or sequence (i.e., attachment, penetration, uncoating, replication, assembly, release and maturation) but with differences in the various steps specific to different classes of viruses. The difference in the replication of HTLV has to do with the fact that it contains enzyme known as reverse transcriptase. This enzyme functions by converting the RNA genome of HTLV, which is single-stranded to double-stranded DNA genome. By this process, there is a reverse flow of genetic information (the flow is typically from DNA to RNA). There is then the transportation of the DNA copy into the nucleus of the host cell, subsequently there is circularization and integration into the host cell chromosome. HTLV 1 principally infects CD4+ T-cells but can also infect an extensive range of cells including but not limited to CD8+ T-cells and B-lymphocytes among other cells. The large choice of cells infected by HTLV is to some extent due to the capability of HTLV glycoprotein *Env* to interact with cellular surface receptors that are extensively distributed (Martinez et al., 2019).

A matured HTLV 1 virion that is infectious first gets attached to a target cell and then fuses with the target cell membrane by interacting with the cell surface receptors GLUT1/HSPG/NRP-1 through the transmembrane domains of *Env* protein (figure 2.3). The HTLV 1 capsid core that enters the infected cell has 2 clones of the viral RNA genome (gRNA) alongside viral protease, RT (reverse transcriptase) and also IN (integrase). Subsequently, the gRNA is passed into the cytoplasm of the infected cell. The reverse transcriptase then converts the RNA genome that is single stranded to DNA genome that is double stranded. The required promoter and enhancer components to start RNA transcription are located within the HTLV-1 provirus LTRs. Also within the 3' end of LTR is situated polyadenylation signals.

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The principal enhancer of virus transcription is *Tax*, which powerfully triggers virus transcription throughout the initial part of infection through CREB interaction. *Tax* additionally interacts with multiple cellular transcription elements and starts transcriptional pathways, like nuclear factor-kappa B (NF-κB), AP-1 and serum response factor (SRF). Initiation of nuclear factor-kappa B pathway promotes the transcription of varied cytokines and their receptor genes and also anti-apoptotic genes like *bclxL* and *survivin*. The initiation of nuclear factor-kappa B pathway is essential for tumour formation either in vitro or in vivo. *Tax* additionally interacts with the functions of p53, p16 and MAD1. These interactions permit HTLV-I-infected cells to flee from apoptosis and therefore induce genetic instability (Masao Matsuoka, 2005).

The double stranded viral DNA is then transported into the host cell nucleus and incorporated into the host cell genome. Host cellular RNA polymerase II then transcribes the new provirus and is modified post transcription. Post-transcription, *Rex* is a very important regulator for splicing and transportation of HTLV 1 mRNA. Both full-length and spliced virus mRNA is transferred from the nucleus to the cytoplasm. The translation apparatus of the host cell translate viral proteins and also the *Gag, Gag-Pol* and *Env* proteins are conveyed to the cell membrane of the host cell along with two duplicates of the gRNA genome. Most probably, the whole length virus gRNA is moved to the cell membrane, where it dimerization can take place as well as interaction with *Gag* polyprotein and hence packaging into assembling units. The double spliced mRNA are translated by ribosomes and go on to express enzymatic proteins and unspliced mRNA express structural proteins after translation by free ribosomes. Membrane-bound ribosomes likewise translate single spliced mRNA to express *Env*. In order to create immature virus particles, the virus proteins and gRNA along with cellular elements convene at a virus budding point along the cell membrane. In the assembly and budding of virus particles, Gag-

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gRNA, Gag-Gag and Gag-membrane interactions are all necessary. The budding virus disengages from the cell membrane and goes through a maturation procedure via the action of viral protease. Throughout and soon after the discharge of immature virus particles, the viral protease cleaves the Gag and Pol polyproteins. HTLV-1 membrane antigens (MA) remain closely associated with the plasma membrane; A capsid shell containing reverse transcriptase, integrase, and HTLV-1 nucleocapsid coated gRNA is formed by the HTLV-1 capsid. If infectious, the mature virus particle is able to infect a permissive target cell (Fox et al., 2016; Martin et al., 2016). The steps involved in HTLV viral replication are illustrated in figure 2.3.

Beyond initial sites of viral replication, it is presumed that initial infection occurs locally within the regional lymphatics. The resulting infection is capable of inducing malignant, autoimmune, and neurological illnesses. A latent infection, during which the virus is dormant or in persistent infections, in which low-levels of the virus are continually being produced is also possible. These properties justify the life-long nature of retroviral infections and render the maladies induced by HTLV exceptionally troublesome to treat.

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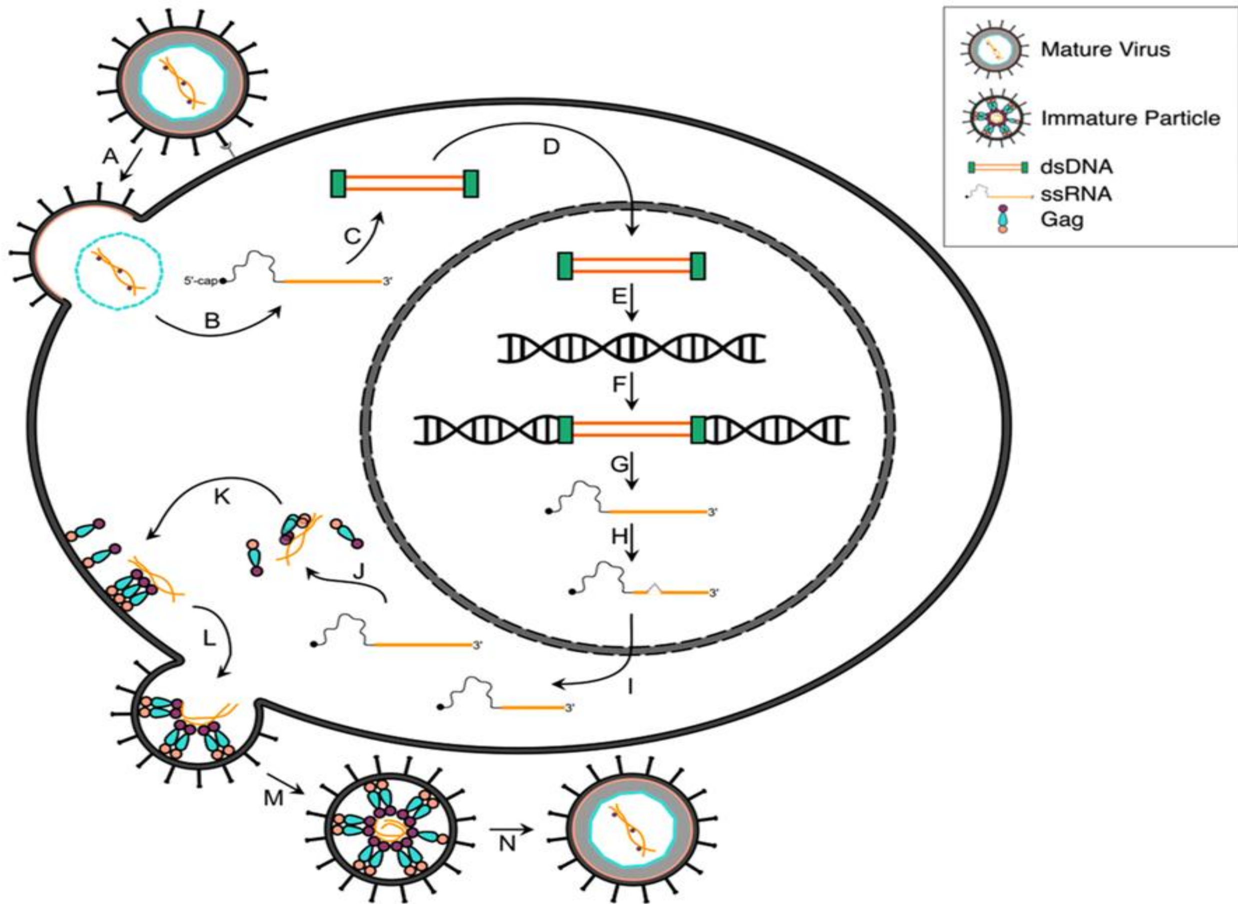

Figure 2. 4: Steps involved in HTLV 1 replication: the process commences from A through to N.

Adapted from Martin et al., 2016.

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2.7 HOST IMMUNE RESPONSES TO HTLV

An incessant viral infection strikes a balance between the immunity of the host and viral virulence. The host's effort to sterilize immunity should be prevented by viruses that maintain chronic infection. When the degree of inflammation and viral replication prevents excessive tissue damage from occurring, a equilibrium is achieved. Strategies for moderating the host immune system and tempering viral replication have also been developed for viruses which cause chronic infection (Verdonck et al., 2007).

For HTLV 1, P12, an accessory viral protein, physically responds to the heavy chains of class I human major histocompatibility complex (MHC), triggering MHC degradation. This causes the host immune system to flee virally. Similarly, it appears that moderate replication of HTLV 1 results from in vivo suppression of the expression Levy. Cytotoxic T lymphocytes (CTLs) regulated by CD8 + cells are partly responsible for this event because the loss of CTLs increases the in vivo tax expression. These CTLs tend to target the Tax protein directly because the HTLV 1 proviral load in people with HAM / TSP decreases when valproate, the histone deacetylase inhibitor, is used to induce Tax transcription. Therefore, the CTL-response of the host targets tax-expressing cells, decreasing the number of cells infected in vivo. The function of HTLV-1 antibodies, by comparison, remains largely unknown (Bangham & Osame, 2005).

In ATLL patients, cell-mediated immunity becomes progressively impaired, this is indicated by reduced reactivity to *Mycobacterium tuberculosis* recombinant antigen. Similarly, *Strongyloides stercoralis* infection is also noted for widespread dissemination in ATLL patients. Alternatively, Strongyloides infection in HTLV-1 carriers is associated with increased risk for ATLL development (Bosqui et al., 2019; Weatherhead & Mejia, 2014). This result may be connected to findings that HCV co-infection in HTLV-1 carriers increases the risk of liver cancer, indicating

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that chronic inflammation caused by multiple agents and T cell activation, as well as tax-induced T cell proliferation, contributes to the in vivo promotion of ATLL. In about two thirds of ATLL cases, ATLL cells have been reported to express FoxP3. This result indicates that ATLL may be a regulatory T cell neoplastic disease because Foxp3 is a master gene that regulates regulatory functions of regulatory T cells. These phenotypes of immunosuppressive ATLL cells can account for the immunodeficiency found in ATL patients. The surface chemokine receptor, CCR4, and CCD4 and CCL22 ligands are expressed by regulatory T cells; these cells are increased in HTLV-1 infected individuals. Increased development of CCL22 could mechanistically lead to increased regulatory T cells in people infected with HTLV-1 (Bangham & Osame, 2005; Furuta et al., 2017; Tarokhian et al., 2018).

All patients with HTLV-1 diseases make humoral antibodies to different HTLV-1 antigens. The major viral gene items perceived by sera of infected people are those of the *Tax* gene, *Env* and *Gag*. As in all retroviruses, the gag proteins are the significant immunogens and are answerable for the most punctual antibodies to show up. Sera from infected people ordinarily perceive every one of the three gag proteins, pl5, p24 and p19. There is extensive cross-reactivity between HTLV-1 and HTLV-2, especially in the district encoding p24. Serologic profile of HTLV-infected people differs impressively, including a few people who basically show monospecific trend of antibodies. Normally, invulnerability to HTLV gives off an impression of being unique in relation to that of different retroviruses; in that human serum interceded virolysis was not viable for HTLV virions, utilizing either ordinary human serum or human serum from a HTLV immune antibody carrier. Subsequently the infection may have characteristic protection from humoral safe components (Bangham & Osame, 2005).

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2.8 VIRAL PATHOGENIC PROCESSES

HTLV-1 is known to transform human primary T-cells in vitro and in vivo, but cancer develops after an extended latency phase in just a minute number of infected individuals (just around 5 percent of HTLV-1 infections). This implies that during this time a multistep oncogenic process may occur, leading to cell proliferation and also to the accumulation of genetic mutations. Many studies suggest that tax plays an important part in this oncogenic process (Johnson et al., 2001; M. Matsuoka & Jeang, 2011)

HTLV 1 encodes a tax oncoprotein, which gives infected cells survival and proliferative properties. Post-translation tax is modified by phosphorylation, ubiquitination and acetylation. Through experiments, these post-translation modifications have proved vital to the function of *Tax* protein. *Tax* expression alone was hypothesized to be adequate for immortalization of human T cells, but not for transformation. *Tax's* ability to alter cells in vivo has been meticulously scrutinized using transgenic mouse models; the results indicate the expression of *Tax* alone will competently drive formation of tumour in vivo. Nonetheless, expression of *Tax* only is not adequate for human ATL formation (Akbarin, Shirdel, Bari, & Mohaddes, 2017; Barbeau & Mesnard, 2011; Cavanagh et al., 2006; Mesnard et al., 2015).

The 3 'LTR produce an antisense transcript encoding for a protein known as HBZ. Within the U5 sequence of the 3' LTR is located the promoter for the HBZ gene. Although *Tax* transcripts are found in only a few transformed ATLL cells, all ATLL cells have HBZ mRNA. The HBZ protein has been delineated to work with transcription factors like CREB, CREB-2, cyclic AMP-responsive element modulator (CREM-Ia), the activating transcription factor ATF-1, as well as c-Jun, JunB, and JunD via its basic Zip (bZIP) domain and was initially thought to be subject to Tax-mediated viral transcription. Additionally, HBZ by selection inhibits the classical NF-κB

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pathway by preventing DNA binding of p65 and stimulating its degeneration (Vernin et al., 2014).

