SEROPREVALENCE OF HUMAN T-CELL LYMPHOTROPHIC VIRUS AND ITS ASSOCIATION WITH WILMS TUMOUR ANTIGEN AMONG WOMEN ATTENDING POST-NATAL CLINICS IN ZARIA NIGERIA.

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JULY, 2018

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DEPARTMENT OF MICROBIOLOGY,

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DECLARATION

I declare that the work in this dissertation entitled **"Seroprevalence of Human T-Cell Lymphotrophic Virus and its Association with Wilms Tumour Antigen among Women Attending Post-Natal Clinics in Zaria, Nigeria"** has been carried out by me in the Department of Microbiology, Ahmadu Bello University under the supervision of Dr. E. E. Ella and Prof. M. Aminu. The information derived from the literature has been duly acknowledged in the text and a list of references provided. No part of this dissertation was previously presented for another degree or diploma at this or any other Institution.

Hyelakidati Samuel HANANIYA …… ……

Signature Date

CERTIFICATION

This dissertation entitled **"Seroprevalence of Human T-Cell Lymphotrophic Virus and its Association with Wilms Tumour Antigen among Women Attending Post-Natal Clinics in Zaria, Nigeria"** by Hyelakidati Samuel HANANIYA meets the regulations governing the award of the degree of Masters of Science in the Department of Microbiology, Ahmadu Bello University, Zaria and it has been approved for its contributions to knowledge.

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DEDICATION

This work is dedicated to God Almighty for His mercy, love and grace.

ABSTRACT

Human T-cell lymphotrophic virus (HTLV) is reported to be associated with Adult T-cell leukaemia (ATL) and a spectrum of inflammatory diseases which mainly include HTLV-associated Myelopathy/ Tropical Spastic Paraparesis, (HAM/STP). Adult T-cell leukaemia like every other cancer present with variety of tumour markers such as overproliferation of Wilms tumour antigen. This study was aimed at determining the seroprevalence of HTLV and its association with Wilms tumour antigen among women attending post-natal clinics in Zaria, Nigeria. Blood samples were collected from 190 consenting women and each was analysed for HTLV antibodies and Wilms tumour antigen using ELISA. White blood cell, lymphocyte and platelet counts were measured using an automated haematological analyzer. At the end of the assay the seroprevalence of anti-HTLV antibodies (IgG) was found to be 3.2% (6/190). Results of analysis of anti-HTLV antibodies based on some demographic and risks factors showed that only history of previous transfusion was significantly associated with HTLV infection (p=0.010*).* The Wilms tumour antigen ELISA results showed that the HTLV positive women had a higher mean Wilms tumour antigen concentration of 116.22±18.39pg/ml compared to the HTLV negative women (66.41±20.13pg/ml). The difference was statistically significant (p=0.00). Haematological analysis showed a non-significant increase (p=0.213, 0.305) in the mean concentration of white blood cell (HTLV positive=6.98±0.132 10^9 cells/L, HTLV negative=6.85±0.93 10^9 cells/L) and lymphocyte count (HTLV positive=2.92±1.114 10^9 cells/L, HTLV negative=2.52±0.93 10^9 cells/L) but showed a significant increase (p<0.017) in the mean platelet count(HTLV positive=249.5±80.35 10^9 cells/L, HTLV negative=178.8±70.45 cells/L) in infected women. Thus the study showed that HTLV is circulating among the women in the study area with high concentration of Wilms tumour antigen among the positive women. In addition, blood transfusion was a risk factor for HTLV infection. Hence it is recommended that screening of blood for HTLV be incorporated in the routine test for blood donors.

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LIST OF ABBREVIATIONS

AML: Acute Myeloid Leukaemia

ATL: Adult T-cell Leukaemia

ATLL: Adult T-cell leukaemia/lymphoma

DDS: Denys–Drash Syndrome

ELISA: Enzyme Linked Immunosurbent Assay

HAM/TSP: HTLV-1 associated myelopathy/tropical spastic paraparesis

HAU: Human T-cell Lymphotrophic Virus Associated Uveities

HBZ: HTLV-1 Basic Leucin Zipper Factor

HIV: Human Immunodeficiency Virus

HTLV: Human T-Lymphotrophic Virus or Human T-cell Lymphotrophic Virus

IFN: Interferon

LTR: Long Terminal Repeats

MDS: Myelodysplastic Syndromes

MIP: Macrophage Inflammatory Protein

MTCT: Mother to Child Transmission

Tax: Transactivator x Protein

WT: Wilms' Tumour

WT1: Wilms Tumour Gene

CHAPTER ONE

1.0 INTRODUCTION

1.1 Background to the Study

The Human T-cell lymphotropic viruses or Human T-lymphotrophic viruses (HTLV) belong to the *Retroviridae* family, genus Deltaretrovirus. They are classified into four types: 1, 2, 3 and 4 (Kannian and Green, 2010). The HTLV-1 was described in 1980 by Poiez *et al*. (1980) and since then it has been reported in all the five continents, with an estimated 15 to 20 million infected people worldwide (Qayyum and Choi, 2014). The virus is considered as endemic in countries where the prevalence ranges from 0.5 to 20% in the population, depending on age and gender, and non-endemic, where the prevalence is less than 0.1% (Proietti *et al*., 2005). The sero-prevalence increases with age and is higher in females than males (Verdonck *et al*., 2007). Areas of high prevalence for HTLV-1 include Japan, sub-Saharan Africa, and the Caribbean basin, South America, Melanesia and the Middle East. The HTLV-2 was described in 1982 by Kalyanaraman *et al*. (1982) and it is endemic in African and Ameridian populations, but its worldwide distribution has been ascribed to transmission among intravenous drug users. HTLV Types 3 and 4 were discovered in a rural area of southern Cameroon (Wolfe *et al*., 2005; Mahieux and Gessain, 2011). However, only HTLV-1 has been convincingly linked to human diseases at present (Goncalves *et al*., 2010). Human T-cell lymphotropic virus Types 1 and 2 are closely related yet distinct human retroviruses that share approximately 70% of their overall nucleotide sequence (Gallo, 2005).

Human T-cell lymphotropic virus type 1 has been shown to be associated with at least two well-defined clinical syndromes, namely Adult T-cell leukaemia/lymphoma (ATLL) and HTLV-1 associated myelopathy/tropical spastic paraparesis (Costa *et al*., 1995; Blattner, 2009). Human T-

cell lymphotropic virus type 2 was initially isolated from a patient with hairy cell leukaemia but its pathogenicity is not clearly understood (Shimotohno *et al*., 1984). HTLV type 2 has also been linked with other neurological syndromes (Roucoux and Murphy, 2004). Adult T-cell leukaemia/lymphoma (ATLL) has 4 subtypes: acute, lymphomatous, chronic, and smoldering. Clinically, the first 2 variants are classified as aggressive, and the latter two are classified as indolent (Qayyum and Choi, 2014). The overwhelming majority of ATLL cases occur in patients infected during the early years of life, presumably because of a less efficient immune response in this age group. In addition, the prolonged infection may increase chances of acquiring subsequent mutations, and ultimately malignant transformation (Bangham and Toulza , 2011).

Infection with HTLV-1/2 can be transmitted by vertical route (mother –to-child), breast milk, sexual intercourse and parenteral routes (blood transfusion and intravenous drug use). In Nigeria, transmission of HTLV infection via blood transfusion resulting in leukaemia/lymphoma is well documented (Kaplan and Khabbaz, 1993; Re *et al*., 2004). Transmission via breastmilk has been also documented and associated with duration of breastfeeding and maternal history of transfusion (Luiz *et al*., 2010). The viral transmission rate from infected mother to child is approximately 20% and depends on HTLV-1 proviral load, duration of breast feeding, and maternal-foetal concordant human leukocyte antigen (HLA) class I type (Goncalves *et al*., 2010; Journo and Mahieux, 2011). Interestingly, breast feeding is more correlated with ATLL, and blood transfusion is more correlated with HTLV-1–associated myelopathy/tropical spastic paraparesis (Goncalves *et al*., 2010). Vertical transmission is also possible via other routes, probably intrauterine or perinatal, although it is less common (Luiz *et al*., 2010).

Wilms tumour gene (WT1) is expressed at high levels in haematopoietic malignancies, such as leukaemias and myelodysplastic syndromes (MDS), and in various kinds of solid tumours,

including lung cancer, and it exerts an oncogenic function in these malignancies (Olga *et al*., 2002). Wilms tumour gene was initially discovered as a tumour suppressor in Wilms' Tumour (WT), a paediatric kidney malignancy that affects approximately 1:10, 000 children (Little, 1997). Mutations of WT1 associated with WT are found almost exclusively in the sporadic form of the disease at a low frequency (15%) (Little, 1997). The study of WT1's involvement in malignant cells by Yang *et al.* (2007) has unexpectedly revealed a potential role for WT1 as an oncogene.

1.2 Statement of the Research Problem

Infections of HTLV-1 and HTLV-2 are life-long with high asymptomatic carrier state (Ahmadi *et al*., 2013). Over 20 million persons are infected with HTLV-1 and HTLV-2 globally with varying levels of sero-prevalence reported in almost every region of the world (Adekunle *et al*., 2015). Recent studies by Abu *et al.* (2014) showed a high sero-prevalence of 14.3% among pregnant women in Zaria. Human T-cell lymphotrophic virus causes leukaemia, also known as cancer of the blood and a malignancy that is dreaded by many humans in the natural setting. In developing countries leukaemia is seen as a death sentence as the management is still poor and survival rate remains low. It must also be noted that knowledge about the virus and its mode of transmission is low, being a retrovirus and sharing similar transmission route with HIV.

The increase in people indulging in multiple sexual activities expose a great number to the virus since it is not routinely screened for as with HIV. Therefore sex with HIV negative individual considered safe may transmit the virus in the population. The issue of exclusive breastfeeding is welcomed by most and the danger of spread via breastfeeding has not been considered. Mothers are not screened for the virus as to advice on the danger of transmission from mother to child. It

has been shown by Namen-Lopes *et al.* (2009) that approximately 25% of infant breastfed by HTLV-1 seropositive mothers acquire the infection.

Overexpression of Wilms tumour gene was shown to be required for the uncontrolled proliferation and defective differentiation of acute leukaemia cells, suggesting involvement of WT1 in leukaemogenesis (Van Driessche *et al*., 2005).

1.3 Justification of the Study

Identifying high risk groups remains one of the greatest ways to help arrest the spread of the virus thereby reducing its prevalence.

This research would create awareness among nursing mothers and eventually to the general public on HTLV and its association with leukaemia with consequent reduction in prevalence because most women are screened for HIV and Hepatitis B during anti-natal period to safeguard the health of the child upon delivery. The screening for HTLV is not routinely done. However it could provide data for care givers and law makers on the prevalence of HTLV and the need to screen mothers for HTLV infection during anti-natal or post-natal before breastfeeding commences.

Wilms tumour antigen can serve as a marker for disease burden and diagnosis of minimal residual disease. The limited expression of Wilms Tumour antigen in normal tissues in adults suggests that Wilms Tumour antigen can be an immunological target for leukaemia diagnosis and therapy.

1.4 Aim of the Study

The aim of the study was to determine the sero-prevalence of human T-cell lymphotrophic virus antibodies and its association with Wilms tumour antigen among women attending post natal clinics in Zaria, Nigeria.

1.5 Objectives of the Study

The objectives of the study were to:

1. determine the prevalence of human T-cell lymphotrophic virus antibodies among women attending post natal clinics in Zaria using ELISA.
2. determine the risk factors associated with human T-cell lymphotrophic virus using structured questionnaire.
3. determine the level of Wilms tumour antigen and its association with human T-cell lymphotrophic virus infection among women attending post natal clinics in Zaria using ELISA technique.
4. To investigate some haematological indices that might be associated with T-cell viral infections.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Viruses

Viruses are known to be abundant, ubiquitous, and to play a very important role in the health and evolution of life organisms. However, most biologists have considered them as entities separate from the realm of life and acting merely as mechanical artifacts that can exchange genes between different organisms (Herrero-Uribe, 2011). “Viruses are Viruses”, Lwoff made this most famous definition of viruses in 1957 (Lwoff, 1957), who also wrote that the definition of a virus is somewhat arbitrary, and many definitions have been offered, a proof of the difficulty of the task. He proposed the definition of viruses as strictly intracellular and potentially pathogenic entities, with an infectious phase, and possessing only one type of nucleic acid, multiplying in the form of their genetic material, unable to grow and to undergo binary fission and devoid of a “Lipmann system” (i.e. a system of enzymes for the production of energy) (Herrero-Uribe, 2011). Gelderblom (2008) defined virus as small obligate intracellular parasite, which by definition contain either a RNA or DNA genome surrounded by a protective, virus-coded protein coat. Viruses may be viewed as mobile genetic elements, most probably of cellular origin and characterized by a long co-evolution with the host. For propagation, viruses depend on complex metabolic and biosynthetic machinery of eukaryotic or prokaryotic host cells (Gelderblom, 2008).

A complete virus particle is called a virion. The main function of the virion is to deliver its DNA or RNA genome into the host cell so that the genome can be expressed (transcribed and translated) by the host cell. The viral genome, often with associated basic proteins, is packaged inside a protein coat called a capsid. The nucleic acid-associated protein, called nucleoprotein,

together with the genome, forms the nucleocapsid. In enveloped viruses, the nucleocapsid is surrounded by a lipid bilayer derived from the modified host cell membrane and studded with an outer layer of virus envelope glycoproteins (Gelderblom, 2008).