The Tax gene was revealed to enable the alternative and classical NF-B pathways in a comparative way. Since the 2 pathways regulate gene expression in cancer cell lines with anti-apoptotic functions, preferential activation of the choice pathway by Tax and HBZ may be involved in the proliferation of ATL cells. It was apparently suggested by an earlier study that HBZ mRNA itself is also important for promoting the proliferation of cells infected with HTLV-1. HBZ also escalates the reverse transcriptase behavior of human telomerase (hTERT). All knowledge obtainable supports the idea that HBZ protein and RNA play vital functions in the promotion of viral replication and cell propagation (Mesnard et al., 2015).

Following infection, HTLV-1 proviral load increases primarily by propelling the proliferation of infected cells (mitotic division). New infection may not considerably add to the provirus load within the chronic phase of infection. HBZ and *Tax* perform crucial functions in preserving clonal longevity. In vivo, the HBZ gene is expressed constantly at a low level in most of the infected cells. Infected cells may flee robust CTL response and additionally be safeguarded from clonal dominance due to genetic instability within the infected cells. On the opposite hand, HTLV-1-activated alterations in cellular gene expression are proposed to promote leukaemogenesis. Thus, *Tax* was established as the principal source of the genetic instability associated with HTLV 1. A recent research by Vernin *et al*., 2014 revealed that HBZ promotes onco-miR expression and also DNA-strand breaks by downregulating the expression of OBFC2A protein by post-transcriptional activation of miR17 and miR21.

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Persistent stress exerted by the host immune response restricts infectious spread during the chronic phase of infection and selects the optimal pattern of proviral expression for survival of the clones.

The *Tax* protein, has for several years been known to be essential and adequate for malignant transformation of HTLV 1 infected cells both in vitro and in vivo hence elevated levels or constant expression of *Tax* is oncogenic. Nonetheless, more recent studies propose that, in natural HTLV-1 infection, HBZ is a more crucial component of oncogenesis than *Tax*. The presence of the monoclonally integrated HTLV 1 provirus is the foremost proof of HTLV 1 infection in ATLL cells. As a result, analysis of the genomic structure of HTLV 1 proviruses as well as their genomic integration sites in ATLL cells can give crucial clues on HTLV 1 leukaemogenesis. Studies of HTLV 1 proviruses and viral gene transcripts have shown that ATLL cells express *Tax* in about half of ATLL cases. On the contrary, HBZ is expressed in all ATL cases, suggestive of the fact that HBZ is crucial in HTLV 1 leukaemogenesis. Throughout the normal course of infection, DNA methylation of the 5'LTR accrues that in the end silences transcription of the 5'LTR sense strand. Nevertheless, DNA methylation does not spread out to the "pX" region and the 3'LTR, which are necessary for transcription of HBZ (Bangham & Matsuoka, 2017).

Recently, within the pX region, a CTCF-binding region has been identified that could account for the arrest of DNA methylation before pX and 3'LTR, and guarantee continuous expression of HBZ. In some cases of ATL, the cancerous clone also has a defective provirus that does not contain the 5'LTR (type 2 defective provirus) that is produced before the provirus is integrated in the genome. The dearth of the 5'LTR prevents expression of *Tax* in some cases.

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A protracted latency is crucial prior to the commencement of ATLL, suggestive of the fact that multiple genetic and epigenetic changes are required for ATLL. Apparently, genetic modifications accumulate in the genes related to pathways which are targeted by both HBZ and *Tax*. Thus HTLV 1 infected clones persevere and proliferate in vivo by the actions of *Tax* and HBZ all through the asymptomatic phase of viral infection. Subsequently, genetic and epigenetic changes repair or enhance these changes. For instance, HBZ induces the expression of CCR4. CCR4 gene gain-of-function mutations are linked to the proliferation and infiltration of ATL cells and are present in approximately 20 percent of ATL cases (Bangham & Matsuoka, 2017; Mesnard et al., 2015). Additionally, Tax stimulates the NFkB pathway very strongly. In ATL cells, miR31 expression potentiates NFkB even when tax is absent. HBZ 's newly assigned roles give new insights into the relationship between HTLV and its host, which can be used to treat and prevent HTLV 1-induced diseases. Pathogenesis of HTLV 1 are highlighted Figure 2.4.

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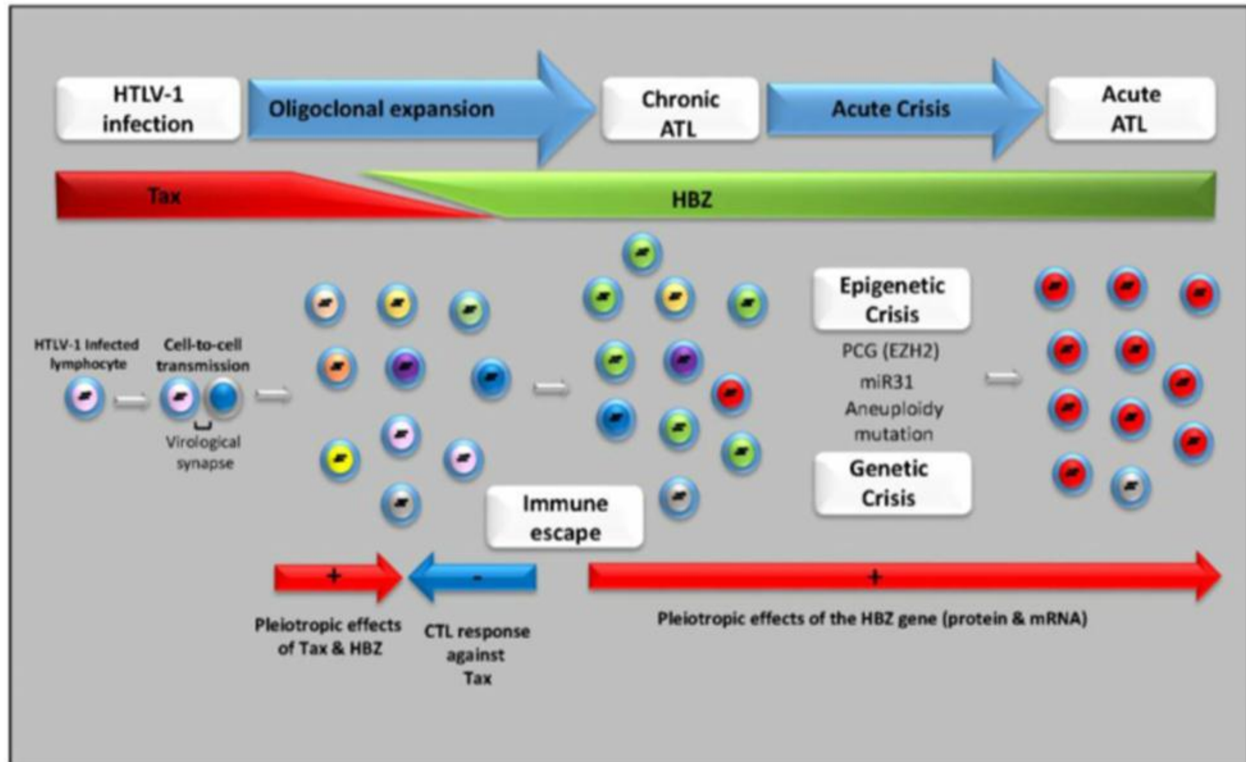

Figure 2. 5: Pathogenesis of HTLV 1

Adapted from (Mesnard et al., 2015)

2.9 LABORATORY DIAGNOSIS OF HTLV INFECTIONS

Diagnosis can be done during blood donation screening, tests carried out due to a family history of the infection, or work-up for a condition associated with HTLV-1 such as ATLL or HAM / TSP. Patients with HTLV infections can either be screened serologically by enzyme immunoassay (EIA, also referred to as enzyme-linked immunosorbent assays - ELISA) or molecule agglutination test. One downside with these tests is the incidence of indeterminate results, when samples react to at least one or more antigens incorporated. The first generation EIAs were based on viral lysate that affected the sensitivity of the tests because of the resultant false positive reactions recorded. Second generation EIAs were later developed that performed

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extremely better than the first generation ones because synthetic HTLV-1 peptides and / or recombinant proteins were utilised in developing them. A repeat assay with the same specimen is conducted if the initial screening immunoassay is reactive. If either or both repeat tests are reactive, the sample is graded as reactive repeatedly and additional tests are conducted to validate and distinguish between the various forms of HTLVs (Verdonck et al., 2007).

The more up to date ELISAs recombinant proteins as well as manufactured HTLV-1 peptides and perform superior to original ELISAs which depended on viral lysate and every now and again brought about bogus positive responses. In any case corroborative testing is still prescribed to take out bogus positive responses and to separate between the diverse HTLV types (Verdonck et al., 2007). Serology-based affirmation tests, for example, indirect immunofluorescence assays (IFA) and Western Blot (WB) examination and line immunoassays are accessible. WB and line immunoassay can distinguish between HTLV-1 and HTLV-2 due to the addition of recombinant gp46-1 and gp46-2 to the blots. These corroborative tests may now and then be lacking; the event of vague results and powerlessness to consistently recognize HTLV-1 and HTLV-2 (Gonçalves et al., 2010). In these uncertain or untypable cases, PCR can give the unmistakable determination of HTLV infection.

Since the development of PCR technology, PCR have been employed in the diagnosis of infectious agent even to the minute detectable levels. Generic and/or type-specific HTLV PCRs have been developed for diagnosing HTLV infections; they are often based on the most conserved region of the genome, *Tax*. Using PCR assays, proviral HTLV-1 DNA is amplified to a detectable level and hence can be quantified. The proviral load (pVL) is expressed as the number of HTLV-1 DNA copies per hundred (100) peripheral blood mononuclear cells (PBMC) or in percentage. It is frequently used for prognosis and disease progression in HTLV infected

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persons. The PCR can conveniently detect all the types of HTLV infections that is HTLV-1, HTLV-2 and the recently discovered HTLV-3 and HTLV-4 as well as their simian counterparts STLVs (Gessain & Cassar, 2012).

The qualitative PCR method (qPCR) can be reliably used to confirm HTLV-I associated lymphomas. When used it can uncover 100% of several pVL in lymphoma cells or other tissue sample. The screening tests like ELISA are cheap and easy to perform. ELISA testing can be conducted on several samples at once, so it can be used for quick testing scaled up to test larger numbers of patients and highly reproducibly whilst the PCR on the other hand are quite expensive and require sophisticated equipment and special conditions to be met. The new recombinant ELISAs give very good specificity and sensitivity rates making them good for both diagnostic and research purposes. In this research a recombinant MP Diagnostics HTLV I ELISA 4.0 was used.

2.10 CLINICAL IMPORTANCE OF HTLV-1

In Japan, it is estimated that nearly 1.2 million individuals are infected with HTLV-I, and more than 800 cases of ATL are diagnosed each year. The cumulative risks of ATL among HTLV-I carriers in Japan are estimated to be about 6.6% for men and 2.1% for women, indicating that most HTLV-I carriers remain asymptomatic (Masao Matsuoka, 2005). Diseases associated with HTLV-1 can generally be grouped into three broad categories: malignant/neoplastic diseases, inflammatory syndromes and opportunistic infections. Two major diseases have been epidemiologically linked to HTLV-1, they are ATLL and HAM/TSP an inflammatory disease of the central nervous system (Gessain & Cassar, 2012). The development of ATLL and other diseases however occurs in about 5% of people with HTLV-1 infection after a very long latency period (Gonçalves et al., 2010; Gross & Thoma-kress, 2016). There are four (4) known types of

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ATLL; they are the acute, chronic, smoldering and T-cell Non-Hodgkin's lymphoma types. Seventy-five percent (75%) of all cases of ATLL are the acute types and are very deadly. The principal genes that are implicated in the development of ATLL are the *Tax* and HBZ genes (Gessain & Cassar, 2012). ATLL may be distinguished by malignant proliferation of CD25+ and CD4+ T-lymphocytes diagnosed by HTLV-1 seropositivity, the presence of morphologically distinct CD3+/4+/25+ lymphocytes with cleaved nuclei (flower cells), and clonal incorporation of HTLV-1 proviruses within the tumour cells. The integration of HTLV-1 proviruses can detected by Southern blotting, inverse PCR, or newer technologies like ultra-deep sequence analysis (M. Matsuoka & Jeang, 2011; Paola Miyazato, Misaki Matsuo, 2016). The primary cells involved in ATLL malignancy are CD4+ T cells in which the HTLV-1 provirus is incorporated. The genes most implicated in the development of ATLL are *Tax* and HBZ genes. HTLV-1 uses *Tax* (a regulatory protein) to actuate anomalous development of infected T cells through a series of pathways (Verdonck et al., 2007). Side effects of ATLL comprise lymphadenopathy (practically 100% of patients), hepatosplenomegaly (about half of patients) and skin sores are additionally normal, which as a rule may go before different manifestations. The illness can likewise influence different organs including the organs of the central nervous system, lungs, and gastrointestinal tract. In about 70% of the patients, the illness comes with hypercalcaemia as an outcome in lytic bone sores (Mahieux & Gessain, 2003; Marçais, Suarez, Sibon, Bazarbachi, & Hermine, 2012).

The primary distinguishing feature of TSP/HAM is protracted inflammation of the grey and white matter of the spinal cord. The clinical attributes of the disease are ceaseless neuromyelopathy, slowly progressive weakness and spasticity of one or both legs, exaggerated reflexes (hyperreflexia), stiff muscles, muscle contractions in the ankle (ankle clonus) and lower

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back pain. The main disability of HAM/TSP patients is spasticity of the lower legs, which leads to patient requiring wheelchairs. The principal laboratory diagnosis of HAM/TSP is raised HTLV-1 antibodies titres in the cerebrospinal fluid (CSF) and serum. The sickness happens all the more habitually in more established patients (individuals 40 years or more) and the evaluated lifetime danger of building up the malady is still under 5% of individuals carrying the disease (Caskey et al., 2007).

Other exceptional inflammatory ailments related with HTLV-1 are infective dermatitis, intermediate uveitis, polymyositis, HTLV-1 - related joint inflammation, aspiratory infiltrative pneumonitis and persevering lymphadenopathy (Mahieux & Gessain, 2003). Expanded viral burden has been recognized in both ATLL and HAM/TSP patients, contrasted with asymptomatic infected people, and is probably the best indicator for illness improvement; however, patients with high proviral burdens can stay asymptomatic for a considerable length of time. Some studies suggest that there are increased opportunistic infections such as Herpes Zoster, Cytomegalovirus (CMV), *Mycobacterium avium*, *Cryptococcus* and *Pneumocystis jirovecii* in HTLV-1 patients (Masao Matsuoka & Gallo, 2019; Verdonck et al., 2007). HTLV-2 has not been authoritatively connected with malignancy; notwithstanding, it has indicated uncommon relationship with a neurological malady taking after HAM/TSP that advances all the more gradually and with milder side effects. Although most HTLV-2 infections symptomless, studies suggest increased occurrence of infectious diseases (such as bronchitis, pneumonia, tuberculosis, and kidney and/or bladder infections) in HTLV-2-infected persons (Afonso, Cassar, & Gessain, 2019; Martinez et al., 2019). Literature suggests that HTLV-1 is more pathogenic than HTLV-2.

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Both HTLV-1 and HTLV-2 are linked epidemiologically to Hairy T-Cell Leukaemia and ATLL since their discoveries in the early 1980s. Various reports have concluded that haematologic malignancies other than ATLL are linked with HTLV-1 infections. Instances of T-cell non-Hodgkin's lymphoma, Sezary's disorder, Mycosis fungoides, enormous granular lymphocytic T-prolymphocytic leukaemia, and T-gamma lymphoproliferative disorder have all been portrayed to be serologically or molecularly connected to HTLV-1 infection (Adedayo & Shehu, 2004; Monavari et al., 2011). The relationship between haematological malignancies and retroviruses is depicted very well by HIV and HTLV-1 infections by causing high level of lymphoproliferative disorders. HTLV-1, initiates leukaemogenesis straightforwardly, in spite of the fact that the process is not fully comprehended. It is viewed as the *Tax* gene items of HTLV-1 actuate the expression of variables as IL-2 receptors, IL-2, granulocyte-macrophage colony stimulating factors (GM-CSF), and other transcription factors. The lifetime danger of developing ATL following HTLV-1 infection is less than 10% if the infection happens very early on in life.

2.**10.1 HIV AND HTLV CO-INFECTIONS**

HTLV and HIV have similar routes of transmission and cellular tropism. HIV/HTLV co-infection has oftentimes been reported. An early study on HIV/HTLV co-infection reported that roughly 7% of persons with AIDS or AIDS-related malady were also HTLV seropositive. All through the later part of the 1980s, many HIV/HTLV co-infections were described in Europe, America, and Africa, particularly in haemophiliacs, intravenous drug users, homosexuals and sex workers (Futsch et al., 2018).

HTLV infected cells produce a variety of chemokines, like MIP-1 α (Macrophage Inflammatory Protein-1 alpha), MIP-1 β (Macrophage Inflammatory Protein-1 beta) or RANTES (Regulated on Activation, Normal T-cell Expressed and Secreted), that act in an autocrine loop to interfere with

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HIV replication. The outcomes of HIV/HTLV co-infections on the pathologic disorders appear clearer with deterioration of symptoms related to HIV or HTLV infection. Though the number of reported HIV/HTLV co-infected cohorts are low, HTLV has been shown to worsen HIV infection by hastening progress to AIDS or snowballing death. Compared to HIV mono-infected people, most people co-infected with HIV / HTLV are more likely to develop myelopathy, thrombocytopenia, bronchitis, urinary tract infection and/or other opportunistic infections, regardless of age, ethnicity or T-cell count. Many reports indicate that co-infection with HIV / HTLV may impact the development of HTLV-related disorders, with an aggravating effect of HIV on the development of ATLL or TSP / HAM. This may result from the increased development of IL-2 and IFN-π found in HIV / HTLV co-infected individuals compared to HIV or HTLV mono-infected individuals in combination with the up-regulated rates of RANTES in HIV / HTLV co-infected cells. This cytokine profile may therefore favour a more rapid onset of myelopathies and neurological disorders in co-infected persons (Futsch et al., 2018).

2.10.2 ADULT T-CELL LYMPHOMA/LEUKAEMIA

ATLL was delineated before HTLV 1 detection, however the association between HTLV 1 infection and the malady was reported two years after HTLV-1 was discovered (Gallo, 2005). ATLL is an aggressive and sometimes incurable malignancy of HTLV-1 infected CD4+ T-cells that develops after long-term chronic infection of up to 30–50 years. ATLL progresses rapidly with leukaemic cell penetration into several organs including skin, spleen, liver, and lungs (Barbeau & Mesnard, 2007). ATLL is typically recognized by the appearance of lymphocytes with lobulated nuclei (flower cells) consistent with mature T lymphocytes with a helper or inducer phenotype ($CD2^+$, $CD3^+$, $CD4^+$, $CD8^-$). The disease is grouped into four clinical

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subtypes based on a wide range of diagnostic criteria like lymphadenopathy, splenomegaly, hepatomegaly, hypercalcemia, skin and pulmonary lesions or infiltration of organs.

Acute subtype: This aggressive kind of ATLL might develop speedily and symptoms may comprise enlarged lymph nodes in the neck, underarm, or groin other include fatigue and skin rash. Additionally lymphadenopathy is a prominent feature of this type of ATLL and a leukaemic depiction. High lactate dehydrogenase (LDH) and hypercalcemia often involving lytic bone lesions may occur in about half of the patients.

Lymphoma subtype: This is also an aggressive kind of ATLL is established mainly in the lymph nodes however it may cause elevated white blood cell (WBC) counts with no proof of blood involvement. Organomegaly is usually involved with circulating leukemic cells usually below 1% and elevated LDH. Hypercalcaemia may be possible.

Chronic subtype: This slow-growing kind of ATLL with lymphocytosis, skin, lung, liver or node involvement that may be stable for several months. There is absence hypercalcemia and LDH may be normal or only marginally increased.

Smoldering subtype: This kind of ATLL is associated with very mild symptoms like skin and/or lung infiltrates with no other organ involvement. The lymphocyte count is usually normal with about 3% atypical lymphocytes in circulation. Serum calcium and LDH are within normal ranges.

The Smoldering and chronic stages of the disease can progress quickly into acute ATLL within months. The subtypes; acute and lymphoma are more severe than Smoldering and chronic subtypes, with an average survival of about 10 months and 43 months respectively, comprising

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60% and 20% of all subtypes. Symptoms are varied and are largely subtype dependent. Symptoms can involve pleural effusion, abdominal pain, ascites, diarrhoea, jaundice, nausea, cough, sputum, unconscious and/or opportunistic infections (Carneiro-proietti et al., 2005; Futsch et al., 2018; Matutes, 2007). Analysis of ATLL cells revealed that each leukaemic cell contained proviral DNA incorporated in its chromosomal DNA showing monoclonal expansion, signifying that leukaemic cells originated from a single infected T lymphocyte (Barbeau & Mesnard, 2007).

The possibility of HTLV-I-associated diseases amongst carriers varies greatly across different geographical regions and according to additional population characteristics. Notwithstanding extensive geographical dissemination, data regarding ATLL occurrence and prevalence rates are rare and thus the reported rates could be an underestimation, particularly for the lymphomas. The accumulative occurrence of ATLL amongst HTLV-I-infected individuals is projected at 1–5% in endemic areas (Carneiro-proietti et al., 2005) with no gender preference. People infected in childhood (vertical transmission) and likewise HTLV-I carriers with high anti-HTLV-I titer and a low anti-*Tax* reactivity might be at considerable risk of developing ATLL.

Differential mRNA expression, up-regulation and down-regulation of various micro-RNAs, cell signaling alterations, somatic mutations, epigenetic deregulations or aneuploidy are processes involved in HTLV-1-induced leukaemogenesis. In the leukaemogenic processes, the *Tax* viral protein plays a crucial role. Above all, *Tax* is able to regulate the expression of viral and cellular genes by triggering the dependent pathways of CREB / ATF, SRF and NF-κB. Subsequently, *Tax* averts cell-cycle arrest and prevents both DNA damage repair and apoptosis pathways. Therefore, *Tax* favours both the propagation of infected cells and the buildup of genetic changes.

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HBZ likewise partakes in the transformation of HTLV-1 infected cells. HBZ stimulates T-cell proliferation, subdues Tax-mediated viral transcription via the 5'LTR, inhibits NF-κB activity, apoptosis and autophagy, disturbs host genomic stability via miRNA expression and weakens Th1 (T-helper 1) mediated antiviral immune response. *Tax* and HBZ function together in transforming infected CD4+ T-cells. Although HTLV-1 regulatory proteins *Tax* and HBZ both have oncogenic attributes, changes within the micro-environment of infected cell may additionally play a role in the development cancer. Indeed, viral oncogenesis follows protracted infection, which shows that HTLV-1 viruses can avoid immune reactions from the host. In addition, a dampened chronic inflammatory response may lead to immunosuppression and thus favour cancer development.

2.10.3 TREATMENT AND PROGNOSIS OF ATLL

Standard treatment, that is effective against other lymphoid cancers, is ineffective for treating aggressive types of ATLL. The treatment of ATLL has therefore become the subject of many scientific and clinical researches with the aim of improving treatment results. Patients with smoldering and chronic types of ATLL have a prolonged course, are usually symptomless and little proof that an aggressive treatment is of any beneficial to them (Carneiro-proietti et al., 2005). There has been an immense testing of many different treatment options over the past 30 years (Gonçalves et al., 2010). It is evident that patients presenting with aggressive ATLL additionally show multidrug resistance of malignant cells, an enormous tumour encumbrance, organ failure, hypercalcemia and/or opportunistic infections because of intense T-cell immunodeficiency and thus leading to poor prognosis of their condition (Gonçalves et al., 2010; Taylor & Matsuoka, 2005).

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After three decades of the initial designation of ATLL as a distinct clinical entity, this condition continues exhibit poor prognosis. Recent appraisals quote median survivals of not more than a year notwithstanding the progresses made in both chemotherapy and palliative care. Cyclophosphamide, adriamycin, vincristine, and prednisolone (CHOP) has been, and perhaps remains, the standard first-line therapy for ATLL and a lot of patients do show either partial or complete remission (Mesnard et al., 2015; Taylor & Matsuoka, 2005). More recently, the use of arsenic trioxide, zidovudine and alpha interferon has exhibited phenomenal remission rate with minimal toxicity (Kchour et al., 2009). To attain good results, treatment approach ought to be based on ATLL subtypes and prognostic factors. The approach usually may include watchful expectant approach, chemotherapy, antiviral therapy and in some cases stem cell transplantation (Marçais et al., 2012; Mesnard et al., 2015). The foremost facets driving prognosis comprise hypercalcemia, age of above 40 years, high levels of LDH and additionally, having three or more lesions. Another independent poor prognostic factor is the involvement of bone marrow (Mesnard et al., 2015). Previous reports have suggested that high doses of corticosteroids in patients with smoldering ATL without concomitant antiretroviral therapy might increase the risk of acute ATLL.

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CHAPTER THREE

3.0 METHODOLOGY

3.1 STUDY DESIGN

The study was a cross-sectional study. Random sampling was used to select consented participants diagnosed with a haematological malignancy at the Haematology Day Care Unit at the Department of Haematology. Data abstraction forms were used to obtain demographic data and clinical history from the study participants.

3.2 STUDY SITE

This research was carried out at the Haematology Department of Korle-Bu Teaching Hospital located in the capital of Ghana, Accra. Korle-Bu Teaching Hospital is the premier health care institution in Ghana established by the colonial administration over one hundred years ago. The hospital started as a general Hospital beginning with bed space of two hundred but over the years, it has seen several major transformations and transitions as well as remoulding and it is now a 2000-bed capacity teaching hospital. It is currently Africa's third-largest hospital, and Ghana's foremost national referral centre. The hospital further attracts many clients internationally especially within the West African sub region. The hospital has 17 Departments / Units for clinical and diagnostic purposes. It has an estimated daily enrolment of 1,500 patients and about 250 admissions to the hospital.

With the establishment of University of Ghana Medical School (UGMS) in 1962 for the purpose of training medical and dental students Korle Bu was granted a teaching hospital status. Currently School of Biomedical and Allied Health Sciences and University of Ghana Medical

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School which are under the College of Health Sciences of the University of Ghana are situated at Korle Bu Teaching Hospital to train medical doctors, dental surgeons and variety of allied health professionals. The schools under the College of Health Sciences undertake their clinical trainings and some academic research at the Hospital. The study participants were recruited at the Haematology Department and the laboratory investigations were conducted at the Microbiology Department all at Korle-Bu Teaching Hospital.