2.2 Retroviruses

The retroviridae family is divided into seven genera (Overbaugh *et al*., 2001). Retroviridae is a family of enveloped viruses that replicate in a host cell through the process of reverse transcription **(**Qadri *et al*., 2016**).** A retrovirus is a positive-sense, single-stranded RNA virus with a DNA intermediate and, as an obligate parasite, targets a host cell. Once inside the host cell cytoplasm, the virus uses its own reverse transcriptase enzyme to produce DNA from its RNA genome. This new DNA is then incorporated into the host cell genome by an integrase enzyme. The retroviral DNA is referred to as a provirus. The host cell then treats the viral DNA as part of its own genome, translating and transcribing the viral genes along with the cell's own genes, producing the proteins required to assemble new copies of the virus. It is difficult to detect the virus until it has infected the host. At that point, the infection will persist indefinitely (Perez and Nolan, 2001).

Retroviruses exist in two different forms: (i) Exogenous viruses: these bear genetic information necessary for the generation of replication of competent viral particles and transferrable from one organism to another. (ii) Endogenous viruses are vertically transmitted through the germ line of its host and most of them have lost the genetic information to produce viral particles. All infectious retroviral particles show a similar structure with a diameter of about 100nm. The viral membrane of retroviruses is built up mainly by its host cell cytoplasm membrane, associated with the viral envelope proteins. The retroviral genome differ in sizes from 7000 to 12000 base pairs depending on the type of virus (HIV: 9000bp, FeLV-A 8500bp), encoded by a single

stranded RNA consisting of a 5`cap structure and a 3´ polyadenylation signal. Retroviral genomes encode for proteins Gag (group specific antigens), Pol (enzymatic activities) and Env (envelope glycoproteins). More complex retroviruses such as lentiviruses, spumaviruses and HTLV encode additional regulatory and accessory proteins. The coding regions are flanked by regulatory control sequences termed long terminal repeats (LTR) (Lower, 1999). These sequences of repetitions contain three different regions termed U3, R and U5 which are located at the 3´ and the 5´end in the provirus genome with the same orientation. The LTR bears all cis active sequences as well as elements of the promoter and enhancer sequences capable of controlling the gene expression. In addition the LTR´s are essential for the process of reverse transcription and integration of the provirus DNA into the host cell genome. Cellular proteins transactivate the transcription of viral protein sequences mainly by binding to the U3 region (Lower, 1999).

2.3 Human T-Cell Lymphotrophic Virus

The discovery of retroviral reverse transcriptase in 1970, was followed by a flurry of activity, sparked by the "War on Cancer," to identify human cancer retroviruses (Baltimore, 1970, Temin, 1970). This resulted in many false claims resulting from various artifacts but the Gallo laboratory carried on, developing both specific assays and new cell culture methods that enabled them to report in the accompanying 1980 PNAS paper, identification and partial characterization of human T-cell leukemia virus (HTLV; now known as HTLV-1) produced by a T-cell line from a lymphoma patient (Coffin, 2015). This discovery had a number of consequences beyond providing dramatic proof for the provirus theory of retrovirus replication put forward by Temin years earlier and discussed by Vogt (Temin, 1964, Vogt, 1997). It provided an important tool for molecular biologists, enabling them to make DNA copies of RNA at will for studies using

molecular hybridization, and, eventually, cloning, PCR, and sequencing. Secondly, because of its presence in virus particles (virions), RT provided a simple means of detecting and quantitating viruses particularly ones that lacked oncogenes, which often had no visible effect on cell cultures far superior to the cumbersome and insensitive electron microscope techniques then in use (Temin, 1964, Vogt, 1997).

The discovery of the first human retrovirus proceeded rather independently in Japan and the United States. Poiesz *et al.* (1980) identified human T-cell leukaemia virus (HTLV) in a T-cell line from a patient with cutaneous T-cell lymphoma, and in 1982 independently, Yoshida *et al*, also Identified adult T-cell leukaemia virus (ATLV). Soon, HTLV and ATLV were shown to be identical at the sequence level and were named HTLV type 1 (HTLV-1) (Gallo, 2005). After the discovery of HTLV-1, a second human retrovirus HTLV-2 was described (Kalyanaraman *et al*., 1982). Prevalent in Central and West Africa, in native Amerindian populations, in North, Central, and South America, and among cohorts of intravenous drug users in the United States and Europe, HTLV-2 has a similar genome structure and shares approximately 70% nucleotide sequence homology with HTLV-1 (Manns and Blattner, 1991). In 2005, two more related viruses, HTLV-3 and HTLV-4, were reported in central Africa (Mahieux and Gessain, 2009). However, only HTLV-1 has been convincingly linked to human diseases at present (Goncalves *et al.,* 2010).

Human T-cell lymphotrophic virus type-1 has six reported subtypes (subtypes A to F). Diverse studies have been performed on HTLV-1 subtyping but present a minor role in the epidemiological status of the virus (Goncalves *et al.,* 2010). The great majority of infections are caused by the cosmopolitan subtype A, and there is no report of subtype influence on the pathogenic potential of HTLV-1 (Goncalves *et al.,* 2010).

Approximately 20 million people worldwide are estimated to be infected with HTLV-1(Qayyum and Choi, 2014). Similarly 90% of the infected individuals remain asymptomatic carriers during their live time. In reality, the immunopathogenesis of this retrovirus is intriguing, since its lifelong persistence in CD4 lymphocytes is considered to result from a prolonged interaction between the virus and the immune system, and may result in the broad spectrum of diseases associated with HTLV-1. This may be related to the direct action of the virus on the immune system or a consequence of the response of the immune system to the virus. In 1993, HTLV-1 screening of blood donors was already being performed in all developed countries and in many developing countries where HTLV-1 is endemic. Moreover, haemovigilance studies were performed, where patients who had received blood from HTLV-positive donors were traced and tested for the virus (Namen-Lopes *et al*., 2009).

2.3.1 Replication of Human T-cell Lymphotrophic Virus

Human T-cell lymphotrophic virus establishes a chronic infection, usually with a long incubation period between infection and first symptoms of the diseases associated with the virus. The principal tropism is for CD4+T cells, but also can infect other cell types such as CD8+ T cells, dendritic cells, macrophages, nerve cells, and haematologic stem cells (Martins *et al*., 2012).

A number of studies have shown that cell-free infection is poorly efficient compared to cell-to-cell virus transfer (Derse *et al*., 2001; Futsch *et al*., 2018), suggesting that HTLV-1 spread *in-vivo* relies more on a cellular intermediate than on the virion itself. Viral particles produced by HTLV-1 infected T-cells have been shown to cross the epithelium by transcytosis, i.e., the transit of a virion incorporated into a vesicle from the apical to the basal surface of an epithelial cell (Martin-Latil *et al*., 2012). Alternatively, HTLV-1 can also infect an epithelial cell and produce

new virions that are then released from the basal surface (Pique *et al*., 2012). Finally, HTLV-1 infected cells can directly bypass a disrupted mucosa (Pique *et al*., 2012)

Having crossed the epithelial barrier, HTLV-1 infects mucosal immune cells directly or via APCs such as DCs or macrophages (Jones *et al.,* 2008). Cell-to-cell transfer of HTLV-1 virions then potentially involves several non-exclusive mechanisms (Pique *et al*., 2012): a virological synapse (Nejmeddine *et al*., 2009), cellular conduits (Van Prooyen *et al*., 2010), or extracellular viral assemblies (Jones and Green, 2010). Infection of resident cells occurs either in the mucosa or in secondary lymphoid organs (Carpentier *et al*., 2015).

After primary infection, HTLV-1 attempts to expand by colonizing new targets by cell-to-cell transfer, reverse transcription of the viral RNA, integration of the provirus into the chromosome, expression of viral proteins and budding of new virions. Another mode of replication involves mitotic division of a cell containing an integrated provirus since an antiviral immune response is also quickly initiated; the efficacy of the infectious cycle is severely attenuated soon after infection, although likely not completely abrogated later on. On the other side, clonal expansion and cell proliferation also require expression of viral factors such as Tax (Boxus *et al.,* 2012). Survival of infected progeny cells therefore requires silencing of viral expression before immune-mediated destruction (Carpentier *et al*., 2015).

It is considered that during primary infection, the virus has a period of active replication by reverse transcription, but that subsequent proliferation occurs mainly via clonal expansion of infected cells, or by viral sinapse, which transmit viral genome through infected cell-to-uninfected cell via contact (Igakura *et al*., 2003). Therefore, HTLV displays low levels of intra-individual genetic variation unlike other retroviruses (Martins *et al*., 2012).

2.3.2 Geographical distribution of Human T-Cell Lymphotrophic Virus

The human T-lymphotropic virus type 1 (HTLV-1) and type 2 (HTLV-2) although closely related, have different geographical distributions (Gessain and Cassar, 2012), pathogenesis (Murphy *et al.,* 2004; Montanheiro *et al.,* 2008) and clinical manifestations. The geographic distribution of the virus has been studied in the almost 30 years since its initial description, with Japan, Africa, the Caribbean islands, and Central and South America emerging as the areas of high prevalence in the world (Proietti *et al.,* 2005). Human T-lymphotropic virus type 1 (HTLV-1) is found in indigenous people of the Pacific Islands and the Americas, whereas type 2 (HTLV-2) is widely distributed among the indigenous people of the Americas, where it appears to be more prevalent than HTLV-1, and in some tribes in Central Africa (Paiva, and Casseb, 2015).

Japan remains the country where HTLV-1 is most endemic a (Goncalves *et al*., 2010). The estimated prevalence in the general population varies from zero in areas where the virus is not found to selected areas with seroprevalences of up to 37%, such as the southwestern isles of Shikoku, Kyushu, and Okinawa (Goncalves *et al*., 2010). In South America, the virus is present in all countries (Goncalves *et al*., 2010). Data obtained from over 2 million blood donors examined in North America for the period of 2000 to 2009 for both viruses, suggests a prevalence of HTLV in the general population of 0.1% to 0.2% and showed a higher prevalence of HTLV-2 (14.7/100,000) than HTLV-1 (5.1/100,000) in the western and southwestern United States (Chang *et al*., 2014). This outcome could be attributed to endemic foci among American Indians (Cook and Taylor, 2014). A previous study among blood donors had already demonstrated a prevalence of HTLV infection of 0.72/1,000 in New Mexico (most cases of which were attributed to HTLV-2) and, in turn, a higher prevalence among American Indian blood donors (1.0%-1.6%) than among non-Hispanic white donors (0.009%-0.06%)(Hjelle *et*

al., 1991). In Venezuela, HTLV-2 was most reported with a prevalence reaching 61% among Guahibo and Yaruro (Paiva and Casseb, 2015). However in central and southern Bolivia, only HTLV-1 was detected among the Quechua and Aymara indigenous people, with a prevalence of 6.8% and 5.3%, respectively (Paiva and Casseb, 2015). In Africa, the seroprevalence increases from the north to the south, varying from 0.6% in Morocco to greater than 5% in several sub Saharan African countries of Benin, Cameroon, and Guinea-Bissau (Dumas *et al*., 1991). In Nigeria Abu *et al*. (2014) reported a seroprevalence of 14.3% among pregnant women in Zaria, Kaduna state.

2.3.3 Transmission of Human T-Cell Lymphotrophic Virus

The routes of transmission of HTLV-1 and HTLV-2 are identical to those of human immunodeficiency virus (HIV); however, free infectious HTLV-1 particles are rarely found in plasma (Paiva and Casseb, 2014). Human T-cell lymphotropic virus transmission primarily occurs through: vertically from mother to child (during transplacental transfer, delivery, or breastfeeding), sexual contact (primarily from men to women) and parenterally through the transfusion of blood and blood components or through contaminated needles (Paiva and Casseb, 2014).

Human T-cell lymphotrophic virus type-1 is primarily transmitted vertically from mother to child. Data from Nagasaki, endemic areas in Japan, were the first to demonstrate transmission in breast milk (summarized in Hino, 2011). Several data has demonstrated the presence of HTLV-1 antigen in breast milk obtained from infected mothers, it has also been established that the oral administration of fresh human milk obtained from HTLV-1-infected mothers led to HTLV-1 infection in uninfected marmosets (Moriuchi *et al*., 2013). Others also reported a significantly increased HTLV-1 infection rate in breastfed children as compared with bottle-fed counterparts

and long-term prospective data showing that mother to child transmission (MTCT) rates were 20.5% in infants breastfed for 6 months or more, 8.3% in those breastfed for <6 months and 2.4% in infants exclusively formula-fed. These data indicate that breastfeeding is the most prevalent, but not the sole route of MTCT of HTLV-1, and that a longer duration of breastfeeding increases the risk of MTCT (Moriuchi *et al*., 2013).

Human T-cell lymphotrophic virus is present in the genital secretions of infected individuals and could be transmitted through sexual intercourse, which is the second most common mode of HTLV transmission (WHO, 2012). Infection in endemic areas is maintained by horizontal transmission though unprotected sexual intercourse. High-risk behaviors, such as unprotected sex (Gotuzzo *et al*., 1994), multiple partners (La Rosa *et al*.,2009), sexual intercourse with injection drug users (Guimarães *et al*., 2011), sexual partners from HTLV-endemic areas (Zehender *et al*., 2004), certain sexual practices and a history of other sexually transmitted diseases have been identified as risk factors for HTLV infection (Zunt *et al*., 2006).

2.3.4 Immunopathogenesis of Human T-Cell Lymphotrophic Virus

After transmission, reverse transcription generates proviral DNA from genomic viral RNA, and the provirus is integrated into the host genome by viral integrase. HTLV-1 infection is thought to spread only through dividing cells, with minimal particle production. Therefore, the quantification of provirus reflects the number of HTLV-1-infected cells, which defines the proviral load. In this regard, an increase in numbers of HTLV-1-infected cells using cell division, by actions of accessory viral genes, especially tax, may provide an enhancement of infectivity (Taylor and Matsuoka, 2005). Tax expression induces proliferation, inhibits the apoptosis of HTLV-1-infected cells and, conversely, evokes the host immune response, including cytotoxic T cells, to kill virus-infected cells. HTLV-2 is not known to have a precise pathologic role. It is not

associated with any malignancies, but only with rare cases of subacute myelopathy like HAM/TSP, that have a more slow progression (Araujo and Hall, 2004).