3.3 STUDY POPULATION

Patients with haematological malignancies including but not constrained to Hodgkin Lymphoma, non-Hodgkin Lymphoma (NHL), Chronic Lymphocytic Leukaemia (CLL), Acute Lymphoblastic Leukaemia (ALL) and Acute Myeloid Leukaemia (AML). Also Multiple Myeloma (MM) as well as Multiple Myeloma (MM), Myelodysplasia (MDS) and Myeloproliferative Neoplasm who present at the Haematology Day Care Unit of Korle-Bu Teaching Hospital as well as inpatients with haematological malignancies were included in this study. Haematological malignancies were diagnosed by routine procedures including clinical examinations, full blood counts, bone marrow aspirates, trephine biopsies and lymph node histology. The Haematology Department of Korle-Bu Teaching Hospital attends to patients with haematologic malignancies all over the country and even patients within West Africa sub region including but not limited to Togo and Sierra Leone.

Study participants were served with a consent form and nature of the study including the associated risks and benefits explained to them before enlisting them to join the study. The sampling technique used to select the participants to be included in the study was random sampling. Only eligible participants using the inclusion and exclusion criteria who also consented to be part of the study were enlisted into the study.

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3.4 SAMPLE SIZE

This was determined based on the formulary: $n = \underline{z^2 \times P\ (1\text{-}P)} e^2$

Interpretation:

n = least sample size

z = 1.96, the standard score at 95% certainty interval

P= estimated prevalence

e = 5% allowable error

Prevalence of HTLV-1, 5.1% (Akinbami et al., 2014)

The least sample size (n) = $\frac{(1.96)^2(0.051)\ (1\text{-}0.051)}{(0.05)^2} = 74$

The sample size was adjusted upwards and a total of 200 patients with haematological malignancies were recruited for the study.

3.5 INCLUSION CRITERIA

Participants diagnosed with a haematological malignancy at the Department of Haematology, Korle-Bu Teaching Hospital.

Participants who are 13 years old or more and have consented to do the study.

3.6 EXCLUSION CRITERIA

Participants diagnosed with benign haematological disorders.

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3.7 SAMPLE COLLECTION

Sampling was done by random sampling following informed consent from the research participants. A venous blood sample of 5mls was collected from the antecubital fossa into a clean, plain tube. The blood permitted to coagulate completely before centrifugation at 3,000 rpm for 10 minutes. The serum was then be collected into sterile cryovials and stored in aliquots at -70°C prior to analysis. The sample collection was carried out under aseptic conditions. Standard precautions were followed in the handling and transportation of the specimens. Repeated freezing and thawing was not done.

3.8 VARIABLES

3.8.1 DEPENDENT VARIABLES

The dependent variable for this study were persons with haematological malignancies.

3.8.2 INDEPENDENT VARIABLES

The independent variables for this study are; age, gender, marital status, previous history of blood transfusion and HTLV infection.

3.9 DATA COLLECTION TECHNIQUES/METHODS AND TOOLS

Data abstraction forms were used for collecting demographic and other clinical and laboratory data among the study participants. A structured survey questionnaire on the research topic was designed and allotted to the study participants to collect answers from participants on demographics. Data abstraction forms were administered to the consenting participants to obtain information on type of haematological malignancy, previous history of blood transfusion, date of diagnosis and some laboratory data (Hb, lymphocyte count among others). Researcher administered questionnaire method was employed in filling out data abstraction forms to ensure

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consistency in asking the questions. The questionnaires were designed and reviewed with guidance from supervisors as well as reference materials. All questionnaires were scrutinized for mistakes and completion. Any questionnaire with with indistinct answers or missing data that could not be explained was barred. Double entry of data was done to reduce data entry errors and validate authenticity.

3.10 HTLV 1 ANTIBODY SEROLOGY ASSAY

Sera from study participants were tested using commercial immunoassays for Anti-HTLV-1 specific for HTLV-1 by enzyme-linked immunosorbent assay (ELISA) method utilizing anti HTLV antibody pack (MP Diagnostics HTLV I ELISA 4.0). The testing was done strictly as indicated by the test manufacturer's guidelines and instructions. The testing was done in duplicates. All reagents trays and flasks used during the procedure were washed thoroughly with distilled water and dried. All pipettes including multichannel pipette were calibrated and working accurately prior to use for the testing procedures. The incubator was also calibrated and working properly prior to use and was set to 37°C.

All test reagents and samples were brought to equilibrium at room temperature before use. The working conjugate was prepared fresh prior to use at 1:200 dilution factor with diluent. Similarly, the diluted plate wash (1X PLATE WASH) was diluted using one (1) volume of 20X PLATE WASH with nineteen (19) volumes of distilled water (reagent grade quality). Importantly absorbance was measured within 10 minutes after addition of the STOP SOLUTION.

ELISA optical density (OD) measurements were performed using a Spectra II microassay plate reader at dual wavelength of 450nm-620nm. Cut-offs were calculated as recommended by the

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manufacturer (Domínguez, Salcedo, & García-vallejo, 2015). Standard operating procedures and safety precautions were adhered to strictly. Quality control measures recommended by the manufacturer were adhered to. The testing procedures and result interpretation were done according to the manufacturer's instruction.

3.10.1 PRINCIPLES OF THE ASSAY

The HTLV ELISA is a direct sandwich, solid-phase enzyme-linked immunoassay that uses a composition of recombinant proteins and a tri-fusion recombinant protein labeled with horseradish peroxidase. This test composition guarantees concurrent recognition of different specific IgA, IgG and IgM antibodies against HTLV-I in serum or plasma.

The wells of the polystyrene microplate strips are covered with a mixture of three distinctive HTLV recombinant proteins, which compare to the exceptionally antigenic fragments of HTLV-I viruses (p19, gp21, gp46). The conjugate is based on a tri-fusion recombinant protein, which is labeled with horseradish peroxidase. The cloning into a single vector of three cDNA fragments that encode the three HTLV recombinant proteins produces the tri-fusion antigen. Within the wells, human sera or plasma, dissolved into the diluent comprising the conjugate, is incubated. If present, HTLV 1-specific antibodies (IgA, IgG and IgM) can bind both the antigens immobilized in the solid phase and the conjugate's tri-fusion antigen. The wells are thoroughly washed after incubation, to get rid of unbound materials.

A colourless substrate solution containing 3,3',5,5' – tetramethylbenzidine (TMB) chromogen is then added to each well. The presence of specific antibodies is indicated by the presence of a blue colour after incubation, which changes to yellow when sulphuric acid is added to terminate the colour reaction. The intensity of the resulting yellow product is measured with a dual (A_{450}–

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A_{620}) or single (A_{450}) wavelength microassay plate reader and corresponds to the measure of antibodies present in the sample.

3.10.2 ASSAY PROCEDURE

The assay was carried out carefully as indicated by the manufacturer's guidelines.

All kit elements and test samples were brought to equilibrium at room temperature as well as test samples and control vials were mixed thoroughly before commencing the assay. The freshly prepared working conjugate was poured into a reagent reservoir, utilizing a multichannel pipettor, 50µl of freshly made working conjugate was added to all wells. Fifty microliters (50µl) of diluent only is added to well A1. Fifty microliters (50µl) of each test specimen was added into the individual wells starting at well H1 using a separate pipette tip for each specimen. This gave a final specimen dilution of 1:2. This was then mixed by pipetting up and down at least once and repeated until all test specimen were added. The specimens were gently added to avoid overflowing in order not to contaminate adjacent wells. Fifty microliters (50µl) of non-reactive control was added per well in triplicates (wells B1, C1 and D1) also mixing by pipetting up and down at least once. The reactive control was also dispensed in a similar manner into wells E1, F1 and G1. This was repeated for all the microplates used.

Proper mixing of the specimens and controls was ensured by gently tapping on all sides of the microplates. The microplates were then carefully covered with adhesive microplate covers to prevent evaporation during incubation, labeled and incubated for 60 minutes at +37˚C in an incubator. After this incubation period, the covers were removed and discarded. The contents of the microplates were discarded from the wells by inverting the microplate and tapping dry on paper towel. The microplates were washed with diluted plate wash. The washing was done six (6) times with at least 300µl per well per wash, taking care not to scratch the inside of the well

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surface. After the last wash, the microplates were tapped onto absorbent paper several times to remove any residue remaining, taking care not to have dislodged the microplate strips from the holder.

The substrate was poured into a reagent reservoir, using a multichannel pipettor, 100µl of substrate was added to each well. The microplates were covered with new adhesive microplate covers and incubated 30 minutes in the dark at 37˚C. The substrate is light sensitive so exposure to light was only minimal. After incubation, the microplate covers were removed and discarded. The stop solution was poured into a reagent reservoir. Using a multichannel pipettor, 100µl of the stop solution was added to each well just as was done for the addition of the substrate. The addition of the stop solution stops the colour reaction. The microplates were then mixed by gently tapping them.

The absorbance for each well was then measured at 450nm to 620nm wavelength using a dual (A_{450}–A_{620}) wavelength microassay plate reader. The readings were carried out within 10 minutes of addition of the stop solution. See Appendix V and VI for calculation of cut of point and interpretation of results.

3.11 DATA HANDLING

Confidentiality of data obtained for all study participants was adequately ensured. Demographic, laboratory and clinical data were captured on data abstraction forms, which were transcribed to digital format and stored electronically. All hard copies of case investigation forms were kept in locked file with access limited to the investigators and electronic data password protected. Samples were coded prior to analysis. Computer assisted generation was used to clean data to manage outliers and missing data. Data verified for accuracy was transferred into SPSS version 23.0 for analysis.

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3.12 STATISTICAL ANALYSIS

Data analysis was performed with Excel and Statistical Package for the Social Sciences (SPSS) software version 23.0 (SPSS, Chicago, IL). Descriptive statistics were used to analyze demographic characteristics and the other information provided by the respondents. Univariate analysis including frequency and percentages for categorical variables, means and standard deviations for continuous variables after testing for normality. The association between different factors were evaluated by chi-square test (x^2), or Fisher's exact test when an expected value was less than 5. Multiple logistic regressions were used. P values below 0.05(5%) were considered statistically significant. The results were presented using tables and graphs.

3.13 ETHICAL ISSUES

Ethical approval was sought from the Ethical and Protocol Review Committee of College of Health Sciences, University of Ghana (appendix I). Approval was also be sought from the management of Haematology Day Care Unit of Korle-Bu Teaching Hospital. Written informed consent was also sought from the study participants. 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 numbers of the researcher and supervisors for clarification as well as actions established to guarantee confidentiality of the research data. The general and specific objectives of the research plus the risks and merits of the study were explained to study participants. Every consenting participant was made to endorse the informed consent form (see Appendix II) with his or her signatures/thumbprints.

Venous blood samples were taken from consented study participants. Venous blood sampling involves the risk of minimal pain at the site of puncture, discomfort and infection thus qualified

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and well trained laboratory personnel was involved during the sampling and as well as sterile syringes, and needles were used to ensure these risks are reduced if not avoided.

Study identification numbers were assigned to all respondents and data analysis was done based on these numbers without referring to any particular participant's name. This was password protected and used by only named investigators. Only a summative report of the results of this study were given out. Participation in the study was solely voluntary.

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CHAPTER FOUR

4.0 RESULTS

4.1 DEMOGRAPHIC CHARACTERISTICS

The study randomly surveyed 200 clinically diagnosed patients with haematological malignancy. The youngest respondent was 16 years and the oldest 92 years old. The average age of the respondents in this study was 49.1±17.7 years. About a third 30.5% (n=61) of the respondents were 60 years and above, 19.5% (n=39) were 50-59 years while only 5% (n=10) were less than 20 years. Fifty-four percent (54.5%) (n=109) of the respondents were females. Most of the respondents 63.5% (n=127) were employed, 12% (n=24) were students while 19% (n=38) were retired. Of the respondents, 59.5% (n=119) were married while 15% (n=30) were widowed. Most of the respondents 40.5% (n=81) had secondary level education, 36.5% (n=73) had tertiary level education while 13.5% (n-27) had no formal education.

4.2 CLINICAL CHARACTERISTICS

Clinical characteristics of respondents in this study is presented in Table 4.1. The most prevalent haematological malignancies of respondents in this study was Chronic Myeloid Leukaemia, it constituted a third 31.5 (n=63) of the cases. About 15.5% (n=31) of the respondents had Multiple Myeloma, 15% (n=30) had Non-Hodgkin Lymphoma, 13.5% (n=27) had Chronic Lymphocytic Leukaemia and 10.5% (n=21) had Hodgkin Lymphoma. Other comorbidities observed among respondents were hypertension 19.5% (n=39) and 7% (n=14) diabetes. About a third 35.5% (n=71) had blood transfusions.

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Table 4. 1: Clinical Characteristics of Participants

Clinical characteristics	**Frequency**	**Percent (%)**
Haematological malignancies		
Acute Lymphoblastic Leukaemia	4	2.0
Acute Myeloid Leukaemia	6	3.0
Chronic Lymphocytic Leukaemia	27	13.5
Chronic Myeloid Leukaemia	63	31.5
Hodgkin Lymphoma	21	10.5
Multiple Myeloma	31	15.5
Myelodysplastic syndrome	6	3.0
Myeloproliferative Neoplasm	11	5.5
Non-Hodgkin Lymphoma	31	15.5
Total	200	100
Other comorbidities		
Asthma	1	0.5
DM	14	7.0
DM, Asthma	1	0.5
HEP B Infection	1	0.5
HPT	39	19.5
HPT, DM	7	3.5
HPT, DM, Asthma	1	0.5
NIL	136	68.0
Total	200	100
Blood Transfusion		
No	129	64.5
Yes	71	35.5
Total	200	100

DM=diabetes mellitus HPT=hypertension NIL=no comorbidity

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4.4 SEROPREVALENCE OF HTLV-1

Two hundred (200) blood samples from patients with malignant haematologic disorders were tested for HTLV-1 using the ELISA technique. Out of the 200 samples, 14 of them were positive for HTLV-1 infection including 3 males and 11 females resulting in a seroprevalence of 7.0%. Figure 4.3 shows the prevalence of HTLV-1 among respondents in this study.