Adult T-cell leukemia (ATL), transformation of HTLV-1–infected cells depends on numerous factors, of which the most important are host immune status and viral-encoded genes (Pillat *et al*., 2011). Two HTLV-1 transactivator x (Tax) protein and HTLV-1 basic leucine zipper factor (HBZ), appear to play a central role (Matsuoka and Jeang, 2011), Tax protein is a transactivator of viral RNA and also has a profound role in immortalization through multiple mechanisms (Bangham and Toulza, 2011). HTLV infection is considered to be a 2-phase process. The initial phase is Tax mediated, characterized by production of 500 to 5000 clones of T cells in an infected host. Once immunity develops, CD8beta-cell mediated cytotoxic T lymphocytes can eliminate the host Tax-expressing cells, thus containing the infection (Qayyum and Choi, 2014). The maintenance phase follows, with low immunogenicity and clonal expansion of infected cells mainly driven by HBZ (Matsuoka, 2010). This is consistent with the observation that Tax expression is depleted and HBZ is persistently detectable in ATLL (Bangham and Toulza, 2011).

Human T-cell lymphotrophic virus type-1 can infect different cell types, including T cells, B cells, fibroblasts, dendritic cells, and macrophages. However, regulatory T cells expressing CD25 and transcription factor forkhead box P3 (FOXP3) are considered to be the cell type that transforms to ATLL (Matsuoka and Jeang, 2011). Regulatory T cells control and suppress the cytotoxic T-lymphocyte function: therefore, HTLV-1-infected regulatory T cells have a survival advantage over other HTLV-1 infected cells that are often eradicated by cytotoxic T lymphocytes. As a result, there is a profound increase in these cells over a period of time, as seen in ATLL (Matsuoka and Jeang, 2011; Wang and Ke, 2011). HTLV-1 is proposed to induce and maintain high levels of FOXP3 through chemokine CCL22 expression (Wang and Ke, 2011).

Human T-cell lymphotrophic virus type-1 has been associated with HAM/STP by some studies but a definite conclusion is yet to be drawn on the matter, but HTLV-1 tax viral load, genetic background or immune disturbances have been implicated as major causes (Pillat *et al*., 2011). There is also a mounting evidence for high levels of serum/cerebrospinal fluid (CSF) inflammatory cytokines in HAM/TSP patients. Macrophage inflammatory protein (MIP-1) levels are higher in HAM/TSP patients than in asymptomatic carriers (Montanheiro *et al*., 2007). Thus, in addition to the proinflammatory cytokines, such as interferon (IFN-), IL-2, and IL-15 (Azimi *et al*., 2001), and the high activity of cytotoxic T lymphocytes (CTL), MIP-1released by peripheral blood mononuclear cells (PBMC) may play an important role in HAM/TSP pathogenesis. This process can also influence the inflammatory activity in the spinal cord and thus contribute to the HAM/TSP progress (Pillat *et al*., 2011).

Alternatively, type 3 CXC chemokine receptors (CXCR3), which are expressed at high levels on activated and memory T lymphocytes, selectively respond to some of these chemokines. Those memory cells are inducible by IFN-, which is found at high levels in HTLV-1-infected subjects (Montanheiro *et al*., 2009) these cells may in turn migrate to the spinal cord, leading to a potential damage to the myelin membrane (Guerreiro *et al*., 2006). Thus, two pathways could be implicated in HAM/TSP development, including: (i) memory cells directly involved with cytolytic damage driven by IFN-, and (ii) RANTES and MIP-1(alpha) acting as chemotactic factors, all produced mainly by CD8+T cells. MIP-1 may have an important role in mediating tissue-specific leukocyte recruitment and T-cell stimulation. This may result in damage to the myelin membrane, a major characteristic of myelopathy in HTLV-1-infected patients (Pillat *et al*., 2011). A chemokine-induced activation of T cells via CCR5 leads to activation of focal adhesion kinases, which also have important roles in cell motility including cell spread and

migration (Ganju *et al*., 2000). These CCR5 ligands are critical for T cell proliferation and the transcriptional activation of cytokine genes (Oppermann, 2004). The predilection of neuroinflammation by HTLV-1 in the thoracic cord may be due to slower local blood flow, allowing a better opportunity for cells expressing adhesion molecules to transmigrate (Izumo *et al*., 1997), while other, more watershed areas of the central nervous system usually remain clinically silent (Pillat *et al*., 2011).

2.3.5 Human T-Cell Lymphotrophic Virus associated diseases

It has been a little more than 20 years since the relationship between the HTLV virus and countless systemic diseases has been known (Goncalves *et al*., 2010). New perspectives have opened up on the pathophysiology of neoplastic, autoimmune and infectious diseases and even on some conditions hitherto considered degenerative. HTLV-associated diseases differ in their clinical presentation, clinical course and response to treatment. (Romanelli *et al*., 2010).

The association of HTLV-1 infection with ATLL, HAM/TSP, and HTLV-associated uveitis (HAU) has been established (Proietti *et al*., 2005). Other inflammatory diseases such as uveitis, polymyositis, arthritis and alveolitis, as well as infective dermatitis and some types of skin lesions are also associated with HTLV-1 (Romanelli *et al*., 2010). HTLV-2 is not clearly associated with disease, but it has been associated with increased susceptibility to bacterial infections, with significant impact on the morbidity of carriers (Goncalves *et al.,* 2010). HTLV-2 also appears to be associated with an increased susceptibility of pneumonia, asthma and bronchitis, bladder and kidney infection, inflammatory conditions, such as arthritis, and with increased mortality, being suggested that HTLV-2 may inhibit immunologic responses to respiratory infections and induce inflammatory or autoimmune reactions (Roucoux and Murphy,

2004). Approximately 95% of HTLV-1 carriers remain asymptomatic throughout life, whereas about 5% develop diseases associated with the virus (Martins *et al*., 2012).

Adult T-cell leukemia/lymphoma

Adult T-cell leukemia/lymphoma (ATLL) is an uncommon lymphoproliferative disorder of mature CD4 T-cells that is caused by the retrovirus HTLV-1 (Jabbour *et al*., 2011). ATLL was originally described by Takatsuki *et al*. (1977) (Qayyum and Choi, 2014). ATL is an aggressive lymphoproliferative malignancy of peripheral T-cells, with short survival in its acute form and an incidence of less than 5% in HTLV-1-infected people (Shimoyama *et al.*, 1991). It was initially described in Japan and later in the Caribbean region and South America (Qayyum and Choi, 2014). In Europe and the United States, ATL was diagnosed in immigrants from regions of endemicity. Its occurrence is associated with vertical transmission through breastfeeding (Luiz *et al*., 2010).

The World Health Organization Classification of Tumours of Hematopoietic and Lymphoid Tissues in 2008 subclassified ATLL into 4 distinct variants according to the Shimoyama classification: acute (60%), lymphomatous (20%), chronic (15%), and smoldering (5%). There are no “sine qua non” features for each variant, and overlap is seen.

The acute variant manifests as marked leukocytosis with atypical lymphocytes and eosinophilia. Hypercalcemia with or without osteolytic lesions is present in more than 70% of patients, which may result in renal dysfunction and neuropsychiatric disturbances. Elevated lactate dehydrogenase level is not uncommon. Patients present with constitutional symptoms, massive lymphadenopathy sparing mediastinum, cutaneous lesions, and organomegaly (Bazarbach *et al*., 2011). Central nervous system involvement in ATLL ranges from 0% to 25%, characterized by

multiple ring-enhancing lesions. These lesions are mostly asymptomatic and are seen more frequently in acute and lymphomatous variants, essentially during relapse or systemic ATLL (Dungerwalla *et al*., 2005). Respiratory complications are more frequent in acute ATLL secondary to tumour cell infiltration or opportunistic infections, such as cytomegalovirus, Pneumocystis jiroveci-pneumonia, toxoplasmosis, and bacterial abscess or sepsis (Tanosaki and Tobinai, 2011). The lymphomatous variant is an aggressive advanced disease resembling acute-onset subtype. Marked lymphadenopathy without leukaemia is a prominent feature. Skin involvement and hypercalcemia are less frequent (Jabbour *et al*., 2011). The chronic variant typically presents with skin rash, leukocytosis with absolute lymphocytosis, mild lymphadenopathy, and hypercalcemia (Matutes, 2007; Jabbour *et al*., 2011). The smoldering variant is asymptomatic and is characterized by normal white blood cell count with less than 5% circulating atypical lymphoid cells and without associated hypercalcemia or organomegaly (Jabbour *et al*., 2011). Skin and pulmonary involvement is common. Progression to acute variant can occur. The cutaneous variant presents with skin lesions without systemic involvement (Bittencourt *et al*., 2007).

Typical ATL cells have convoluted nuclei with homogeneous and condensed chromatin, small or absent nucleoli, and agranular and basophilic cytoplasm. These cells are called flower cells and are considered characteristic of ATL (Matutes, 2007).

HTLV-1-associated myelopathy/tropical spastic paraparesis

Human T-cell lymphotrophic virus type 1 (HTLV-1) is the ethiologic agent of the neurological disorder HTLV-1-associated myelopathy/tropical spastic paraparesis (HAM/TSP). Although the majority of HTLV-1–infected individuals remain asymptomatic during their lifetime, approximately one percent of this population develops a myelopathy consisting of a chronic

inflammation of the white and gray matter of the spinal cord (Micheli *et al*., 2011). HAM/TSP was first described in Jamaica in the nineteenth century, but the aetiology of the condition, infection with the retrovirus HTLV-1, was only identified in the 1980s (Gessain *et al*., 1985; Osame *et al*., 1986). HAM/TSP causes chronic disability and, accordingly, imposes a substantial health burden in areas where HTLV-1 infection is endemic. Since the discovery of the cause of HAM/TSP, considerable advances have been made in the understanding of the virology, immunology, cell biology and pathology of HTLV-1 infection and its associated diseases. However, progress has been limited by the lack of accurate animal models of the disease (Charles *et al*., 2015).

There is no accurate number of HAM/TSP or ATL cases since these diseases are not considered reportable by the World Health Organization (WHO), although reports from Japan documented approximately 800 cases of ATL yearly (Micheli *et al*., 2011). The major histopathological characteristic of HAM/TSP is a chronic inflammation of the white and gray matter of the spinal cord followed by a degenerative process that preferentially affects the white matter in the lower spinal cord (Bhigjee *et al*., 1993). HAM/TSP is characterized by a chronic slowly progressive spastic paraparesis with bladder disturbances, absent or mild sensory loss and low back pain, with seropositivity for HTLV-1 antibodies, in the absence of spinal cord compression. Despite the more usual presentation characterized by a slow progression, 21.5% of the patients may experience a rapid progression, with severe disability two years after the onset of symptoms (Charles *et al*., 2015). This phenomenon is related to older age of onset, parenteral HTLV-1 transmission route, high viral loads, and high antibody titers (Micheli *et al*., 2011).

In contrast to ATL, the incidence of HAM/TSP decreases with age. Thus, HAM/TSP progression is similar to multiple sclerosis (MS), with few reported cases among people over 60 years of age

(Ebers, 2005). This could be explained by age-related lack of CD8+ cell hyperactivity and associated lower risk for clinical development of HAM/TSP (Effros *et al*., 2005).

The signs and symptoms of HAM/TSP are caused by focal inflammatory lesions in the CNS (Charles *et al*., 2015), but are found most frequently in the upper thoracic spinal cord. The infiltrate is dense around blood vessels, but can spread diffusely throughout the parenchyma. The cause of this distribution of lesions is not completely understood. T-cells are thought to enter the CNS in 'watershed' areas of the circulation, where the blood flow rate is low, especially at the borders between regions supplied by different spinal arteries. The mononuclear cell infiltrates consist primarily of T lymphocytes; CD4+ T-cells predominate in early, active lesions and the proportion of CD8+ T-cells rises progressively as the lesion progresses (Izumo, 2010). The inflammatory process culminates, after months or years, in macroscopic changes in the CNS in particular, with a loss of spinal cord volume (Shakudo *et al*., 1999).

HTLV-1-associated Uveitis

Human T-cell lymphotrophic virus -1-associated uveitis (HAU) is a condition associated with HTLV infected adults of both gender (Goncalves *et al*., 2010). It is characterized by a granulomatous or nongranulomatous reaction accompanied by vitreous opacities and retinal vasculitis with rare exudative retinochoroidal alterations in one or both eyes (Mochizuki *et al*., 1992). Qualitative lachrymal film defects have been shown to be frequent (Pinheiro *et al*., 2006). Sicca syndrome associated with HTLV-1 differs from ocular manifestation in primary or secondary Sjo¨gren's syndrome, because it does not reveal any of the immunological alteration related to a rheumatologic disease (Goncalves *et al*., 2010).

The characteristic symptoms have been described as “flying flies” and visual blurring of acute or subacute installation (Pinheiro *et al*., 2006). In patients with HAM/TSP, uveitis was more frequent among those with an earlier onset of HAM/TSP and in patients with severe motor disability (Pinheiro *et al*., 2006). HAU has clinical characteristics that can vary according to geographical area. The corneal disease was only observed in patients from Caribbean and Brazil but not observed Japanese patients (Goncalves *et al*., 2010).

Dermatological manifestations

Human T-cell lymphotrophic virus-associated infective dermatitis is a chronic, recurrent, eczema that predominantly affects children and adolescents (Goncalves *et al*., 2010). It is characterized by erythematous, scaly and crusty lesions affecting the scalp and the retroauricular, cervical, perioral, nasal and inguinocrural areas. Mild to moderate itching, chronic nasal secretions and nasal crusting are often present and patients are generally infected by *Staphylococcus aureus* and/or *Streptococcus* beta *haemolyticus*. Dermatitis is accompanied by an exaggerated Th1 response and elevated proviral load. Patients with HTLV and infective dermatitis are at a high risk of developing HAM/TSP (30%) and ATL (Nascimento *et al*., 2009). Other dermatological conditions that have exhibited heightened prevalence in studies of asymptomatic HTLV carriers are dermatophytosis, seborrheic dermatitis and acquired ichthyosis (Goncalves *et al*., 2010). Disseminated and recurrent forms of scabies have also been described in association with HTLV infections (Blas *et al*., 2005).

2.3.6 Other HTLV-associated abnormalities

Polymyositis, inclusion body myositis and fibromyalgia

Muscle inflammation has been associated with HTLV-1 (Goncalves *et al*., 2010). It can be the single manifestation of the disease, or it can be associated with HAM/TSP. Patient complaints

are myalgia and proximal muscle weakness. Laboratory tests show an elevated erythrocyte sedimentation rate and creatine kinase level. Muscle biopsy specimens show a myopathic pattern of inflammation (Goncalves *et al*., 2010).

Myopathy can affect patients irrespective of the presence of other neurological manifestations. In one region of Japan, between 1986 and 2006, Matsuura *et al.* (2008) observed higher prevalence for the combination of inclusion body myositis and HTLV (11 out of 21 patients, 52.3%), than for polymyositis (27.5%), with a seropositivity of 11.6% in the general population. A high proviral load and immune susceptibility was the principal factor in emergence of the pathological process. Patient response to immunosuppressor treatment was considered unsatisfactory in management implying a possible association with HTLV infection (Luiz *et al*., 2010). An association has been demonstrated between fibromyalgia and chronic viral infections. Cruz *et al*. (2006) found fibromyalgia in 38% of people infected with HTLV and in 4.8% of controls, demonstrating a significant association.

Arthritis, synovitis and autoimmune disease

There have been reports of connective tissue disease patients with HTLV-1 infections (Romanelli *et al*., 2010). HTLV-associated arthritis has similar clinical characteristics to idiopathic rheumatoid arthritis. Yakova *et al.* (2005) have demonstrated that patients with rheumatoid arthritis and other connective tissue diseases have greater proviral loads than asymptomatic carriers, in common with HAM/TSP patients. The proviral load is elevated in synovial fluid obtained from patients with arthritis and diseases of the connective tissues.

Other autoimmune conditions associated with HTLV-1 include endemic polymyositis, bronchoalveolar pneumonitis, and autoimmune thyroiditis (Kawai *et al*., 1992).

Polyneuropathy

Peripheral neuropathies in HTLV-1-infected patients with and without HAM/TSP have been documented by Araujo and Silva, (2006). Polyneuropathy is usually asymmetric, sensory motor, axonal, or of a mixed type. The major complaints are hypoesthesia in a stock and-glove distribution, paresthesias, and burning of the lower limbs. For this reason, Goncalves *et al*. (2010) stated that HTLV infection should be investigated for individuals with polyneuropathies of unknown origin.

Electroneuromyography of these patients shows abnormalities in peripheral nerves that consist of minimal increases in the distal latencies with moderated slowing of the proximal waves and of the distal conduction velocities along the peroneal and sural nerves (Goncalves *et al*., 2010).

Infectious and parasitic diseases

Some studies suggest that HTLV infection may be associated with immunosuppression and so increases the risk of other infectious comorbidities (Goncalves *et al*., 2010). *Strongyloides stercoralis* hyperinfection of patients infected with HTLV is the result of immunoresponse abnormalities that make systemic dissemination of the infestation possible, leading to recurrent chronic infection and poor clinical response to the usual treatments (Freites, 2008). Porto *et al*. (2005) highlighted a possible protective effect of strongyloides against HAM/TSP, caused by a modulated Th1 response. Patients with tuberculosis have a higher rate of HTLV-1 infection than the general population.

Scabies crustosa, also known as Norwegian scabies, is a rare form of presentation that is characterized by its severity and dissemination. When diagnosed it should arouse a high degree of suspicion of an HTLV coinfection, particularly if there are no other identifiable reasons for

immunosuppression and in endemic areas. *Scabies crustosa* is correlated with poor prognosis and increased mortality (Blas *et al*., 2005).

Psychiatric disorders

Among asymptomatic HTLV-1 carriers, a high rate of depression was found compared to non-infected individuals. Depression could be related to the psychological impact of the incurable state or could be related to a biological effect of the virus (Stumpf *et al*., 2008).

2.4 Prevention of Human T-Cell Lymphotrophic Virus

The prognosis for ATL and HAM/TSP is poor, and no vaccine is yet available. For HAM/TSP, a long-lasting, progressive disease, the financial cost for the individual, the family, and the health system is very high. In this sense, public health interventions such as counseling and education of high-risk individuals and populations are of paramount importance (Carneiro-Proietti *et al*., 2006).

Retrospective and prospective epidemiological studies revealed the mother-to-child transmission rate was around 20% (Yoshimitsu *et al*., 2013). Prevention of mother-to-child transmission has the most significant impact on the occurrence of HTLV-1 infection and associated diseases. Avoidance of breastfeeding is recommended as it is the major form of vertical transmission of this virus (Hino, 2011). A prefecture-wide intervention study in Nagasaki Prefecture in Southern Japan to refrain from breast-feeding by carrier mothers revealed a marked reduction of HTLV-1 mother-to-child transmission from 20.3% to 2.5%. Thus, prenatal screening for HTLV-1 was recommended to be employed in endemic areas, combined with relevant counseling of carrier mothers regarding transmission of HTLV-1 through breastfeeding. Although children breast-fed for less than 6 months has significantly lower incidence of HTLV-1 infection than those breast-

fed for more than 6 months, their chances of infection are significantly higher than those of bottle-fed children. Thus exclusive bottle-feeding was recommended (Hino, 2011). Even with exclusive bottle-feeding, 2.5% of infants born to carrier mothers where infected with HTLV-1 (Yoshimitsu *et al*., 2013).

Intrauterine transmission of HTLV-1 is rare, transplacental transmission during delivery is most likely as is the case for other viruses, i.e. HBV and HCV (Yoshimitsu *et al*., 2013).Thus prenatal screening for HTLV-1 was recommended in specific geographical areas, combined with counseling of seropositive mothers regarding transmission through breastfeeding. Carneiro-Proietti *et al.*, (2002) stated that due to risk of malnutrition in developing countries, public health policies should consider this adverse effect in less developed countries and recommend an alternative feeding formula for children at risk of acquiring HTLV-1 infection through mother's milk. In the case of pregnancy, a cesarean section was recommended, to minimize the risk of perinatal transmission (Goncalves *et al*., 2010).

Human T-cell lymphotrophic virus type-1 can also be spread through contact with bodily fluids such as whole blood or whole blood products. The development of ATLL related to transfusion is exceptional. Thus, the purpose of prevention of horizontal transmission is mainly to reduce HTLV-1 carrier population (Yoshimitsu *et al*., 2013). HTLV-1 screening program to prevent transfusion-related transmission of HTLV-1 has been developed since 1986 and many countries in endemic areas started to employ systematic screening of all blood donors (Osame *et al*., 1990). Screening of blood donor candidates has been shown to be an effective strategy in preventing HTLV-1 transmission (Yoshimitsu *et al*., 2013). HTLV-1-seropositive individuals were advised not to donate blood, semen, organs, or milk, where milk banks are available (Goncalves *et al*., 2010).

Most of sexual transmission of HTLV-1 is from men to women. Recommendations to prevent sexually transmitted infections were emphasized, including condom use, avoiding multiple and unknown sexual partners and paying or receiving money for sex (Yoshimitsu *et al*., 2013). When one of the partners in a stable relationship is negative, the need for condom use was emphasized (Goncalves *et al*., 2010).

There have been reports by Goncalves *et al*. (2010) that counseling and education of intravenous drug users (IDU) to implement harm reduction practices may be effective in preventing HTLV-1 infection in any population group. Access to correct information about HTLV infection is very important. Confusion with HIV is common, even in health care settings, and leads to unnecessary stress on the patient, which is frequently associated with self-destructive thoughts (Guiltinan *et al*., 1998). Counseling including orientation about the transmission of the virus and the possible source of the infection and an offering of testing to the partner/spouse and children was recommended. This is especially true when counseling blood donor candidates, because they are usually young, asymptomatic, and of a reproductive age (Goncalves *et al*., 2010).

2.5 Wilms' Tumour Gene 1 (WT1)

Wilms' tumour (WT), also called nephroblastoma, was first characterized by the German pathologist and surgeon Dr Carl Max Wilhelm Wilms (1867-1918) in 1899. His diagnosis of WT was based on clinical and histological appearance, the latter typically being a triphasic renal tumour consisting of blastemal, epithelial and stromal elements (Zantinga and Coppes, 1992). Max Wilms was unfortunately infected with diphtheria and died in May 1918. Almost 100 years after the publication of his monograph on the pathology of the childhood kidney tumours, it was discovered that a deletion of chromosome region 11p13 was linked to WT (Haber *et al*., 1990; Call *et al*., 1990). The gene was later isolated and named Wilms' tumour gene 1 (WT1). WT1

gene was found to encode a putative zinc finger transcriptional regulator with crucial functions in embryonic development and was originally described as a tumour suppressor gene (Haber *et al*., 1990). However, owing to WT1 protein overexpression in a variety of solid cancers that normally do not express WT1, it has later been suggested that WT1 might play an oncogenic role (Hohenstein, and Hastie, 2006). The WT1 protein has been demonstrated as a promising target for cancer immunotherapy. Clinical trials of WT1 peptide vaccination in patients with myeloid malignancies and several solid cancers has resulted in positive outcomes (Oka *et al*., 2008)

2.5.1 Wilms' Tumour Gene 1 structure

The WT1 gene spans approximately 50 kb DNA at chromosome locus 11p13. The gene consists of 10 exons and encodes an mRNA transcript of about 3 kb. The mRNA translates into a 449-amino-acid protein with a proline- and glutamine-rich amino terminus harboring defined functional domains that exert transcriptional repression, activation, self-association, DNA binding, RNA recognition and nuclear localization signals (Scharnhorst *et al*., 2001 ; Yang *et al*., 2007). The carboxy terminal domain of WT1 contains four Krupple-like, cysteine2-histidine2 zinc fingers encoded by exon 7-10, which are involved in RNA and protein interactions that permit binding to DNA sequences. This DNA-binding domain of WT1 shares high homology with the zinc finger region of early growth response protein 1 (Rauscher *et al*., 1993; Menke *et al*., 1998). In addition to binding DNA and some proteins, zinc fingers can regulate RNA targets and mediate nuclear localization (Yang *et al*., 2007). Besides binding to other proteins, WT1 can also self-associate, and the major domain required for this self-association has been mapped to the first 182 amino acids of WT1 (Menke *et al*., 1998).

In mammals, exons 5 and 9 of WT1 pre-mRNA are alternatively spliced, giving rise to four different splice isoforms designated A, B, C and D (Haber *et al*., 1991; Gessler *et al*., 1992**).** The

molecular weight of the WT1 proteins has been variously reported between 49 and 54 kDa (Reddy and Licht, 1996). The first alternative splicing event affects the entire exon 5 and leads to the presence or absence of 17 amino acids (AA) between the proline/glutamine-rich terminal domain and the carboxy terminal zinc finger domain of WT1. The second alternative splicing event generates either inclusion or exclusion of three AA—lysine, threonine and serine (KTS)—at the end of exon 9, affecting the conformation of zinc fingers three and four in the WT1 protein. These two isoforms (+KTS and −KTS) are conserved in all vertebrates and fish, and non-mammalian vertebrates appear to express only these two variants (Hastie, 2001). The mRNA isoform containing both splice inserts is the most prevalent variant in both human and mouse, whereas the least common is the transcript missing both inserts (Haber *et al*., 1991). Studies have demonstrated that WT1 isoforms lacking the KTS insertion (−KTS) bind to DNA more strongly and act as transcriptional regulators (Roberts, 2005). The gene product that contains the insertion (+KTS) also acts as a transcriptional regulator, in addition to being associated with post-transcriptional processes. The use of an upstream CTG start codon or an internal ATG start codon at the end of exon 1 result in a truncated isoform (Hohenstein, and Hastie, 2006). Another WT1 isoform, AWT1, arises from the use of an alternative promoter that resides within exon 1 (Dallosso *et al*., 2004).

Alternative WT1 mRNAs are generated through RNA editing at nucleotide 839 where leucine 280 is replaced by proline (Sharma *et al*., 1994). The WT1 gene may thus produce different mRNA isoforms, suggesting that each isoform has a distinct contribution to the function of the WT1 gene and that balanced expression of the isoforms is essential for proper WT1 function (Scharnhorst *et al*., 2001). The gene structure is presented in figure 2.1. Blue shading indicates alternatively spliced domains.

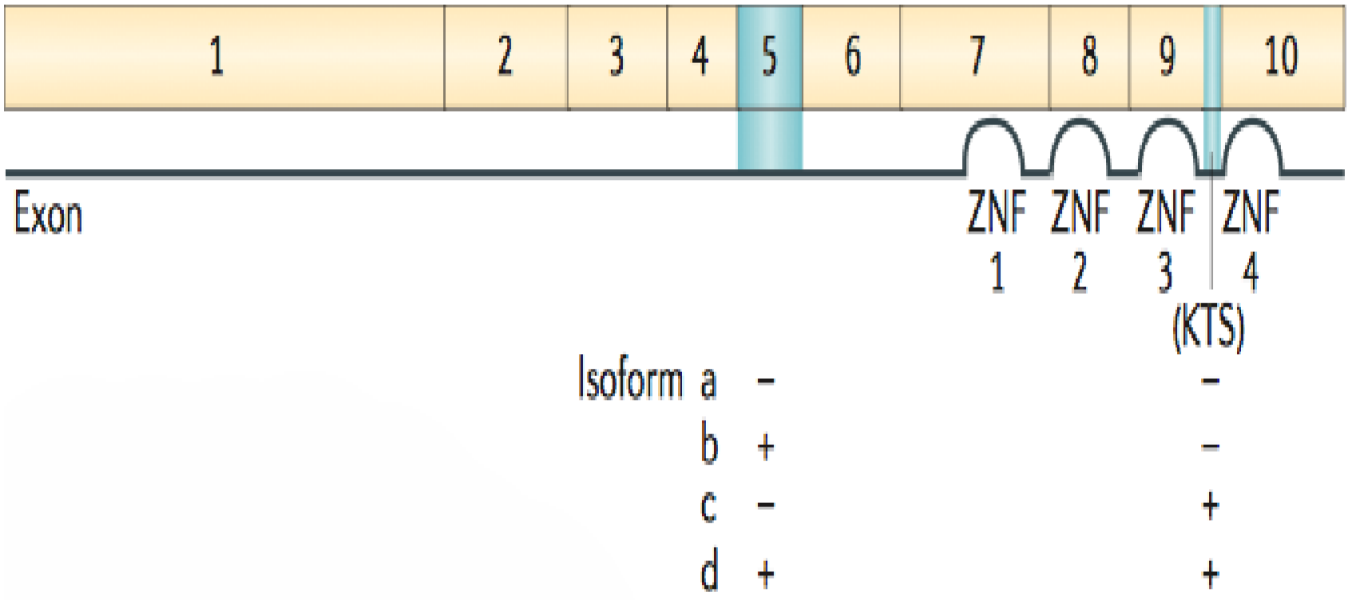

Figure 2.1. WT1 gene and protein structure. Adapted from Huff, 2011 Nature Reviews Cancer (Huff, 2011).