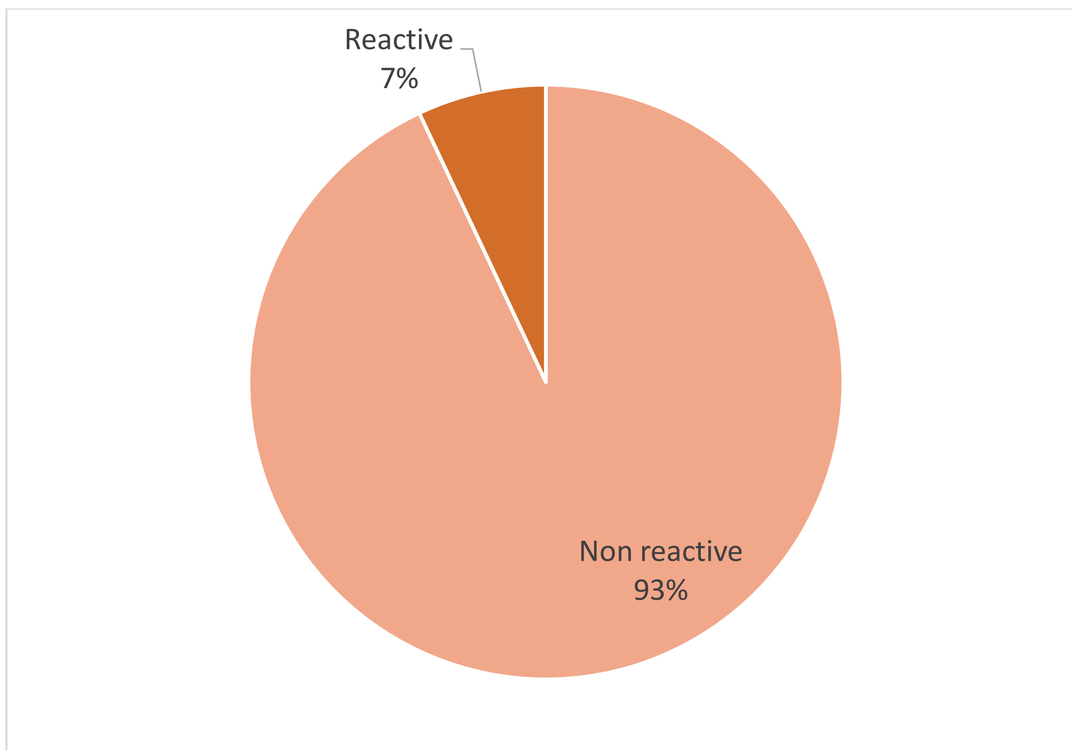

Figure 4. 1: Seroprevalence of HTLV-1

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4.5 ASSOCIATION BETWEEN HTLV-1 INFECTION AND HAEMATOLOGICAL MALIGNANCIES

Association between HTLV-1 infection and haematological malignancies is presented in Table 4.2. No significant association was found between HTLV-1 infection and haematological malignancies (χ^2 p=0.081).

Table 4. 2: haematological malignancies and HTLV-1 status

Haematological malignancy	**HTLV 1 Non-Reactive (%)**	**HTLV 1 Reactive (%)**	**Total no. of participants**	**p-value**
Acute Lymphoblastic Leukaemia	4 (100)	0 (0)	4 (100)	0.081
Acute Myeloid Leukaemia	6 (100)	0 (0)	6 (100)	
Chronic Lymphocytic Leukaemia	26 (96.2)	1 (3.8)	27 (100)	
Chronic Myeloid Leukaemia	59 (93.6)	4 (6.3)	63 (100)	
Hodgkin lymphoma	21 (100)	0 (0)	21 (100)	
Multiple Myeloma	29 (93.5)	2 (6.5)	31 (100)	
Myelodysplastic Syndrome	4 (66.7)	2 (33.3)	6 (100)	
Myeloproliferative neoplasm	11 (100)	0 (0)	11 (100)	
Non-Hodgkin Lymphoma	26 (83.3)	5 (16.7)	31 (100)	
Total	186 (93.0)	14 (7.0)	200 (100)	

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4.6 ASSOCIATION BETWEEN DEMOGRAPHIC CHARACTERISTICS AND HTLV STATUS

Association between demographic characteristics and HTLV infection is presented in Table 4.3. Among respondents who were 30-39 year, 12.5% were reactive to HTLV, 10.8% of those 40-49 years were reactive while only 4.9% of those ≥ 60 years. A higher proportion of females 10.1% were HTLV-1 positive compared with 3.3% males. In this study, 10% of respondents who were unemployed were reactive for HTVL compared with 7.9% of those who were retired. About 7.8% of respondents who were single and 7.6% of those married were reactive to HTLV infection. Among those with primary level education, 15.8% were reactive to HTLV while 6.8% of those with tertiary level education were reactive to HTLV infection. There was no significant association between age group (p=0.406), gender (p=0.061), occupation (p=0.963) marital status (p=0.693), educational level (0.513) and HTLV infection.

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Table 4. 3: Demographic Characteristics and HTLV status

Characteristics	**HTLV Status** **NON-REACTIVE**	**REACTIVE**	**Total no. of participants**	**χ^2**	**p-value**
Age group (years)					
<20	10 (100.0)	0 (0.0)	10 (100.0)	5.081	0.406
20-29	21 (100.0)	0 (0.0)	21 (100.0)		
30-39	28 (87.5)	4 (12.5)	32 (100.0)		
40-49	33 (89.2)	4 (10.8)	37 (100.0)		
50-59	36 (92.3)	3 (7.7)	39 (100.0)		
≥60	58 (95.1)	3 (4.9)	61 (100.0)		
Total	186 (93.0)	14 (7.0)	200 (100.0)		
Gender					
Female	98 (89.9)	11 (10.1)	109 (100.0)	3.518	0.061
Male	88 (96.7)	3 (3.3)	91 (100.0)		
Total	186 (93.0)	14 (7.0)	200 (100.0)		
Occupation					
Employed	119 (93.7)	8 (6.3)	127 (100.0)	0.282	0.963
Unemployed	10 (90.9)	1 (9.1)	11 (100.0)		
Student	22 (91.7)	2 (8.3)	24 (100.0)		
Retired	35 (92.1)	3 (7.9)	38 (100.0)		
Total	186 (93.0)	14 (7.0)	200 (100.0)		
Marital status					
Married	110 (92.4)	9 (7.6)	119 (100.0)	0.733	0.693
Single	47 (92.2)	4 (7.8)	51 (100.0)		
Widowed	29 (96.7)	1 (3.3)	30 (100.0)		
Total	186 (93.0)	14 (7.0)	200 (100.0)		

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Education level					
No Education	25 (92.6)	2 (7.4)	27 (100.0)	2.299	0.513
Primary	16 (84.2)	3 (15.8)	19 (100.0)		
Secondary	77 (95.1)	4 (4.9)	81 (100.0)		
Tertiary	68 (93.2)	5 (6.8)	73 (100.0)		
Total	186 (93.0)	14 (7.0)	200 (100.0)		

4.7 CHARACTERISTICS OF HTLV-1 SEROPOSITIVE PATIENTS

Table 4.4 shows characteristics of HTLV-1 positive patients in this study. Majority 78.6% (n=11) of the positive patients were females. About 35.7% (n=5) had NHL while 28.6% (n=5) had CML. Also, 42.9% had previous history of blood transfusion. The optical densities of the positive patients are shown in appendix X.

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Table 4. 4: Characteristics of HTLV-1 Positive patients

	Haematological malignancy	Gender	Age	Hb (g/dl)	Transfusion history
1	NHL	Female	47	7.8	Yes
2	NHL	Female	51	10.2	No
3	MM	Female	72	12.3	No
4	MDS	Female	45	4.7	Yes
5	NHL	Male	54	12.4	No
6	MDS	Female	39	8.6	Yes
7	MM	Female	71	10.8	No
8	CML	Female	66	13.3	Yes
9	CML	Female	31	9.7	No
10	CLL	Male	35	13.6	No
11	CML	Female	30	9.6	Yes
12	NHL	Female	42	10.6	Yes
13	CML	Female	53	11.7	No
14	NHL	Male	45	7.7	No

4.8 ASSOCIATION BETWEEN PREVIOUS HISTORY OF BLOOD TRANSFUSION AND HTLV-1 INFECTION

In this study, the association between blood transfusion and HTLV-1 status was investigated. The findings show that, among those with history of blood transfusion, 8.5% were reactive while 6.2% of those with no history of blood transfusion were reactive to HTLV-1 infection. However, this study did not find any significant associations between previous history of blood transfusion and HTLV-1 infection among study participants (p-value = 0.551).

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4.9 TREATMENT OUTCOMES OF HTLV-1 SEROPOSITIVE PATIENTS WITH HAEMATOLOGICAL MALIGNANCIES

The treatment outcomes of the study participants were grouped into those in clinical remission and those who had relapsed. Because the study considered both old and newly diagnosed patients with haematologic malignancies, a third group was introduced as "newly diagnosed". At the time of sampling for this study, the "newly diagnosed" were starting their treatment. Table 4.4 shows treatment outcomes of seropositive patients with hematological malignancies. Among respondents who were reactive for HTLV-1 seropositive, 21.4% (n=3) were newly diagnosed while 21.5% (n=40) of those non-reactive were newly diagnosed. There was no significant association between HTLV-1 seropositive and outcomes after treatment.

Table 4. 5: Outcomes of HTLV-1 seropositive patients with haematological malignancies

		Clinical remission	Relapsed	Newly diagnosed	Total	χ^2	p-value
HTLV ELISA	Non-reactive	137 (73.7)	9 (4.8)	40 (21.5)	186 (100.0)	0.720	0.698
	Reactive	11 (78.6)	0 (0.0)	3 (21.4)	14 (100.0)		
	Total	148 (74.0)	9 (4.5)	43 (21.5)	200 (100.0)		

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4.10 ASSOCIATION BETWEEN HAEMATOLOGICAL PARAMETERS AND HTLV-1 INFECTION

Association between lymphocytosis, anaemia, thrombocytosis and HTLV-1 infection is presented in Table 4.5. There was no significant association between anaemia (p=0.941), HCT (p=0.717), TWBC (P=0.924), PLT (p=0.281) and HTLV-1 infection.

Table 4. 6: Haematological parameters and HTLV infection

Variables		N	Mean	Std. Dev	F-value	p-value
Hb, g/dl	Non-reactive	186	10.27	2.69	0.006	0.941
	Reactive	14	10.21	2.46		
	Total	200	10.27	2.67		
HCT, %	Non-reactive	186	30.70	7.70	0.132	0.717
	Reactive	14	29.93	7.50		
	Total	200	30.65	7.67		
TWBC, x109/L	Non-reactive	186	18.40	37.13	0.009	0.924
	Reactive	14	17.41	34.59		
	Total	200	18.33	36.88		
LYM, x109/L	Non-reactive	186	11.05	29.10	0.465	0.496
	Reactive	14	5.70	12.44		
	Total	200	10.68	28.27		
PLT, x109/L	Non-reactive	186	267.94	184.87	1.170	0.281
	Reactive	14	213.64	115.49		
	Total	200	264.14	181.21		

Hb=haemoglobin TWBC=total white blood cells LYM=lymphocytes PLT=platelets

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CHAPTER FIVE

5.0 DISCUSSION, CONCLUSION AND RECOMMENDATIONS

5.1 DISCUSSION

HTLV-1 is associated with cell-cell transmission and is spread in cells after blood transfusion, sexual contact, or breastfeeding. The information and understanding of HTLV-1 prevalence in different populations and patients groups is crucial because it may be useful in establishing prophylactic measures to decrease rates of viral transmission from infected individuals. HTLV-1 infection is chronic and can lead to untreatable diseases. HTLV-1 infection is a risk factor for certain haematologic malignancies especially ATLL.

This study was aimed at determining the role of HTLV 1 infections in haematological malignancies among patients seen at the Department of Haematology, Korle-Bu Teaching Hospital. The study participants included old and new patients diagnosed with any haematological malignancy. Some of the patients were diagnosed as far back as 2002 and some as recently as 2019 during the period the study was conducted. In this study more women participated 54.5% (n=200), this is consistent with a study on the age and gender distribution of leukaemia patients conducted at the Haematology Department which showed lower male to female ratios in all the types of leukaemia studied (Ekem & Dei-Adomakoh, 2015). In another study conducted in Iran which looked at the prevalence of HTLV 1 infections amongst persons with haematologic and non-haematologic malignancies, 52.0% of the study participants were females (Jalaeikhoo Hasan, Mosayeb Soleymani, Mohsen Rajaeinejad, 2017) which is consistent with findings in this current study.

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This study demonstrated that out of the two hundred (200) study participants, fourteen (14) patients with various haematological malignancies had HTLV-1 infection. This gives a seroprevalence of HTLV-1 infection among the study population of 7.0% (14 out of 200). The results obtained from this study when compared to results from other studies showed that the seropositivity of 7.0% is much lower than what was reported by other researchers. Adedayo & Shehu, 2004 reported a seroprevalence of 38.6% among patients with lymphoid malignancies in Dominica and Monavari *et al*., 2011 also reported a seroprevalence of 12.0% among patients with malignant haematological disorders in Tehran, Iran. In a similar study conducted in Lagos, Nigeria on the seroprevalence of HTLV 1 infection amongst persons with lymphoid malignancies, an overall seroprevalence of 5.1% was reported (Akinbami et al., 2014). This seroprevalence (5.1%) reported in our neighbouring West African nation is quite comparable to the findings of this current study.