2.5.2 Wilms' Tumour gene 1 as a tumour suppressor gene

Wilms tumour gene-1 was initially discovered as a tumour suppressor gene in Wilms' tumour (Willasch *et al*., 2009). It was found that, in the majority of cases, Wilms' tumours expressed wild-type WT1, sometimes to high levels. In fact, in the sporadic form of the disease, only 10% had a WT1 mutation. Those findings suggested that WT1 mutations are important only in a small fraction of cases (Li, 2015). However, three Wilms' tumour-related syndromes were associated with germline mutations or deletions in WT1: WAGR syndrome (Wilms' tumor, ANIRIDIA, genitourinary abnormalities, and mental retardation) (Gessler *et al*., 1990), Denys–Drash syndrome (DDS)(Pelletier *et al*., 1991), and Frasier syndrome (Barbaux *et al*.,1997; Klamt *et al*., 1998). The function of WT1 as a tumour suppressor has been studied with different models *in-vitro* and *in-vivo*. WT1 expression conferred the ability to suppress cell proliferation in the Wilms' tumour cell line, G401, and to induce programmed cell death in osteosarcoma cell lines (Englert *et al*., 1995; Mcmaster *et al*., 1995). WT1 has also been found to induce apoptosis in the Saos-2 cell line and B16F10 melanoma cells (Zamora-Avila *et al*., 2007). In mice, WT1 could induce growth suppression, reduce tumour formation, and suppress tumourigenicity (Fraizer *et al.*, 2004). In addition, over expression of WT1 was found to induce cell cycle arrest and apoptotic cell death in M1 leukaemia cells (Murata *et al*., 1997).

2.5.3 Wilms' tumour gene 1 as an oncogene or a chameleon gene

Whereas WT1 behaves as a tumour suppressor gene in WT, a wealth of data on the overexpression of WT1 in a variety of human cancers of both haematological and non-haematological origin suggests WT1 plays a role as an oncogene. Overexpression of WT1 has been demonstrated in carcinomas from a variety of origins, including lung cancer (Oji *et al*., 2002; Mundlos *et al*., 1993), breast cancer (Loeb *et al*., 2001), colon cancer (Koesters *et al*.,

2004), pancreatic cancer (Oji *et al*., 2004), ovarian cancer (Andersson *et al*., 2014; Shimizu *et al*., 2000), primary astrocytic tumours (Oji *et al*., 2004), sarcomas (Athale *et al*., 2001), malignant melanoma (Rodeck *et al*., 1994), mesothelioma (Amin *et al*., 1995) and other tumours. WT1 has been shown to regulate the cell cycle, by contributing to the pro-proliferative effect (Andersson, 2016). WT1 protein also exerts transcriptional regulation of growth factors and growth factor receptors and has the potential to increase apoptosis, which contributes to WT1-regulated cell survival. In breast cancer cells, ablation of the WT1 protein led to increased apoptosis and cell cycle arrest at G1 (Tuna *et al*., 2005). Studies have also revealed a role of WT1 in angiogenesis and vascularization, cell proliferation and migration, which are important steps for tumour growth (Wagner *et al*., 2008).

The oncogenic role of WT1 in leukaemia has been extensively studied, and the role of WT1 in leukaemia appears to be complex and also contradictory (Andersson, 2016). Overexpression of WT1 has been reported in acute myeloid leukaemia (AML), chronic myeloid leukaemia, acute lymphoblastic leukaemia (ALL) and myelodysplastic syndrome (MDS) (Inoue *et al.*, 1997; Ariyaratana and Loeb**,** 2007). Collectively, these studies strongly suggest a tumour-promoting or oncogenic role of WT1 in leukaemogenesis. However, some findings support a tumour suppressor role of WT1 in leukaemia. A considerable proportion of AML and precursor T-cell lymphoblastic leukaemia (T-ALL) shows WT1 mutations. The first report of somatic WT1 mutations associated with development of AML was published in 1994 by Pritchard-Jones *et al*. (1994). Since then, large cohort studies of cytogenetically normal AML (CN-AML) cases have confirmed the frequency of about 10% mutated WT1 in adult AML (Paschka *et al.*, 2008; Virappane *et al*., 2008). The majority of mutations involve insertions, deletions and missense mutations such as those observed in patients with Denys Drash Syndrome DDS (Huff, 2011**)**.

These data suggest that as in WT, WT1 has a tumour suppressor function in leukaemia. The oncogenic or tumour-suppressive effect of WT1 alterations is likely to be a result of how a cell at a particular stage of development responds to perturbations in the expression of those genes (Huff, 2011**)**. In 2011 Huff suggested retirement of the labels "tumour suppressor" and "oncogene" to describe the WT1 function and introduced the ingenious label "chameleon gene" for WT1 (Huff, 2011**)**.

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Study Area

The study area was Zaria in Kaduna State, Nigeria. Zaria is a major city in Kaduna state, in the northern part of Nigeria and is located on latitude 11^{o} 04'N and longitude 7^{o} 43'E. It is defined by a 15km radius from the PZ post office and is well connected by roads and rail with other regions of the country. Distances from Kaduna, Kano, Jos and Sokoto are approximately; 75km, 176km, 387km, and 404km respectively. Zaria is the second principal town in Kaduna state and home to the Ahmadu Bello University, and a host to several Federal Government institutions and establishments.

The population of Zaria is approximately 975,153 (2006 census). It is made up of two local government area (LGA) councils, Zaria LGA [consisting of two districts; Zaria (walled) city and Tudun–Wada], and Sabon Gari LGA [which consist of three districts; Sabon-Gari, Samaru and the government reservation area (GRA)] (Oladimeji and Ojibo, 2012).

3.2 Study Design

The study was a Health Care Center based cross-sectional study involving the use of self-designed, structured questionnaire and the analysis of blood samples from female patients attending post natal care that have consented.

3.3 Study Population

The study population comprised women of all ages and works of life attending post natal care that reported to some selected Primary Health Care Centers. The selected health care centers were as follows:

i. Samaru Primary Health Care Center, Samaru.

ii. Kwata Primary Health Care Center, Sabon gari.

iii. Babbandodo Primary Health Care Center, Zaria city.

iv. Rimin Doko Primary Health Care Center, Zaria city.

3.4 Ethical Clearance and Consent

Ethical clearance (Appendix I) for the study was obtained from the Ethics Committee of the Kaduna State Ministry of Health and consent (Appendix II) was sought from all participants of the study.

3.5 Sample Size

The sample size for the study was determined using the formula by Kadam and Bhalerao (2010) and a prevalence of 14.3% from a previous study by Abu *et al*. (2014)

$n=Z^2Pq/L^2$

Where

n = number of samples

Z = standard normal deviate at 95% CI = 1.96

P = 14.3% (Abu *et al*., 2014) = 0.143

q = 1 – 0.143 = 0.857

L = allowable error of 5% (0.05)

$n = Z^2Pq/L^2$

$n = 1.96^2 * 0.143*0.857/0.05^2= 0.47059584/0.0025 = 188.31677 \sim 188$ samples

The calculated sample size was 188 which was the least number of samples to be used for the study. However, 190 samples were used in the study for convenience.

3.6 Inclusion Criteria

Women included in the study were breast feeding mothers attending post natal care who consented irrespective of age and education.

3.7 Exclusion Criteria

Women not included in this study were other patients or women attending post natal care but unwilling to consent.

3.8 Data Collection

All consenting patients were each administered a structured questionnaire (Appendix III) which was aimed at obtaining socio-demographic data, family history and risk factors associated with leukemia. Interpretation was offered where necessary.

3.9 Sample Collection

Four (4) ml of venous blood samples was collected from each consenting breast feeding woman after filling the questionnaire and was aliquoted into EDTA (2ml) and plain tubes (2ml) respectively and processed by centrifuging at 3000rpm for 3-5minutes to obtain the serum following the method used by Adekunle *et al.* (2015).

3.10 Haematological Analysis

Full blood counts such as white blood cell count, platelet count and lymphocyte count were obtained using a haematology auto analyser (BC3600, Mindray, China), strictly following manufacturer's instructions.

3.11 Human T-cell Lymphotrophic Virus 1 and 2 Enzyme Linked Immunosorbent Assay

The Human T-Cell Lymphotropic Virus types 1 and/or 2 (HTLV-1/2) test kit (Diagnostic Automation and Cortex Diagnostics Inc USA) is an enzyme-linked immunosorbent assay (ELISA) for the qualitative determination of antibodies for HTLV in serum or plasma. It is used for the diagnosis of clinical conditions related to infection with HTLV-1 and/or HTLV-2, intended for screening blood donors.

3.11.1 Test principle

This kit used is an antigen "sandwich" enzyme immunoassay (ELISA) method, with polystyrene microwell strips pre-coated with recombinant HTLV antigens expressed in *E.coli*. The patient's serum sample is incubated in the microwells together with second recombinant HTLV antigens conjugated to horseradish peroxidase (HRP-Conjugate). The pre-coated antigens express the same epitopes as the HRP-Conjugate antigens, but are expressed in different hosts. In case of presence of anti-HTLV in sample, the pre-coated and HRP-conjugated antigens will be bound to the two variable domains of the antibody and during incubation; the specific antigen-antibody immunocomplex is captured on the solid phase (Diagnostic Automation and Cortex Diagnostics Inc USA, 2017).

3.11.2 Test procedure

All test reagents and serum specimens were brought to room temperature (30^0C) before use and positive and negative controls were tested with each series of test sera. Using the disposable pipette provided, 50µl HRP-Conjugate were added to each well except the blank, followed by the addition of 50µl of Positive control, Negative control, and specimen to their respective wells and gently mixing. The plate was then covered with the plate cover and incubated for 60minutes at 37°C. At the end of the incubation, the plate cover was removed and discarded. Each well was

washed 5times with diluted Wash buffer each time allowing the microwells to soak for 30-60 seconds. After the final washing cycle, the plate was then turned onto blotting paper, and tapped as to remove any reminders of the wash buffer.

Exactly 50µl of Chromogen A and 50µl Chromogen B solutions were dispensed into each well including the blank after which the plate was covered with plate cover and mixed by tapping gently and then the plate was incubated at 37°C for 30minutes in the dark. The enzymatic reaction between the chromogen solutions and the HRP-Conjugate produced blue color in positive control and HTLV 1/2 Positive sample wells. The plate cover was removed and discarded. Using a multichannel pipette, 50µl stop Solution was added into each well and mixed gently. Intensive yellow color developed in positive control and HTLV 1/2 positive sample wells. The plate reader was calibrated with the blank well and the absorbance was read at 450nm.The cut-off value was calculated and result evaluated. Each microplate was considered separately when calculating and interpreting the results of the assay. The results were calculated by relating each sample's optical density (OD) value to the cut off value (C.O.) as recommended by the manufacturer.

3.12 Quantification and Detection of Wilms Tumour Antigen

The kit used (Wulhan Fine Biological Technology Co.,Ltd.) was a sandwich enzyme immunoassay for *in-vitro* quantitative measurement of Wilms tumour antigen in human tissue homogenates, cell lysates and other biological fluids.

3.12.1 Test principle

The microtiter plate provided in this kit has been pre-coated with an antibody specific to WT1. Standards and samples are then added to the appropriate microtiter plate wells with a biotin-

conjugated antibody specific to WT1. Avidin conjugated to Horseradish Peroxidase (HRP) is added to each microplate well and incubated. After TMB substrate solution is added, only those wells that contain WT1, biotin-conjugated antibody and enzyme-conjugated avidin exhibited a change in color. The enzyme-substrate reaction is terminated by the addition of sulphuric acid solution and the color change is measured spectrophotometrically at a wavelength of 450nm ± 10nm. The concentration of WT1 in the samples is then determined by comparing the optical density of the samples to the standard curve. (Wulhan Fine Biological Technology Co.,Ltd. 2017).

3.12.2 Test procedure

All test reagents and serum specimens were brought to room temperature before use. Estimation of the concentration of target protein in the test sample was done and proper dilution factor to make the diluted target protein concentration fall in the optimal detection range was taken. The plate was washed two (2) times before standard, sample and control were added. Hundred microlitre (100µL) of dilutions of standard was added into the appropriate wells, which was then covered with the plate sealer and incubated for 90 minutes at 37°C. The liquid in each well was discarded and washed two (2) times with wash buffer. Hundred microlitre (100µL) of Biotin-labeled antibody working solution was added to each well and incubated for 60 minutes at 37°C after covering with the plate sealer. The solution was aspirated and washed three (3) times each time allowing the wash buffer to stay in the wells for 1 minute. The remaining liquid was removed from all wells completely by slapping the plate onto absorbent paper.