A recent large scale study conducted in Gabon on the general rural populace revealed an overall HTLV-1 prevalence of 8.7% (Djuicy et al., 2018). In a similar study conducted in Iran among patients with haematologic disorders and non-haematologic malignancies in a tertiary referral hospital by Jalaeikhoo Hasan *et al*., 2017, they reported a seroprevalence of 2.4% among patients with malignant haematologic disorders which is lower than what this study found out. The HTLV-1 seroprevalence obtained from this study is however higher than that reported by Biggar *et al*., 1984, they reported a seroprevalence of 3.6% and 4.0% for urban and rural populations respectively in Ghana. A recent study also reported HTLV-1/2 seroprevalence of 3.3% (unpublished data) among healthy blood donors at the Southern Area Centre of the National Blood Service, Ghana. Another study also conducted in Ghana by Armah *et al*., 2006 reported an overall HTLV-1 seroprevalence of 2.1% among pregnant women in the southern part of the

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country. The seroprevalence in this current study (7.0%) which is much higher than that reported by Fox *et al*., 2016 whose research was among children with childhood malignancy and their healthy mothers in Malawi. They and reported a seroprevalence of 2.6% (11/418) in mothers and 2.2% (12/534) in children.

Studies of HTLV-1 and HTLV-1/2 seroprevalence in Ghana is based largely on healthy blood donors or pregnant women. This study looked at a special cohort of study participants who have malignant haematologic disorders hence the higher seroprevalence being reported in this group. A similar study conducted in Iran on high risks (thalassemia and hemodialysis) patients demonstrated a similar HTLV-1 seroprevalence of 7.6% (Karimi, Nafici, & Imani, 2007). The seroprevalence of 7.0% among patients with haematological malignancies in Ghana in this current study is much higher compared to seroprevalence reported in other cohorts in Ghana.

In this current study, the association between haematological malignancies and HTLV-1 infection was not statistically significant (P-value = 0.081) but Adedayo & Shehu, 2004 and Monavari *et al.,* 2011 reported a significant association between HTLV-1 and haematologic malignancies in Dominica and Iran respectively. This could partly be due to inherent geographical differences, differences in sample size and the level of HTLV-1 endemicity. HTLV-1 infection is endemic in Dominica and Iran compared to Ghana. This current study did not find a significant association between previous history of blood transfusion and HTLV-1 infection. This is consistent with findings of Akinbami *et al*., 2014 in Nigeria. They also did not find statistically significant association of previous history with HTLV-1 in lymphoid malignancies. Nevertheless, it is interesting to note in this study that 8.5% of study participants with previous history of blood transfusion were HTLV-1 seropositive while 6.2% of study

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participants with no previous history of blood transfusion were HTLV-1 seropositive. Although the seropositivity rate of those with previous transfusion history was higher than those without, the association was not a significant one statistically (P-value = 0.551). Even though this study did not investigate risks factors that influence the HTLV-1 seropositivity among patients with haematologic malignancies, other studies such as Jalaeikhoo Hasan *et al*., 2017, Karimi *et al*., 2007 and Gonçalves *et al*., 2010 as well as Djuicy *et al*., 2018 all concluded that previous history of blood transfusion (especially multiple transfusions) is a significant risk factor for HTLV-1 infection.

In this study age specific seroprevalence was not observed in respondents less than 30 years but seroprevalence was observed in age groups above 30 years with the age group 30-39 years having the highest HTLV-1 seropositivity of 12.5% (table 4.2). This is consistent with other studies of HTLV-1 seropositivity that concluded the increasing seropositivity with increasing age (Djuicy et al., 2018). Increasing age is a known risk factor for HTLV-1 infection. However, the high seropositivity among the age groups 30-39 years and 40-49 years may suggest that other transmission routes rather than mother-to-child transmission is more common in Ghana. HTLV-1 can be transmitted by sexual contact or blood transfusion as supported by a lot of studies (Afonso et al., 2019). Also in this study, married respondents showed the highest HTLV-1 seropositivity giving more credence to the sexual contact transmission of HTLV-1. It must be pointed out though that the association of marital status with HTLV-1 seropositivity was not significant statistically.

The treatment outcomes of the participants in this study revealed that majority of the respondents were in clinical remission with only a few relapsed cases. This confirms that the treatment

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regimens in the haematology department are very effective in controlling the malignancies and improving the health of their patients. Out of the 14 HTLV-1 seropositive study participants, 11 representing 78.6% (Table 4.6) were categorized as being in clinical remission. This study could not pinpoint when the HTLV-1 infection started in these patients with malignant haematological disorders. This study did not find any significant association of disease outcomes with HTLV-1 infection (p-value =0.698).

Generally, the means of the haematological parameters of the study participants were very good. This is due to the fact that most of the study participants were in good condition and in clinical remission at the time of the study. This study did not reveal statistically significant association of HTLV-1 seropositivity with the following haematology parameters Hb (p=0.941), total WBC (P=0.924), lymphocytes (p=0.496) and platelet (p=0.281). This picture would have been much different if the participants were recruited at the time of diagnosing their malignant haematological disorders. In another study by Chaturvedi *et al*., 2007 in Jamaica, they found out that HTLV-1 was associated with elevated lymphocyte count, lymphocyte abnormalities, anaemia and decreased eosinophils.

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5.2 CONCLUSION

In conclusion, the results of this study have shown that the seroprevalence of HTLV-1 among patients with haematological malignancies is 7.0%. The HTLV-1 seroprevalence obtained in this study is high. Consequently, it is possible to consider HTLV-1 screening during clinical and laboratory workup of patients with haematological malignancies in Ghana. However, No significant association was found between HTLV-1 infection and haematological malignancies. There was no significant association of HTLV-1 with previous history of blood transfusion as well as some haematologic parameters (Hb, lymphocyte count, total WBC and platelet count) studied.

This study revealed that the seroprevalence of HTLV-1 in hematologic malignancies is higher compared to the seroprevalence of the general healthy population reported in Ghana. Further larger prospective studies are needed to corroborate the current evidence.

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5.3 RECOMMENDATIONS

A large prospective study which is population based should be carried out among the general population including but not limited to healthy people (blood donors, pregnant women) and at risks groups (multi-transfused patients, commercial sex workers) to determine the overall national seroprevalence of HTLV-I infection in Ghana.

A nationwide large-scale study on the role of Human T-Cell Lymphotropic Virus type 1 (HTLV-1) infections in haematological malignancies will be very helpful to corroborate the current evidence.

In addition, a large retrospective study involving blood donors and blood recipients as well as pregnant women and children to positive mothers to determine the rate and duration of seroconversion of HTLV-1 will be helpful.

Outcome from such studies will provide adequate information on the risks factors as well as the transmission routes peculiar to our nation and how to mitigate its effects.

A well-functioning national health research board should be constituted with the mandate to support health related researches. This national health research board be able to support students research to some extent by facilitating the procurement of materials and reagents especially those that need to be imported.

5.4 LIMITATIONS

1. This study was unable to determine risk factors associated with HTLV-I infection and thus could not ascertain the route of infection for the study population.

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REFERENCES

Adedayo, O. A., & Shehu, S. M. (2004). Human T-Cell Lymphotropic Virus Type 1 (HTLV-1) and Lymphoid Malignancies in Dominica : A Seroprevalence Study. *American Journal of Hematology*, *77*, 336–339. https://doi.org/10.1002/ajh.20208

Afonso, P. V, Cassar, O., & Gessain, A. (2019). Molecular epidemiology , genetic variability and evolution of HTLV ‑ 1 with special emphasis on African genotypes. *Retrovirology*, *16*(39), 1–15. https://doi.org/10.1186/s12977-019-0504-z

Akbarin, M. M., Shirdel, A., Bari, A., & Mohaddes, S. T. (2017). Evaluation of the role of TAX , HBZ , and HTLV- 1 proviral load on the survival of ATLL patients. *BLOOD RESEARCH*, *52*(2).

Akinbami, A., Durojaiye, I., Dosunmu, A., John-Olabode, S., Adediran, A., Oshinaike, O., … Okunoye, O. (2014). Seroprevalence of human T-lymphotropic virus antibodies among patients with lymphoid malignancies at a tertiary center in Lagos, Nigeria. *Journal of Blood Medicine*, *5*, 169–174. https://doi.org/10.2147/JBM.S67912

Ampofo, W., Nii-trebi, N., Ansah, J., Abe, K., Naito, H., Aidoo, S., … Ishikawa, K. (2002). Prevalence of Blood-Borne Infectious Diseases in Blood Donors in Ghana. *Journal of Clinical Microbiology*, *40*(9), 3523–3525. https://doi.org/10.1128/JCM.40.9.3523

Armah, H. B., Narter-olaga, E. G., Adjei, A. A., Asomaning, K., Gyasi, R. K., & Tettey, Y. (2006). Seroprevalence of human T-cell lymphotropic virus type I among pregnant women in Accra , Ghana. *Journal of Medical Microbiology*, *55*, 765–770. https://doi.org/10.1099/jmm.0.46426-0

University of Ghana http://ugspace.ug.edu.gh

Bangham, C. R. M., & Matsuoka, M. (2017). Human T-cell leukaemia virus type 1 : parasitism and pathogenesis. *Phil. Trans. R. Soc B*, *372*, 1–10.

Bangham, C. R. M., & Osame, M. (2005). Cellular immune response to HTLV-1. *Oncogene*, *24*, 6035–6046. https://doi.org/10.1038/sj.onc.1208970

Barbeau, B., & Mesnard, J.-M. (2007). Does the HBZ Gene Represent a New Potential Target for the Treatment of Adult T-Cell Leukemia ? *International Reviews of Immunology*, *26*, 283–304. https://doi.org/10.1080/08830180701690843

Barbeau, B., & Mesnard, J. (2011). Making Sense out of Antisense Transcription in Human T-Cell Lymphotropic Viruses (HTLVs). *Viruses*, *3*, 456–468. https://doi.org/10.3390/v3050456

Besson, G., & Kazanji, M. (2009). One-Step , Multiplex , Real-Time PCR Assay with Molecular Beacon Probes for Simultaneous Detection , Differentiation , and Quantification of Human T-Cell Leukemia Virus Types 1 , 2 , and 3 □ . *Journal of Clinical Microbiology*, *47*(4), 1129–1135. https://doi.org/10.1128/JCM.02006-08

Biggar Robert, J., Carl, S., Charlotte, G., E., C. W., H., L. P., W., C. J., … A., B. W. (1984). Type-I HTLV antibody in urban and rural Ghana, West Africa. *International Journal of Cancer*, *34*(2), 215–219. https://doi.org/10.1002/ijc.2910340212

Bosqui, L. R., Silva, T. F., Sanfelice, R. A., Machado, L. F., Steinle, É. C., Bertasso, B. A., … Rogerio, W. (2019). The Influence of Human T-Lymphotropic Virus 1 on Patients Coinfected by the Helminth Strongyloides stercoralis. *International Journal of Tropical Diseases*, *2*(1), 2–7.

University of Ghana http://ugspace.ug.edu.gh

Boxus, M., & Willems, L. (2009). Mechanisms of HTLV-1 persistence and transformation. *British Journal of Cancer*, *101*(9), 1497–1501. https://doi.org/10.1038/sj.bjc.6605345

Carbone, A., & Gloghini, A. (2013). Relationships between lymphomas linked to hepatitis C virus infection and their microenvironment. *World Journal of Gastroenterology*, *19*(44), 7874–7879. https://doi.org/10.3748/wjg.v19.i44.7874

Carneiro-proietti, F., Catalan-soares, B. C., Proietti, F. A., Ba, A., & Murphy, E. L. (2005). Global epidemiology of HTLV-I infection and associated diseases. *Oncogene*, *24*, 6058–6068. https://doi.org/10.1038/sj.onc.1208968

Caskey, M. F., Morgan, D. J., Porto, A. F., Giozza, S. P., Muniz, A. L., Orge, G. O., … Glesby, M. J. (2007). Clinical Manifestations Associated with HTLV Type I Infection: A Cross-Sectional Study. *AIDS Res Hum Retroviruses*, *23*(3), 365–371. https://doi.org/10.1089/aid.2006.0140.Clinical

Cavanagh, M., Landry, S., Audet, B., Arpin-andré, C., Hivin, P., Paré, M.-ève, … Barbeau, B. (2006). HTLV-I antisense transcripts initiating in the 3 ’ LTR are alternatively spliced and polyadenylated. *Retrovirology*, *15*(3), 1–15. https://doi.org/10.1186/1742-4690-3-15

Chaturvedi, A. K., Wilson, M., Sanders-lewis, K. A., Katki, H. A., Urquhart, N., Walters, M. A., … Hisada, M. (2007). Hematologic and Biochemical Changes Associated with Human T Lymphotropic Virus Type 1 Infection in Jamaica : A Report from the Population-Based Blood Donors Study. *Clinical Infectious Diseases*, *45*, 975–982. https://doi.org/10.1086/521932

University of Ghana http://ugspace.ug.edu.gh

Ciminelli, A., Melo, F., Copin, M., Carneiro-proietti, A. B., & Meireles, S. (2016). Hematological manifestations of human T lymphotropic virus type 1 infection : a possible association with autoimmune myelofibrosis. *Brazilian Journal of Hematology and Hemotherapy*, *38*(1), 75–78. https://doi.org/10.1016/j.bjhh.2015.08.006

Confidence, N., Anyanwu, J., Ekah, E., Ohwofasa, A., Aminu, M., & Tsp, H. A. M. (2018). Re-emergence of human T-lymphotropic viruses in West Africa. *Brazilian Journal of Infectious Diseases*, *22*(3), 224–234. https://doi.org/10.1016/j.bjid.2018.05.003

Djuicy, D. D., Mouinga-Ondémé, A., Cassar, O., Ramassamy, J.-L., Mamimandjiami, A. I., Bikangui, R., … Gessain, A. (2018). Risk factors for HTLV-1 infection in Central Africa : A rural population-based survey in Gabon. *PLOS Neglected Tropical Diseases*, *12*(10), 1–16.