Exactly100µL of HRP-Streptavidin conjugate (SABC) working solution was then added to each well and incubated for 30 minutes at 37°C after covering it with the plate sealer. The aspiration/wash process was repeated for total 5 times. Ninety microliter (90µL) of TMB

substrate solution was then added to each well followed by incubated for 15 - 25 minutes at 37°C and protected from light. The liquid turned blue by the addition of substrate solution. Finally 50µL of stop solution was then added to each well. The liquid turned yellow by the addition of stop solution. The liquid was then mixed by tapping the side of the plate. Absorbance was read at 450nm immediately, and standard curve was plotted and concentrations determined.

3.13 Data Analysis

Results were recorded and presented in tables and charts where appropriate. Data obtained from questionnaires as well as those gotten from the test analysis were analyzed using the Statistical Package for Social Sciences (SPSS) version 17 at 0.05 level of significance and at 95% confidence interval. Relationship between variables was determined using Pearson's Chi- square analysis and the prevalence of the infection was calculated in percentage.

CHAPTER FOUR

4.0 RESULTS

Out of the 190 blood samples screened, 6 (3.2%) were positive for Human T-cell lymphotrophic virus (HTLV) IgG (Figure 4.1), with Kwata PHC having the highest prevalence of 4.2% (2/48). There was no statistically significant association between HTLV infection and hospital location (χ^2=0.605, df=3, *P*=0.895**) (**Table 4.1)**.**

The women's socio-demographic data were assessed in relation to HTLV infection. In relation to the type of family, there was no statistically significant association (χ^2=3.017, df=1, *P*=0.082) although women from polygamous families had the highest prevalence of 6.2% (4/64), while women from monogamous families had a prevalence of 1.6% (2/126) as shown in table 4.2. With respect to occupation, there was no statistically significant association with HTLV infection (χ^2=3.878, df=3, *P*=0.275), although women who were self-employed had the highest prevalence of 6.5% (4/62), the lowest prevalence was reported in women who were civil servants (0.0%)(Table 4.2). The distribution of anti-HTLV antibodies in relation to age showed that women in age group 15-25 years had the highest prevalence of 6.2% (5/72) and age group 36-45 years had no prevalence. This difference was however not statistically significant (χ^2=5.492, df=2, *P*=0.064) (Table 4.2).

The association between HTLV infection and level of education of the women was presented in Table 4.2. Though there was no statistically significant association with the level of education and HTLV (χ^2=2.460, df=3, *P*=0.483), women with primary education had the highest prevalence of 5.9% (2/32)while anti-HTLV antibody was not detected in those with no form of formal education.

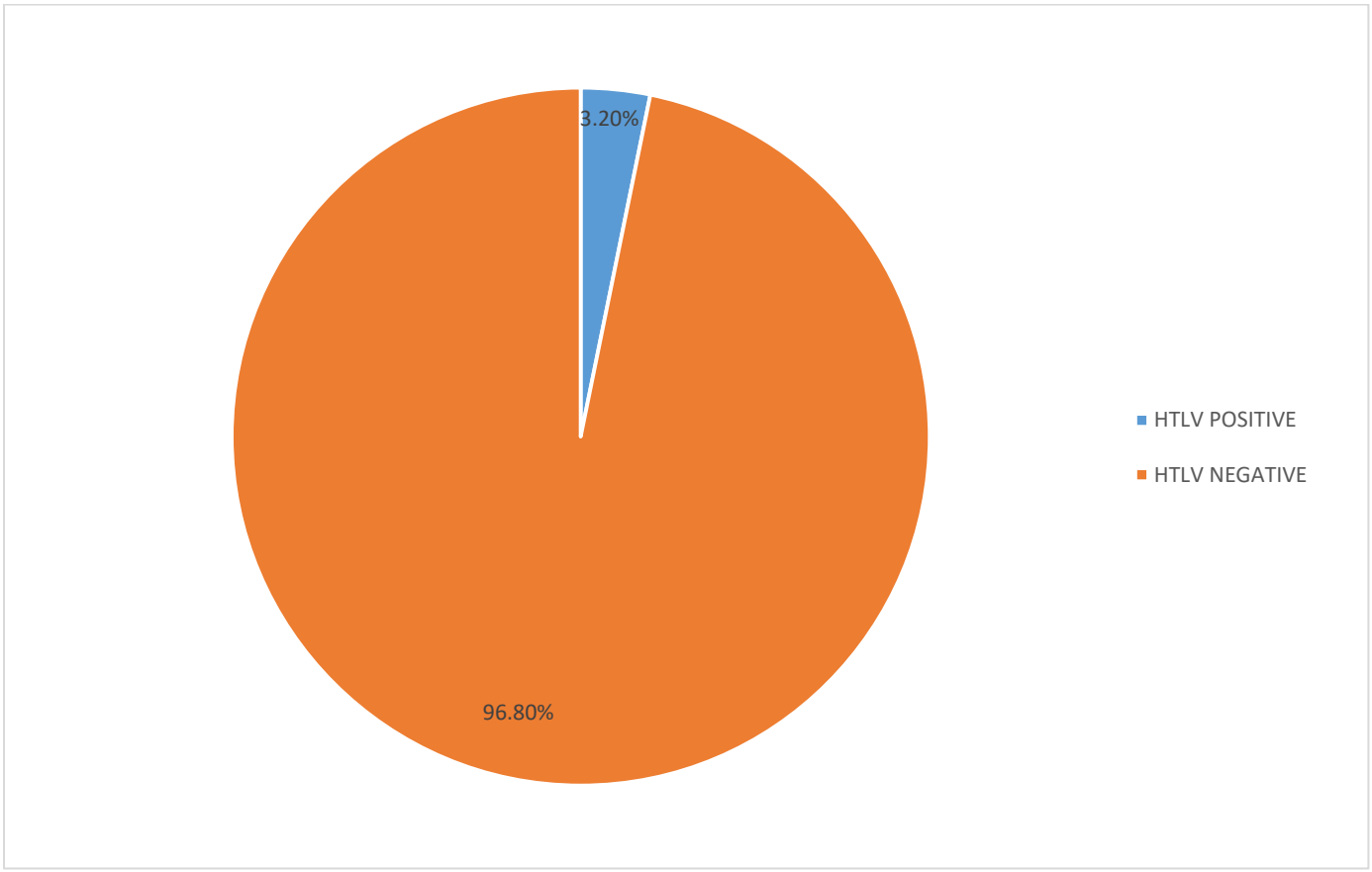

Figure 4.1: Seroprevalence of Anti-Human T-cell lymphotrophic Virus IgG among Women Attending Post-Natal Clinics in Zaria, Nigeria

Table 4.1: Sero-prevalence of Anti-Human T-Cell lymphotrophic Virus IgG According to Primary Health Care (PHC) Center.

Health Center	Number Examined	Number Positive (%)	χ^2	*P*-Value
Samaru PHC	46	1(2.2)	0.605	0.895
Kwata PHC	48	2(4.2)		
Babbandodo PHC	47	1(2.1)		
Rimin Doko PHC	49	2(4.1)		
Total	190	6(3.2)		

Table 4.2: Sero-prevalence of Anti-Human T-Cell Lymphotrophic Virus IgG According to Some Demographic Factors

Factor	Number Examined n=190	Number Positive (%)	χ^2	*P*-Value
Type of Marriage				
Monogamy	126	2(1.6)	3.017	0.082
Polygamy	64	4(6.2)		
Occupation				
Unemployed	80	2(2.5)	3.878	0.275
Self-employed	62	4(6.5)		
Civil servant	40	0(0.0)		
Others	8	0(0.0)		
Age Group(Years)				
15-25	72	5(6.9)	5.492	0.064
26-35	98	1(1.0)		
36-45	20	0(0.0)		
Level of Education				
No Formal Education	31	0(0.0)	2.460	0.483
Primary	34	2(5.9)		
Secoundary	85	2(2.4)		
Tertiary	40	2(5.0)		

Table 4.3 shows the association between Anti-HTLV IgG and some associated risk factors. There was no significant association between HTLV infection and all the risk factors studied such as history of family cancer (χ^2=0.896, df=1, *P*=0.629), intravenous drug use (χ^2=0.167, df=1, *P*=0.682), sharing of sharp objects (χ^2=0.281, df=1, *P*=0.596,) and history of X-ray exposure (χ^2=0.784, df=1, *P*=0.366), except for history of previous blood transfusion which shows significant association (χ^2=10.388, df=1, *P*=0.010).

Table 4.3: Sero-prevalence of Anti-Human T-Cell Lymphotrophic IgG in Relation to Some Risk Factors

Factor	Number Examined	Number Positive (%)	*P*-Value	χ^2	Odds Ratio	Confidence interval 95% Cl
History of cancer						
Yes	24	0(0.0)	0.333	0.896	Undef.	1.007-1.069
No	166	6(3.6)				
Blood Transfusion						
Yes	48	5(10.4)	0.010	10.388	0.065	0.007-0.569
No	142	1(0.8)				
Intravenous Drug Use						
Yes	5	0(0.0)	0.167	0.682	Undef.	1.007-1.061
No	185	6(3.2)				
Period of Breastfeeding						
Yes	92	1(1.1)	0.114	2.501	4.892	0.561-42.696
No	98	5(5.1)				
Sharing of Sharp Object						
Yes	46	2(4.3)	0.596	0.281	0.629	1.111-3.549
No	144	6.3)				
History of X-Ray Exposure						
Yes	24	0(0.0)	0.366	0.784	Undef.	1.007-1.068
No	166	6(3.2)				

Concentration of Wilms tumour antigen ELISA in relation to HTLV infection status is presented in Table 4.4. The results showed a statistically significant increase (p=0.000) in concentration of Wilms tumor antigen in relation to HTLV seropositivity (mean conc. = 116.22±18.39 pg/ml), when compared to HTLV seronegativity (mean conc. = 66.41±20.13 pg/ml).

Findings of haematological investigations in relation to HTLV infection are presented in Table 4.5. There was no statistically significant association between white blood cell count and HTLV infection (HTLV positive=6.98 10^9 cells/L, HTLV negative 6.85 10^9 cells/L, p=0.213) and lymphocytes mean count (HTLV positive=2.92 10^9 cells/L, HTLV negative=2.52 10^9 cells/L, p=0.305) however, there was a general statistically significant increase in platelet count in relation to HTLV infection (HTLV positive 249.5 10^9 cells/L, HTLV negative 178.5 10^9 cells/L p=0.017).

Table 4.4: Concentration of Wilms Tumor Antigen (Pg/ml) in Relation to Human T-Cell Lymphotrophic Virus Infection (Independent Samples T-Test)

HTLV infection status	**mean WT1 antigen concentration (pg/ml)**	***p*-value**
HTLV positive n=6	116.22±18.39	**0.00**
HTLV Negative n=80	66.41±20.13	

Table 4.5: Haematological Investigations in Relation to Human T-Cell Lymphotrophic Virus (HTLV) Infection (Independent Samples T-Test)

Haematological Parameter	HTLV Positive n=6	HTLV Negative n=184	*p*-value	Ref. Range (10^9 cells/L)
WBC	6.98±0.132	6.85±0.93	0.213	4.0-11.0
LMPH	2.92±1.114	2.52±0.93	0.305	1.2-4.0
PLT	249.5±80.35	178.8±70.45	0.017	150-450

WBC= White blood cell. LYMP= Lymphocytes PLT= Platelet

CHAPTER FIVE

5.0 DISCUSION

The assessment of the seroprevalence of HTLV infection is important especially in endemic areas like Nigeria because transmission of the virus could in some cases result in malignancies. In this study, the seroprevalence of Human T-lymphotropic virus among women attending post-natal care was 3.2%. The prevalence found in the study implies that some of the infected women are likely to develop haematological malignancies and also makes this study area fall in the category of HTLV endemic location in Nigeria as the virus is considered as endemic in areas where the prevalence ranges from 0.5 to 20% in the population (Proietti *et al*., 2005). The prevalence recorded in this study is comparable with the findings of Adekunle *et al*. (2015) who reported a prevalence of 2.7% in Osun state, Nasir *et al*. (2015) with a seroprevalence of 4.9% in Abuja and Yuguda *et al*. (2017) with a prevalence of 3.2% in Ibadan. However the prevalence observed in this study was lower than that obtained by Abu *et al*. (2014) in Zaria who reported a prevalence of 14.3% among pregnant women, and Forbi and Odetunde (2007) who reported 22.9% among community sex workers (CSWs) and 16.7% among pregnant women. The reason for the lower prevalence may be due to small sample size used, uncommon nature of the virus and its slow replication.

Several studies in different regions of the world have identified socio-demographic variables like age, gender, socio-economic status and environmental conditions as factors for acquisition of Human T-lymphotropic virus infection (Adekunle *et al*., 2015). In relation to marriage type, the highest prevalence of 6.1% was observed among women who belong to polygamous families compared to 1.6% observed among women from monogamous families, though it was not statistically significant ($P>0.05$). The high prevalence observed among women in polygamous

family may be attributed to multiple sexual partners of the spouse, given transmission from husband to wife to be 60% and from wife to husband to be 0.4% over a 10 year period (Paiva and Casseb, 2014).

This study showed that the seropositivity of HTLV reduced with age in the women studied, with the highest prevalence found in age group 15-25 years, though it was not statistically significant. This finding was in agreement with the work of Nasir *et al*. (2015) who reported higher seroprevalence among patients aged 21-30 and non in subjects aged 31-50 years but disagree with the work of Verdonck *et al*. (2007) which reported that seropositivity increases with age of women. The reason for zero prevalence among women aged 36-50 may be due to fewer participants of the age group.

The test of association between the women's level of education with seroprevalence of HTLV showed no significant association. Women with primary education had the highest prevalence. The limited literature in this area highlights the need for further research around infection control education practice.

Risk factors associated with HTLV infectivity include multiple sexual partners, a history of STD (Zunt *et al*., 2002), intravenous drug use (Fukushima *et al*., 1995) and previous blood transfusion (Manns *et al*., 1992). In relation to intravenous drug use, the present study shows no association between intravenous drug use and HTLV infection ($p>0.05$). All the positive women had no history of intravenous drug usage. This may be attributed to the geographical location of the study and also the study population as it is believed that drug addiction is relatively less common among women than men (Adamson *et al*., 2015). Also the absence of risk factor of intravenous drug abuse points to pattern of transmission that may be different from those in western societies.

A significant association was observed between history of blood transfusion and seropositivity to HTLV among women. Blood transfusion is an important route of transmission. Persons who had received blood transfusion are also more likely to develop possible antibodies to HTLV with consequent development of HTLV-1- associated myelopathy/tropical spastic paraparesis. The result from this study agrees with the findings of Manns *et al*. (1992) that previous blood transfusion increases the risk of acquiring HTLV infections.

Wilms tumour gene though proposed as a promising target gene for cancer immunotherapy, the prognostic value of Wilms tumour gene (WT1) in solid tumours remain inconclusive (Qi *et al*., 2015). From the study, the concentrations of human Wilms tumour antigen were significantly higher in HTLV positive compared to HTLV negative individuals. The reason for the dominance of Wilms tumour antigen in relation to infection is unknown at the moment, but studies have indicated that WT1 might play an oncologic role in haematologic malignancies on a variety of solid tumors, including leukaemia, breast cancer, ovarian cancer (Miyoshi *et al*., 2002; Qi *et al*., 2015). Tatsumi *et al*. (2008) stated that it is possible that WT1 could inhibit cell apoptosis by transcriptional activation and upregulation of proto-oncogenes.

Haematological parameters are measurable indices of blood that serve as markers for disease diagnosis. Changes in some of these haematological indices are some of the common observations that are attributed in cases of infections of Human T-lymphotropic virus (Goncalves *et al*., 2010). White blood cells (leucocytes) play a pivotal role in the defense mechanisms against infections. Findings from the hematological investigations in this study revealed that HTLV infected individuals had higher but statistically insignificant levels for leucocytes and lymphocytes concentrations compared to the non-infected individuals. The HTLV effect on lymphocytes may be related to viral transactivation or immune response (Bartman *et al*., 2008).

Nasir *et al*. (2015) reported that HTLV-1/HIV-1-coinfected individuals had higher but statistically insignificant total leukocyte counts as well as low insignificant total lymphocyte counts when compared with HIV monoinfected patients. The reason for no difference in mean concentrations may be due to the fact that HTLV does not lyse infected cell but rather transform them.

This study also showed significantly higher platelet counts in HTLV infected women compared to HTLV negative women. This agrees with the findings of Bartman *et al.* (2008). The reason for the observed difference in platelet values may be related to up-regulation of either IL-6, thromboprotein (TPO), or its receptor monophosphoryl lipid A through HTLV-1 and HTLV-2 infection (Bartman *et al*., 2008). A report by Majka and colleagues (2002) indicated that both IL-6 and TPO were capable of protecting megakaryocytes from apoptosis and stimulating various cell types responsible for megakaryopoiesis.

CHAPTER SIX

6.0 CONCLUSION AND RECOMMENDATION

6.1 Conclusion

This study established a 3.2% seroprevalence of Human T-cell lymphotrophic virus among women attending some post-natal clinics in Zaria, Nigeria, indicating that HTLV is endemic in Zaria.

There was no significant association between HTLV seroprevalence and the risk factors studied ($p>0.05$), except for history of blood transfusion ($P= 0.01$).

A relationship between HTLV infection and Wilms tumour antigen concentration was established; as the level of WT1 antigen was found to be significantly higher ($P=0.00$) in HTLV seropositive women (116.22±18.39pg/ml) compared to HTLV seronegative women (66.41±20.13 pg/ml).

The study also established a statistically significant effect in the platelet count among the HTLV seropositive women ($P= 0.017$).

6.1 Recommendation

In view of the 3.2% prevalence obtained from this study which indicates endemicity, it is recommended that awareness should be created among population on mode of transmission of the virus.

Due to the significant association found with the history of previous blood transfusion and HTLV infection, it is recommended that HTLV testing should be incorporated in the routine test for blood donors.

Further research should be done on leukocytes sub-sets like CD4+ and CD8+ counts with reference to HTLV infection due to the non-significant effect of HTLV infection on white blood cell and lymphocyte counts.

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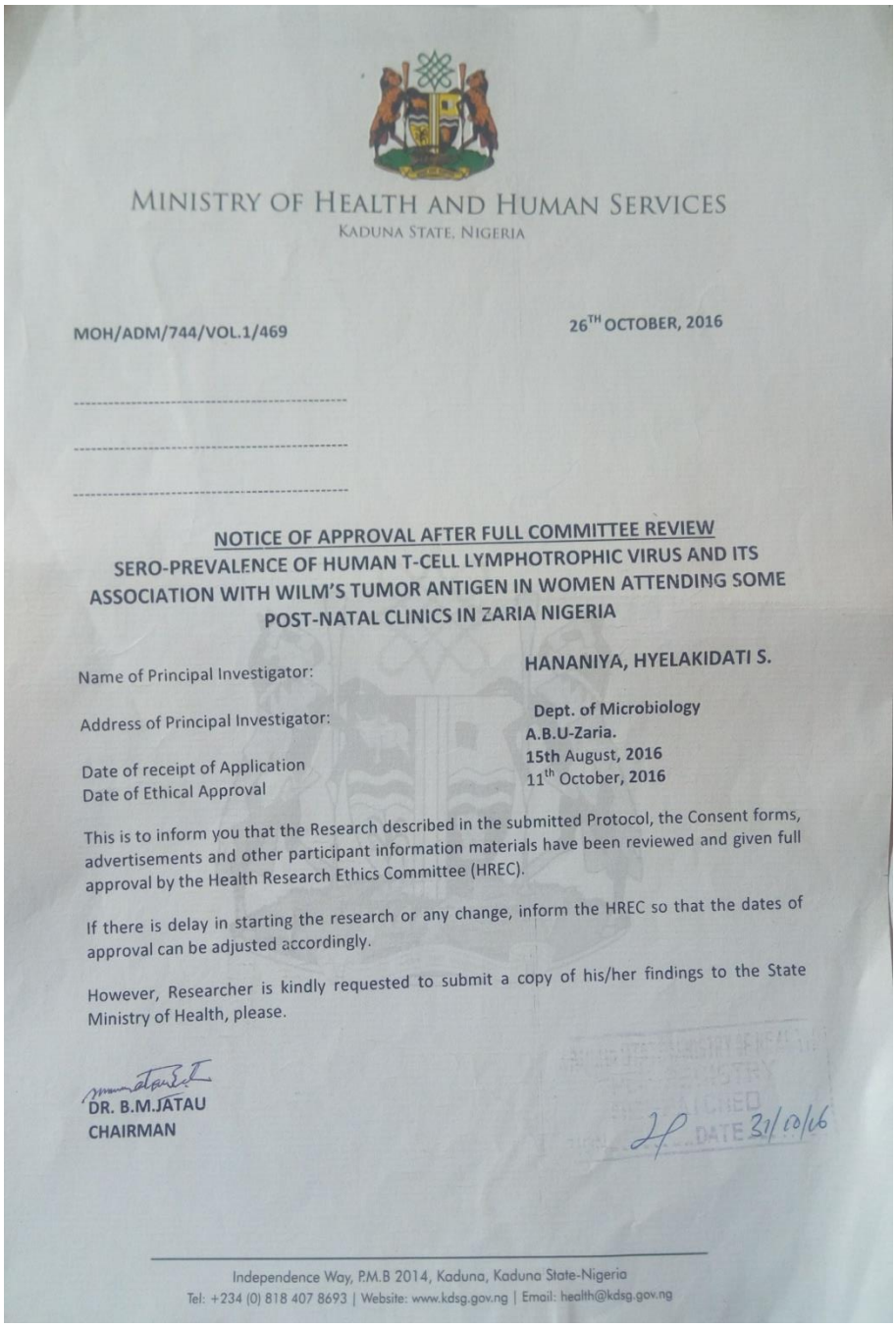
APPENDICES

Appendix I: Ethical clearance obtained from the Ethics Committee of the Kaduna State Ministry of Health.



MINISTRY OF HEALTH AND HUMAN SERVICES
KADUNA STATE, NIGERIA

MOH/ADM/744/VOL.1/469 — 26TH OCTOBER, 2016

NOTICE OF APPROVAL AFTER FULL COMMITTEE REVIEW

SERO-PREVALENCE OF HUMAN T-CELL LYMPHOTROPHIC VIRUS AND ITS ASSOCIATION WITH WILM'S TUMOR ANTIGEN IN WOMEN ATTENDING SOME POST-NATAL CLINICS IN ZARIA NIGERIA

Name of Principal Investigator: **HANANIYA, HYELAKIDATI S.**

Address of Principal Investigator: **Dept. of Microbiology, A.B.U-Zaria.**

Date of receipt of Application: **15th August, 2016**

Date of Ethical Approval: **11th October, 2016**

This is to inform you that the Research described in the submitted Protocol, the Consent forms, advertisements and other participant information materials have been reviewed and given full approval by the Health Research Ethics Committee (HREC).

If there is delay in starting the research or any change, inform the HREC so that the dates of approval can be adjusted accordingly.

However, Researcher is kindly requested to submit a copy of his/her findings to the State Ministry of Health, please.

DR. B.M.JATAU
CHAIRMAN

DATE 31/10/16

Independence Way, P.M.B 2014, Kaduna, Kaduna State-Nigeria
Tel: +234 (0) 818 407 8693 | Website: www.kdsg.gov.ng | Email: health@kdsg.gov.ng

Appendix II: Consent Form

My name is HANANIYA HYELAKIDATI SAMUEL, a student of Microbiology Department, Ahmadu Bello University, Zaria. I am undertaking a study on the prevalence of human T-cell lymphotrophic virus and its association with Wilms tumor antigen in women attending some post natal clinics in Zaria, Nigeria. This study has been reviewed and granted approval by the Ethics Committees of the Federal Ministry of Health Kaduna State.

I would very much appreciate your participation in this study. This information will help the Government to plan health services and it will be strictly confidential and as well not shown to other persons. Should you have any queries, feel free to contact the Chairman of the Ethics Committee of this Hospital.

As part of the study, you will be asked some questions about your life style and 5ml of blood shall be collected from you. All the answers you give shall be confidential. Participation in the study is completely voluntary. Should you come across any question you do not want to answer, you can leave it and go on to the next or you can stop answering questions at any time. However, I hope you will participate in the study since your information is of paramount importance.

_____ _____

Signature of respondent Date

Appendix III: RESEARCH QUESTIONNAIRE

DEPARTMENT OF MICROBIOLOGY

FACULTY OF LIFE SCIENCES

AHMADU BELLO UNIVERSITY, ZARIA

Topic: Sero-prevalence human of T-cell lymphotrophic virus and its association with wilms tumor antigen in women attending some post natal clinics in Zaria.

Dear respondent I am a student carrying out research on the above topic, I request that u please complete the questions objectively as any information collected will be strictly used for research purpose and will be treated confidentially

Instruction: please tick as appropriate and provide answers where applicable.

Hospital location……..

Patient serial no…….

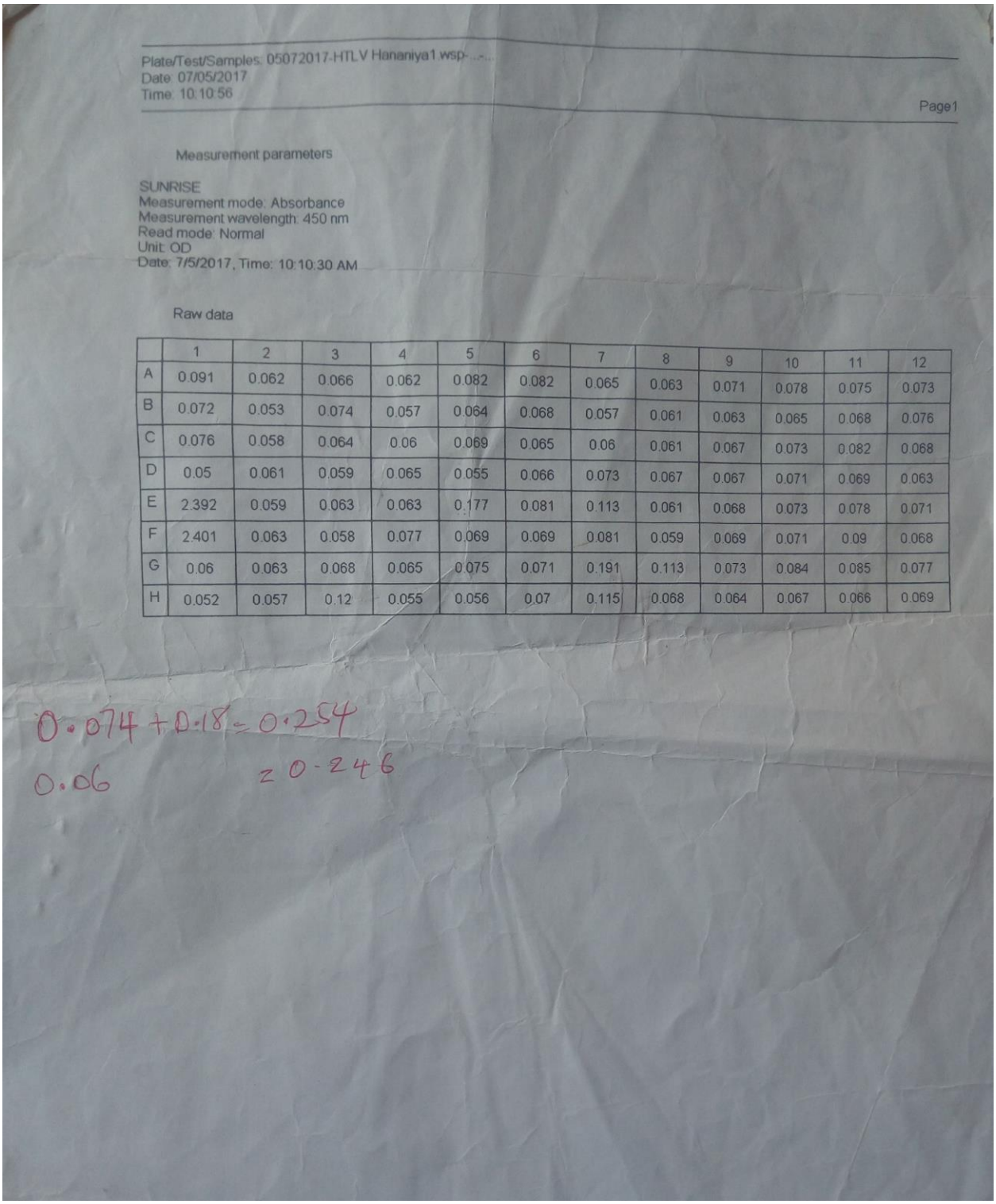
PATIENT"S DATA

1. Age: ( )
2. Level of education: Primary ( ) Secondary ( ) Tertiary ( ) None ( )
3. Marital status: Married ( ) Single ( )
4. Type of marriage: Monogamy ( ) Polygamy ( )
5. Occupation: Self-employed( ) Civil servant( ) Unemployed( ) Farmer( ) other ( )