Domínguez, M. C., Salcedo, M., & García-vallejo, F. (2015). Serological and virological evaluation of human T-lymphotropic virus type 1 infection in family groups from Tumaco , Colombia. *Biomédica*, *35*, 337–346.

Einsiedel, L., Woodman, R. J., Flynn, M., Wilson, K., Cassar, O., & Gessain, A. (2016). Human T-Lymphotropic Virus type 1 infection in an Indigenous Australian population : epidemiological insights from a hospital-based cohort study. *BMC Public Health*, 1–11. https://doi.org/10.1186/s12889-016-3366-5

Ekem, I., & Dei-Adomakoh, Y. A. (2015). Age and Sex Distribution of Leukemia in Patients Seen at a Tertiary Hospital in Ghana. *Jacobs Journal of Haematology*, *1*(4), 4–7.

University of Ghana http://ugspace.ug.edu.gh

Fani, M., Rezayi, M., Meshkat, Z., Rezaee, S. A., Makvandi, M., Abouzari- Lotf, E., & Ferns, G. A. (2019). Current approaches for detection of human T - lymphotropic virus Type 1 : A systematic review. *Journal of Cellular Physiology*, *234*, 12433–12441. https://doi.org/10.1002/jcp.28087

Fox, J. M., Mutalima, N., Molyneux, E., Carpenter, L. M., Taylor, G. P., Bland, M., … Martin, F. (2016). Seroprevalence of HTLV-1 and HTLV-2 amongst mothers and children in Malawi within the context of a systematic review and meta-analysis of HTLV seroprevalence in Africa. *Tropical Medicine and International Health*, *21*(3), 312–324. https://doi.org/10.1111/tmi.12659

Furuta, R., Yasunaga, J., Miura, M., Sugata, K., Saito, A., Akari, H., … Matsuoka, M. (2017). Human T-cell leukemia virus type 1 infects multiple lineage hematopoietic cells in vivo. *PLOS Pathogens*, *13*(11), 1–22. Retrieved from https://doi.org/10.1371/journal.ppat.1006722

Futsch, N., Mahieux, R., & Dutartre, H. (2018). HTLV-1, the other pathogenic yet neglected human retrovirus: From transmission to therapeutic treatment. *Viruses*, *10*(1), 1–25. https://doi.org/10.3390/v10010001

Gallo, R. C. (2005). The discovery of the first human retrovirus : HTLV-1 and HTLV-2. *Retrovirology*, *7*, 1–7. https://doi.org/10.1186/1742-4690-2-17

Gessain, A., & Cassar, O. (2012). Epidemiological Aspects and World Distribution of HTLV-1 Infection. *Frontiers in Microbiology*, *3*, 388. https://doi.org/10.3389/fmicb.2012.00388

University of Ghana http://ugspace.ug.edu.gh

Gonçalves, D. U., Proietti, F. A., Ribas, J. G. R., Araújo, M. G., Pinheiro, S. R., Guedes, A. C., & Carneiro-Proietti, A. B. F. (2010). Epidemiology, treatment, and prevention of human T-cell leukemia virus type 1-associated diseases. *Clinical Microbiology Reviews*, *23*(3), 577–589. https://doi.org/10.1128/CMR.00063-09

Grogg, K. L., Miller, R. F., & Dogan, A. (2007). HIV infection and lymphoma. *Journal of Clinical Pathology*, *60*(3), 1365–1372. https://doi.org/10.1136/jcp.2007.051953

Gross, C., & Thoma-kress, A. K. (2016). Molecular Mechanisms of HTLV-1 Cell-to-Cell Transmission. *Viruses*, *8*(74), 1–22. https://doi.org/10.3390/v8030074

Hewagama, S., Krishnaswamy, S., King, L., Davis, J., & Baird, R. (2014). Human T-Cell Lymphotropic Virus Type 1 Exposures Following Blood-borne Virus Incidents in Central Australia, 2002–2012. *Clinical Infectious Diseases: An Official Publication of the Infectious Diseases Society of America*, *59*(1), 85–87. https://doi.org/10.1093/cid/ciu227

Hoffbrand, A. V., & Moss, P. A. H. (2016). *Hoffbrand's Essential Haematology* (7th editio). Chichester, West Sussex, UK: Wiley Blackwell.

Jalaeikhoo Hasan, Mosayeb Soleymani, Mohsen Rajaeinejad, M. K. (2017). Prevalence of Human T-lymphotropic virus type 1 (HTLV-1) Infection in Patients with Hematologic Disorders and Non-Hematologic Malignancies in a Tertiary Referral Hospital. *Archives of Iranian Medicine*, *20*(4), 224–228.

Johnson, J. M., Harrod, R., & Franchini, G. (2001). Molecular biology and pathogenesis of the human T-cell leukaemia/lymphotropic virus Type-1 (HTLV-1). *International Journal of Experimental Pathology*, *82*(3), 135–147. Retrieved from http://10.0.4.22/j.1365-2613.2001.00191.x

University of Ghana http://ugspace.ug.edu.gh

Karimi, A., Nafici, M., & Imani, R. (2007). Comparison of Human T- cell Leukemia Virus Type-1 (HTLV-1) Seroprevalence in High Risk Patients (Thalassemia and Hemodialysis) and Healthy Individuals from Charmahal - Bakhtiari Province , Iran. *Kuwait Medical Journal*, *39*(3), 259–261.

Kchour, G., Tarhini, M., Kooshyar, M., Hajj, H. El, Wattel, E., Mahmoudi, M., … The, H. De. (2009). Phase 2 study of the efficacy and safety of the combination of arsenic trioxide , interferon alpha , and zidovudine in newly diagnosed chronic adult T-cell leukemia / lymphoma (ATL). *BLOOD*, *113*(26), 6528–6532. https://doi.org/10.1182/blood-2009-03-211821.

Mahieux, R., & Gessain, A. (2003). HTLV-1 and associated adult T-cell leukemia/lymphoma. *Reviews in Clinical & Experimental Hematology*, *7*(4), 336–361. Retrieved from http://search.ebscohost.com/login.aspx?direct=true&db=a9h&AN=12552007&site=ehost-live

Manns, A., Murphy, E. L., Wilks, R., Haynes, G., Figueroa, J. P., Hanchard, B., … Blattner, W. A. (1991). Detection of Early Human T-cell Lymphotropic Virus Type I Antibody Patterns During Seroconversion Among Transfusion Recipients. *Blood*, *77*(4), 896–905.

Marçais, A., Suarez, F., Sibon, D., Bazarbachi, A., & Hermine, O. (2012). Clinical Trials of Adult T-Cell Leukaemia / Lymphoma Treatment. *Leukemia Research and Treatment*, *2012*, 1–8. https://doi.org/10.1155/2012/932175

Martin, J. L., Maldonado, J. O., Mueller, J. D., Zhang, W., & Mansky, L. M. (2016). Molecular Studies of HTLV-1 Replication : An Update. *Viruses*, *8*(31), 1–22. https://doi.org/10.3390/v8020031

University of Ghana http://ugspace.ug.edu.gh

Martinez, M. P., Saleem, J. Al, & Green, P. L. (2019). Comparative virology of HTLV‐1 and HTLV‐2. *Retrovirology*, *16*(21), 1–12. https://doi.org/10.1186/s12977-019-0483-0

Matsuoka, M., & Jeang, K. T. (2011). Human T-cell leukemia virus type 1 (HTLV-1) and leukemic transformation: Viral infectivity, Tax, HBZ and therapy. *Oncogene*, *30*(12), 1379–1389. https://doi.org/10.1038/onc.2010.537

Matsuoka, Masao. (2005). Human T-cell leukemia virus type I (HTLV-I) infection and the onset of adult T-cell leukemia (ATL). *Retrovirology*, *2*, 1–13. https://doi.org/10.1186/1742-4690-2-27

Matsuoka, Masao, & Gallo, R. (2019). 40 years of the human T-cell leukemia virus : past , present , and future [version 1 ; peer review : 2 approved] Yutaka Tagaya. *F1000Research*, *8*(228), 1–14.

Matutes, E. (2007). Adult T-cell leukaemia/lymphoma. *Journal of Clinical Pathology*, *60*, 1373–1377. https://doi.org/10.1136/jcp.2007.052456

Mazurov, D., Ilinskaya, A., Heidecker, G., Lloyd, P., & Derse, D. (2010). Quantitative Comparison of HTLV-1 and HIV-1 Cell-to- Cell Infection with New Replication Dependent Vectors. *PLoS Pathogens*, *6*(2), 1–11. https://doi.org/10.1371/journal.ppat.1000788

Mesnard, J. M., Barbeau, B., Césaire, R., & Péloponèse, J. M. (2015). Roles of HTLV-1 basic Zip factor (HBZ) in viral chronicity and leukemic transformation. potential new therapeutic approaches to prevent and treat HTLV-1-related diseases. *Viruses*, *7*(12), 6490–6505. https://doi.org/10.3390/v7122952

University of Ghana http://ugspace.ug.edu.gh

Monavari, S. H., Keyvani, H., Mollaie, H., Fazlalipour, M., Sadeghi, F., Salehi-Vaziri, M., … Bokharaei-Salim, F. (2011). Detection of human T-cell lymphotropic virus Type-1 among patients with malignant hematological diseases in Capital of Iran , Tehran. *Journal of General and Molecular Virology*, *3*(5), 67–70. https://doi.org/10.5897/JGMV11.021

Murphy, E. L. (2016). Infection with human T-lymphotropic virus types-1 and -2 (HTLV-1 and -2): Implications for blood transfusion safety. *Transfus Clin Biol*, *23*(1), 13–19. https://doi.org/10.1016/j.tracli.2015.12.001

Paiva, A. M., Assone, T., Haziot, M. E. J., Smid, J., M, L. A., Luiz, C., … Casseb, J. (2018). Risk factors associated with HTLV-1 vertical transmission in Brazil : longer breastfeeding , higher maternal proviral load and previous HTLV-1-infected offspring. *Scientific Reports*, *8*, 8–13. https://doi.org/10.1038/s41598-018-25939-y

Paola Miyazato, Misaki Matsuo, H. K. and Y. S. (2016). Transcriptional and Epigenetic Regulatory Mechanisms Affecting HTLV-1 Provirus. *Viruses*, *8*(171), 1–14. https://doi.org/10.3390/v8060171

Pique, C., & Jones, K. S. (2012). Pathways of cell – cell transmission of HTLV-1. *Frontiers in Microbiology*, *3*(378), 1–14. https://doi.org/10.3389/fmicb.2012.00378

Rafatpanah, H., Farid, R., Golanbar, G., & Azad, F. J. (2006). HTLV-I infection: Virus structure, immune response to the virus and genetic association studies in HTLV-I-infected individuals. *Iranian Journal of Allergy, Asthma and Immunology*, *5*(4), 153–166. https://doi.org/05.04/ijaai.153166

University of Ghana http://ugspace.ug.edu.gh

Rende, F., Cavallari, I., Romanelli, M. G., Diani, E., Bertazzoni, U., & Ciminale, V. (2012). Comparison of the Genetic Organization , Expression Strategies and Oncogenic Potential of HTLV-1 and HTLV-2. *Leukemia Research and Treatment*, 14. https://doi.org/10.1155/2012/876153

Sachse, M., Guadagnini, S., Robbiati, V., & Lasserre, R. (2009). Biofilm-like extracellular viral assemblies mediate HTLV-1 cell-to-cell transmission at virological synapses. *Nature Medicine*, *16*(1), 83–89. https://doi.org/10.1038/nm.2065

Sarkodie, F., Adarkwa, M., Adu-Sarkodie, Y., Candotti, D., Acheampong, J. W., & Allain, J. P. (2001). Screening for viral markers in volunteer and replacement blood donors in West Africa. *Vox Sanguinis*, *80*(3), 142–147. Retrieved from http://search.ebscohost.com/login.aspx?direct=true&db=a9h&AN=4538761&site=ehost-live

Satou, Y., & Matsuoka, M. (2012). Molecular and Cellular Mechanism of Leukemogenesis of ATL : Emergent Evidence of a Significant Role for HBZ in HTLV-1-Induced Pathogenesis. *Leukemia Research and Treatment*, *8*(2), 1–8. https://doi.org/10.1155/2012/213653

Shannon-lowe, C., Rickinson, A. B., & Bell, A. I. (2017). Epstein – Barr virus-associated lymphomas. *Philosophical Transactions Royal Society B*, *372*(20160271), 1–15.

Switzer, W. M., Heneine, W., & Owen, S. M. (2015). Human T-Cell Lymphotropic Viruses. In J. H. James & P. A. Michael (Eds.), *Manual of Clinical Microbiology* (11th editi, pp. 1458–1470). Washington: American Society for Microbiology Press.