SOME ASSOCIATED RISK FACTORS

1. History of blood transfusion: Yes ( ) No ( )
2. History of drugs use: Yes ( ) No ( )
3. History of cancer in family: Yes ( ) No ( )
4. Period of breast feeding: 0-3 months( ) 3-6 months ( ) > 6 months ( )
5. Sharing of needles and syringes: Yes ( ) No ( )
6. Number of births. ( )
7. History of Sexually Transmitted Disease: Yes ( ) No ( )
8. History of smoking: Yes ( ) No ( )

Appendix III: HTLV ELISA Printout



Plate/Test/Samples: 05072017-HTLV Hananiya1.wsp- ...-...
Date: 07/05/2017
Time: 10:10:56

Page1

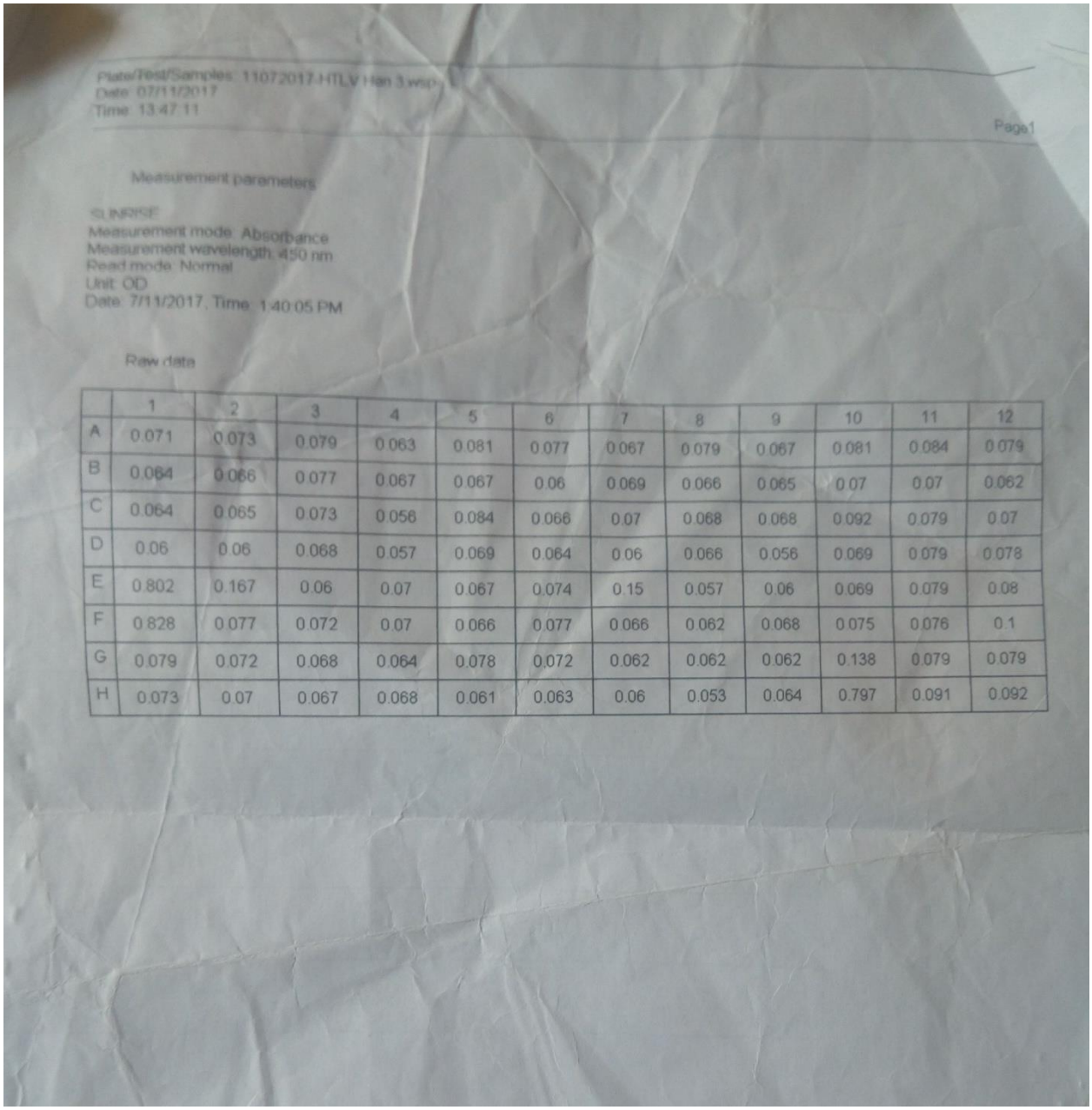
Measurement parameters

SUNRISE
Measurement mode: Absorbance
Measurement wavelength: 450 nm
Read mode: Normal
Unit: OD
Date: 7/5/2017, Time: 10:10:30 AM

Raw data

	1	2	3	4	5	6	7	8	9	10	11	12
A	0.091	0.062	0.066	0.062	0.082	0.082	0.065	0.063	0.071	0.078	0.075	0.073
B	0.072	0.053	0.074	0.057	0.064	0.068	0.057	0.061	0.063	0.065	0.068	0.076
C	0.076	0.058	0.064	0.06	0.069	0.065	0.06	0.061	0.067	0.073	0.082	0.068
D	0.05	0.061	0.059	0.065	0.055	0.066	0.073	0.067	0.067	0.071	0.069	0.063
E	2.392	0.059	0.063	0.063	0.177	0.081	0.113	0.061	0.068	0.073	0.078	0.071
F	2.401	0.063	0.058	0.077	0.069	0.069	0.081	0.059	0.069	0.071	0.09	0.068
G	0.06	0.063	0.068	0.065	0.075	0.071	0.191	0.113	0.073	0.084	0.085	0.077
H	0.052	0.057	0.12	0.055	0.056	0.07	0.115	0.068	0.064	0.067	0.066	0.069

0.074 + 0.18 = 0.254
0.06 = 0.246



Plate/Test/Samples: 11072017-HTLV Han 3.wsp
Date: 07/11/2017
Time: 13:47:11

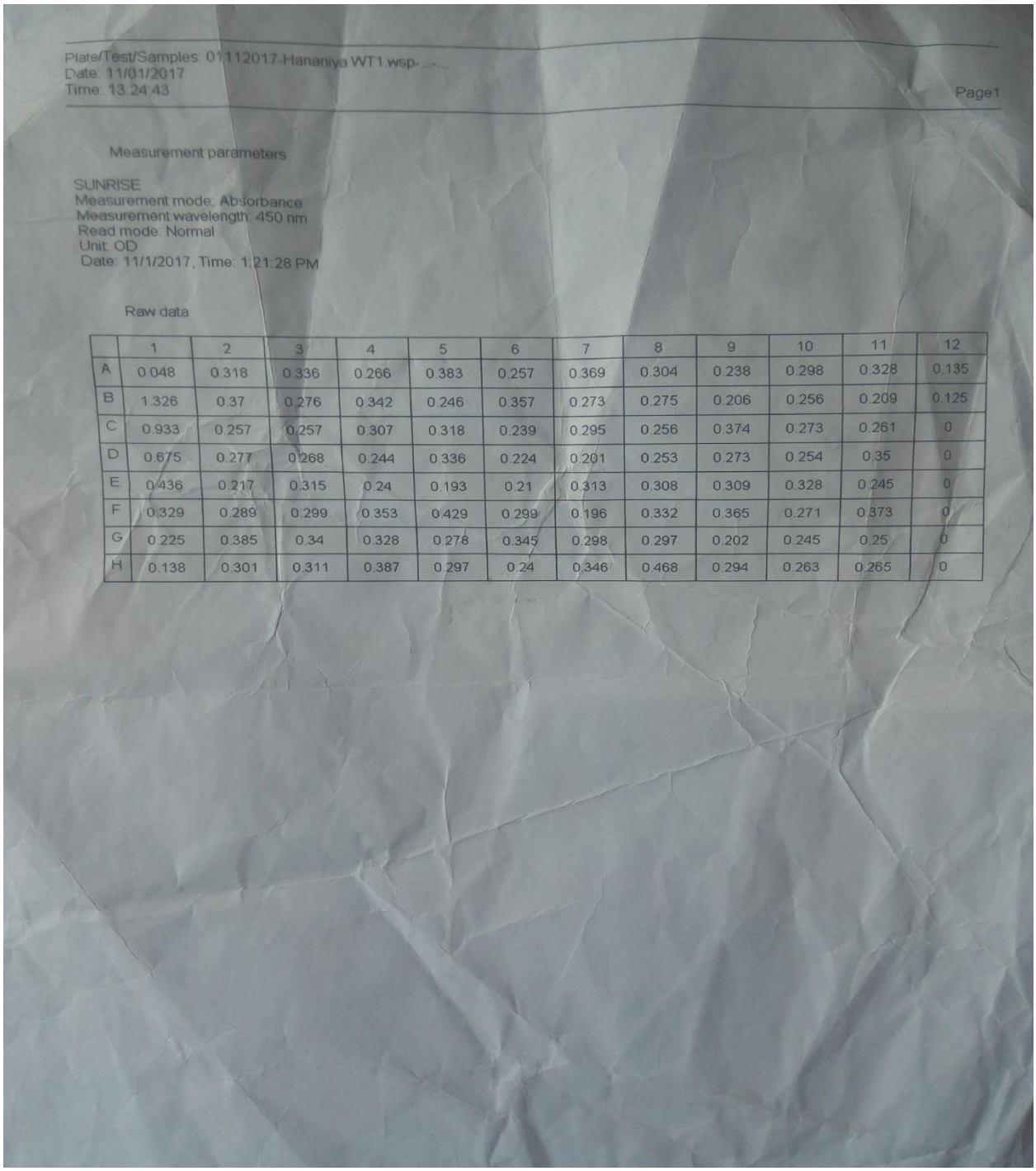
Measurement parameters

SUNRISE
Measurement mode: Absorbance
Measurement wavelength: 450 nm
Read mode: Normal
Unit: OD
Date: 7/11/2017, Time: 1:40:05 PM

Raw data

	1	2	3	4	5	6	7	8	9	10	11	12
A	0.071	0.073	0.079	0.063	0.081	0.077	0.067	0.079	0.067	0.081	0.084	0.079
B	0.064	0.066	0.077	0.067	0.067	0.06	0.069	0.066	0.065	0.07	0.07	0.062
C	0.064	0.065	0.073	0.056	0.084	0.066	0.07	0.068	0.068	0.092	0.079	0.07
D	0.06	0.06	0.068	0.057	0.069	0.064	0.06	0.066	0.056	0.069	0.079	0.078
E	0.802	0.167	0.06	0.07	0.067	0.074	0.15	0.057	0.06	0.069	0.079	0.08
F	0.828	0.077	0.072	0.07	0.066	0.077	0.066	0.062	0.068	0.075	0.076	0.1
G	0.079	0.072	0.068	0.064	0.078	0.072	0.062	0.062	0.062	0.138	0.079	0.079
H	0.073	0.07	0.067	0.068	0.061	0.063	0.06	0.053	0.064	0.797	0.091	0.092

Appendix IV: WT1 ELISA Printout



Plate/Test/Samples: 01112017-Hananiya WT1.wsp-...-...
Date: 11/01/2017
Time: 13:24:43

Page1

Measurement parameters

SUNRISE
Measurement mode: Absorbance
Measurement wavelength: 450 nm
Read mode: Normal
Unit: OD
Date: 11/1/2017, Time: 1:21:28 PM

Raw data

	1	2	3	4	5	6	7	8	9	10	11	12
A	0.048	0.318	0.336	0.266	0.383	0.257	0.369	0.304	0.238	0.298	0.328	0.135
B	1.326	0.37	0.276	0.342	0.246	0.357	0.273	0.275	0.206	0.256	0.209	0.125
C	0.933	0.257	0.257	0.307	0.318	0.239	0.295	0.256	0.374	0.273	0.261	0
D	0.675	0.277	0.268	0.244	0.336	0.224	0.201	0.253	0.273	0.254	0.35	0
E	0.436	0.217	0.315	0.24	0.193	0.21	0.313	0.308	0.309	0.328	0.245	0
F	0.329	0.289	0.299	0.353	0.429	0.299	0.196	0.332	0.365	0.271	0.373	0
G	0.225	0.385	0.34	0.328	0.278	0.345	0.298	0.297	0.202	0.245	0.25	0
H	0.138	0.301	0.311	0.387	0.297	0.24	0.346	0.468	0.294	0.263	0.265	0