University of Ghana http://ugspace.ug.edu.gh

Tarokhian, H., Rahimi, H., Mosavat, A., Shirdel, A., Rafatpanah, H., Akbarin, M. M., … Rezaee, S. A. (2018). HTLV-1-host interactions on the development of adult T cell leukemia/lymphoma: Virus and host gene expressions. *BMC Cancer*, *18*(1), 1–12. https://doi.org/10.1186/s12885-018-5209-5

Taylor, G. P., & Matsuoka, M. (2005). Natural history of adult T-cell leukemia / lymphoma and approaches to therapy Natural history of adult T-cell leukemia / lymphoma and approaches to therapy. *Oncogene*, *24*, 6047–6057. https://doi.org/10.1038/sj.onc.1208979

Tienen, C. Van, Silva, T. I. De, Carlos, L., Alcantara, J., Onyango, C. O., Vincent, T., … Cotten, M. (2012). Molecular Epidemiology of Endemic Human T- Lymphotropic Virus Type 1 in a Rural Community in. *PLoS Negl Trop Dis*, *6*(6), 1–9. https://doi.org/10.1371/journal.pntd.0001690

Verdonck, K., González, E., Dooren, S. Van, Vandamme, A., Vanham, G., & Gotuzzo, E. (2007). Human T-lymphotropic virus 1: recent knowledge about an ancient infection. *Lancet Infect Dis*, *7*, 266–281.

Vernin, C., Thenoz, M., Pinatel, C., Gessain, A., Gout, O., Delfau-Larue, M.-H., … Mortreux, F. (2014). HTLV-1 bZIP Factor HBZ Promotes Cell Proliferation and Genetic Instability by Activating OncomiRs. *Cancer Research*, *74*(21), 6082–6094. https://doi.org/10.1158/0008-5472.CAN-13-3564

Weatherhead, J. E., & Mejia, R. (2014). Immune Response to Infection with Strongyloides stercoralis in Patients with Infection and Hyperinfection. *Current Tropical Medicine Rep*, *1*, 229–233. https://doi.org/10.1007/s40475-014-0032-9

University of Ghana http://ugspace.ug.edu.gh

Wolfe, N. D., Heneine, W., Carr, J. K., Garcia, A. D., Shanmugam, V., Tamoufe, U., … Switzer, W. M. (2005). Emergence of unique primate T-lymphotropic viruses among central African bushmeat hunters. *PNAS*, *102*(22), 7994–7999.

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APPENDICES

APPENDIX I

ETHICAL APPROVAL FROM THE COLLEGE OF HEALTH SCIENCES, UNIVERSITY OF GHANA

UNIVERSITY OF GHANA
COLLEGE OF HEALTH SCIENCES
ETHICAL AND PROTOCOL REVIEW COMMITTEE

Ref. No.: EPRC/APRIL/2019 April 3, 2019

Frank Awuku
Department of Haematology
School of Biomedical and Allied Health Sciences
Korle-Bu

ETHICAL CLEARANCE
Protocol Identification Number: ***CHS-Et/M.8 – 5.16/2018-2019***

FWA: 000185779 **IORG: 0005170** **IRB: 00006220**

The College of Health Sciences Ethical and Protocol Review Committee (EPRC) at its March 28, 2019 full board meeting reviewed and approved your re-submitted research protocol.

Title of Protocol: "The Role of Human T-Lymphotropic Virus Type 1 (HTLV-1) Infection in Haematological Malignancies"

Principal Investigator: Mr. Frank Awuku

This approval requires that you submit six-monthly review report(s) of the study to the Committee and a final full review report to the EPRC at the completion of the study. The Committee may observe, or cause to be observed, procedures and records of the study before, during and after implementation.

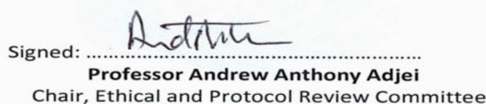
Please note that any significant modification(s) to this project/study must be submitted to the Committee for review and approval before its implementation.

You are required to report all serious adverse events related to this study to the EPRC within seven (7) days verbally and fourteen (14) days in writing.

As part of the review process, it is the Committee's duty to review the ethical aspects of any manuscript that may be produced from this study. You will therefore be required to furnish the Committee with any manuscript for publication.

This ethical clearance is valid till April 4, 2020.

Please always quote the protocol identification number in all future correspondence in relation to this protocol.



Signed: ....
Professor Andrew Anthony Adjei
Chair, Ethical and Protocol Review Committee

cc: Provost, CHS
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Head, Department of Haematology

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APPENDIX II

INFORMATION AND CONSENT FORM

TITLE: **THE ROLE OF HUMAN T-CELL LYMPHOTROPIC VIRUS TYPE1 (HTLV-1) INFECTIONS IN HAEMATOLOGICAL MALIGNANCIES**

INVESTIGATOR: Awuku Frank

INSTITUTION: University of Ghana

College of Health Sciences

School of Biomedical and Allied Health Sciences

Department of Haematology

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Introduction: There is a significant association of Human T-Cell Lymphotropic Virus type 1 (HTLV-1) with haematological malignancies. HTLV 1 causes a lymphoproliferative malignancy of CD4-activated cells called Adult T-Cell Leukaemia/Lymphoma (ATLL) and a chronic myelopathy called HTLV-1 associated Myelopathy/Tropical Spastic Paraparesis (HAM/TSP). This study aims to determine the seroprevalence of HTLV as well as determining the association between HTLV-1 infection and previous history of blood transfusion and evaluate the response to treatment of HTLV-1 seropositive patients with haematological malignancies at Korle Bu Teaching Hospital, Accra. This study aims at providing a better understanding of the association between haematological malignancies and HTLV-1 infection in Ghana.

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In this study, I will require 5 millimetres of venous blood from consented participants which will be collected by venipuncture. Other information on clinical and laboratory data would be abstracted from your folders.

Risk or Discomfort

No harm is expected to occur in the course of the study. However, you may experience or feel pain at the site where the blood sample will be taken. All procedure pertaining to the sample taken will be performed under aseptic conditions to prevent infection.

Benefit

This study may not benefit you by improving or making your medical conditions better. It may benefit patients in future by improving the clinical and laboratory workup of patients with various haematological malignancies.

Confidentiality

Information that will be collected from you will be used solely for the study. Your name and given information that makes it possible for you to be identified will not be used in the writing or reporting of the study. All data abstraction forms will be kept in locked file. Blood samples taken from you will be coded. I, Awuku Frank (the investigator) would hold confidential any information I happen to learn about the patient during the course of this study regarding the health status, drug use and others. Under no circumstances would the information be revealed to a third party.

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Future Use of Biological Specimen

You will be asked if we could store the leftover of your specimen for future studies and testing. Your name will not be recorded on the specimen. You can still participate if you do not want your specimen to be stored.

Voluntary Participation and Right to Leave the Study

Participation in the study is exclusively voluntary. You are at liberty to leave at any time or decide not to answer any question. If you decide not to be part of the study, it will not affect you or negatively influence your course of medical care to be rendered to you or your ward.

Your Right as a Participant

The proposal has been reviewed and approved by the Ethical and Protocol review Committee of the College of Health Sciences of University of Ghana. You can forward your concerns about your right as participant to the study to the chairperson of the committee Prof. Andreaws Adjei (tel:0543065223).

Contacts

If you have any questions about this study or study-related problems, you may contact me the principal investigator, Awuku Frank (tel:0242925994) or Dr. Yvonne Dei-Adomakoh (tel:0243550980) head of Haematology Department, Korle-Bu Teaching Hospital will be responsible for your welfare while you are participating in this study. You may also contact Dr. Amma Benneh (tel:0244365283) Haematology Department, Korle-Bu teaching Hospital for any further questions or clarification on this study. You are free to ask any questions. Thank you.

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APPENDIX III

VOLUNTEER AGREEMENT

The above document describing the benefits, possible risks and procedures for this study entitled **"The role of Human T-Cell Lymphotropic Virus type1 (HTLV-1) infections in haematological malignancies."** has been read and explained to me. I have been given opportunity to ask any question about the research and have been answered to my satisfaction. I willingly agree to participate in this study

_______________________ _______________________________

Date Name and signature or mark of volunteer

If volunteers cannot read the form themselves, a witness must sign here:

I was present while the benefits, risks and procedures were read to the volunteer. All questions were answered and the volunteer has agreed to be part in the study.

_______________________ _____________________________

Date Name and signature of witness

I certify that the nature and purpose, the potential benefits, and possible risks associated with participating in this research have been explained to the above individual.

_______________________ _______________________________________

Date Name Signature of Person Who Obtained Consent

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APPENDIX IV

DATA ABSTRACTION FORM

Personal information	
Study no:	Date:
Name:	Folder no:
Address:	Telephone no:
Age:	Gender: male / female
Occupation:	
Marital status: single divorced/separated widowed	
Highest education: primary secondary tertiary	
Clinical data	
Diagnosis: Age at diagnosis: Date of diagnosis:	
clinical presentation:	
Disease Outcome: A. Clinical remission B. Relapse	
Received blood-product transfusions: YES NO Total units received:	
Laboratory data	
Hb, g/dl:	HCT, %:
TWBC, x10^9/L:	Eosinophils, x10^9/L:
Lymphocytes, x10^9/L:	Platelets, x10^9/L:
Lactate dehydrogenase (LDH): A. Normal B. Abnormal	
LFT: A. Normal B. Abnormal	

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APPENDIX V

DETERMINATION OF CUT OF POINT

CALCULATION OF RESULTS

1.0 Calculation of Non-Reactive Control Mean Absorbance (NRCx)

Well No.	Absorbance
B1	0.004
C1	0.004
D1	0.006
Total	0.014
Mean	= 0.014 / 3 = 0.005

1.0 Calculation of Reactive Control Mean Absorbance (RCx)

Well No.	Absorbance
E1	1.471
F1	1.399
G1	1.412
Total	4.282
Mean	=4.282 / 3 = 1.427

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3.0 Calculation of CUT - OFF value (COV)

CUT - OFF Value = 0.250 + NRCx

NRCx = 0.005

CUT - OFF Value = 0.250 + 0.005 = 0.255

ASSAY VALIDATION

Individual Non-Reactive Control absorbance values should be ≤ 0.100 unit.

Individual Reactive Control absorbance values must be ≥ 0.600 unit.

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APPENDIX VI

MANUFACTURER'S INSTRUCTIONS FOR INTERPRETATION OF ELISA RESULTS

- Specimens with absorbance values less than the CUT-OFF value are considered Non-Reactive.
- Specimens with absorbance values greater than or equal to the CUT-OFF value are considered initially reactive and should be retested in duplicate before interpretation.
- Specimens found Reactive on retesting are interpreted to be repeatedly reactive for antibodies to HTLV.
- Initially reactive specimens which are Non-Reactive on retesting are considered negative.

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APPENDIX VII

DEMOGRAPHIC CHARACTERISTICS OF RESPONDENTS

Variables	Frequency	Percent (%)
Age group (years)		
<20	10	5.0
20-29	21	10.5
30-39	32	16.0
40-49	37	18.5
50-59	39	19.5
≥60	61	30.5
Total	200	100.0
Gender		
Female	109	54.5
Male	91	45.5
Total	200	100.0
Occupation		
Employed	127	63.5
Unemployed	11	5.5
Student	24	12.0
Retired	38	19.0
Total	200	100
Marital Status		
Married	119	59.5
Single	51	25.5
Widowed	30	15.0
Total	200	100.0
Highest education		
No education	27	13.5
Primary	19	9.5
Secondary	81	40.5
Tertiary	73	36.5
Total	200	100.0

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APPENDIX VIII

PREVIOUS HISTORY OF BLOOD TRANSFUSION AND HTLV-1 INFECTION

	HTLV ELISA RESULTS				
	NON-REACTIVE	REACTIVE	Total	χ^2	p-value
Blood Transfusion					
No	121 (93.8)	8 (6.2)	129 (100.0)	0.356	0.551
Yes	65 (91.5)	6 (8.5)	71 (100.0)		
Total	186 (93.0)	14 (7.0)	200 (100.0)		

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APPENDIX IX

HAEMATOLOGICAL CHARACTERISTICS OF RESPONDENTS

	N	Minimum	Maximum	Mean	Std. Deviation
HB, g/dl	200	4.7	17.3	10.26	2.67
HCT, %	200	13.9	54	30.65	7.60
TWBC, $x10^9/L$	200	1.4	279.3	18.33	36.88
LYM, $x10^9/L$	200	0.15	212.7	10.68	28.27
PLT, $x10^9/L$	200	19	1775	264.14	181.20

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APPENDIX X

OPTICAL DENSITIES OF HTLV-1 POSITIVE CASES

HAEMATOLOGICAL MALIGNANCIES	OPTICAL DENSITY (OD)	OPTICAL DENSITY RATIO
Non Hodgkin Lymphoma	0.508	1.972
Non Hodgkin Lymphoma	0.695	2.725
Non Hodgkin Lymphoma	0.805	3.157
Non Hodgkin Lymphoma	1.364	5.349
Non Hodgkin Lymphoma	3.136	12.298
Chronic Myeloid Leukaemia	0.280	1.098
Chronic Myeloid Leukaemia	0.692	2.714
Chronic Myeloid Leukaemia	2.000	7.843
Chronic Myeloid Leukaemia	3.598	14.110
Multiple Myeloma	1.314	5.153
Multiple Myeloma	3.500	13.725
Myelodysplastic Syndrome	2.694	10.564
Myelodysplastic Syndrome	3.598	14.110
Chronic Lymphocytic Lymphoma	3.010	11.804