Measurement of immune response to Hepatitis B vaccine in HIV-1 infected children and Healthy children

Dissertation submitted as part of fulfilment for the M.D. (Branch- IV Microbiology) Degree examination of the Tamil Nadu Dr. M.G.R. Medical University, to be held in May-2019

CERTIFICATE

This is to certify that the dissertation titled **"Measurement of immune response to Hepatitis B vaccine in HIV-1 infected children and Healthy children"** is a bonafide work done by Dr. Sonia.M towards the **M.D. (Branch-IV, Microbiology)** degree examination of the Tamilnadu Dr. M.G.R. Medical University, to be held in May, 2019.

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I hereby declare that this M.D Dissertation entitled **"Measurement of immune response to Hepatitis B vaccine in HIV-1 infected children and healthy children"** is the bonafide work done by me under the guidance of Dr. Rajesh K, Professor, Department of Clinical Virology, Christian Medical College and Hospital, Vellore. This work has not been submitted to any other university in part or full.

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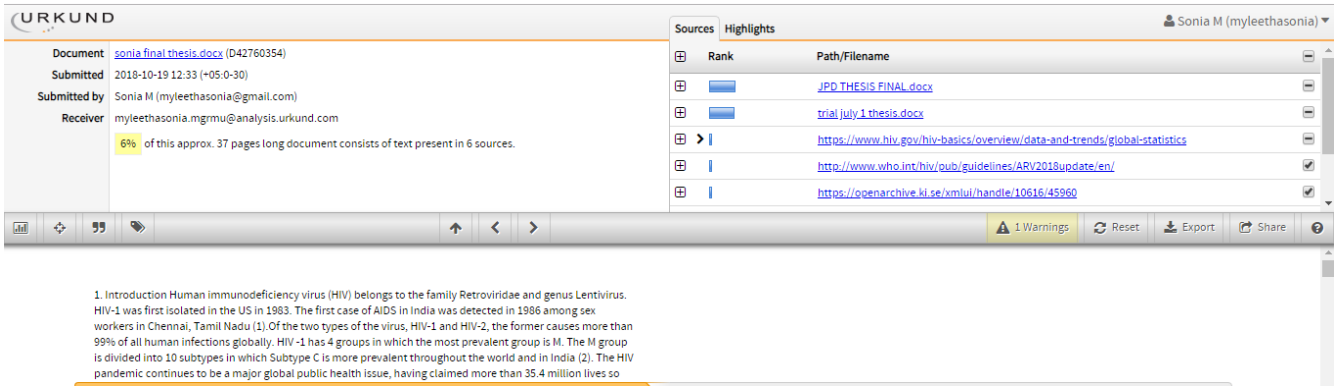

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Contents

1. Introduction

Human immunodeficiency virus (HIV) belongs to the family Retroviridae and genus *Lentivirus.* HIV-1 was first isolated in the US in 1983. The first case of AIDS in India was detected in 1986 among sex workers in Chennai, Tamil Nadu(1).Of the two types of the virus, HIV-1 and HIV-2, the former causes more than 99% of all human infections globally. HIV -1 has 4 groups in which the most prevalent group is M. The M group is divided into 10 subtypes in which Subtype C is more prevalent throughout the world and in India(2).

The HIV pandemic continues to be a major global public health issue, having claimed more than 35.4 million lives so far. Globally, 940 000 people died from HIV-related causes in 2017. There were approximately 36.9million people living with HIV at the end of 2017 with 1.8 million people becoming newly infected with HIV in 2017 globally. Sub-Saharan Africa is the most affected region, with 25.7 million people living with HIV in 2017(3),(4). An estimated 1.8 million children are living with HIV at the end of 2017, mostly (88%) in sub-Saharan Africa(4) According to 2017 UNAIDS reports, there were 160,000 new HIV infections in children at the end of 2016and 120,000 children died of AIDS-related illnesses in the same year. Estimated 43 % of children living with HIV are on ART.(5). As per the recently released, India HIV Estimation 2016 report, the total number of People Living with HIV (PLHIV) in India is estimated at 20.9 lakhs (17.11 lakhs–26.49 lakhs) in 2017 and among this children (< 15 years) account for 6.54% (1.38 lakhs)(6).

More than 90 percent of HIV infections in children result from mother to child transmission.(7). Mother to child transmissionof HIV can occur before (intrauterine), during (intrapartum), or after delivery (through breastfeeding). Transfusion of infected blood or

blood products have accounted for 3-6 % of all paediatric AIDS cases. A higher percentage of HIV infected children acquire the virus intrapartum evidenced by the fact that 70-80% of infected infants do not demonstrate detectable virus until 1 week of age. The mechanism of transport appears to be mucosal exposure to infected blood and cervicovaginal secretions in the birth canal (8). In the absence of any interventions during these stages, rates of HIV transmission from mother-to-child can be between 15–45%(3).

MTCT can be nearly fully prevented if both the mother and the baby receiveappropriate prophylaxis and concerned measures are taken. WHO recommends lifelong ART for all people living with HIV, regardless of their CD4 count, clinical stage of disease, and this includes women who are pregnant or breastfeeding. In 2017, 80% of the estimated 1.1 million pregnant women living with HIV globally received ARV treatments to prevent transmission to their children.They are provided with ARV drugs as early as possible in pregnancy and during the period of breastfeeding.(3).

HIV infects and invades human CD4+ T helper cells which acts as the receptor for the virus. After entry, the reverse transcriptase enzyme transcripts the viral RNA into double-stranded DNA which gets integrated into the genome of the host cell forming an integrated provirus. There is a rapid and persistent replication, depleting this subset of T cells by various direct and indirect mechanisms. As there is an imbalance between the T cell production and destruction, intense immunosuppression occurs(8)

Children living with HIV get sick more severely than adults as their immune systems are not fully developed. Children experience more rapid disease progression than adults. Half of

the untreated children dying within first 2 years of life. The rapid progression is correlated with higher viral burden and faster depletion of CD4 T lymphocytes in infants and children than in adults. Infections occurring in HIV infected children are same like those happening in healthy children, but they cannot fight back since their immune system is deranged. In developing countries tuberculosis, diarrhea, and respiratory illnesses are common infections in HIV-positive children(9)

Changes in the immune system of HIV-infected children are similar to adults infected with HIV. CD4 + T cell depletion may be less dramatic because infants normally have a relative lymphocytosis. A value of 1,500 CD4 cells/mm^3 in children <1 year of age is indicative of severe CD4 depletion and is comparable to <200 CD4 + T cells/mm^3 in adults. Lymphopenia is relatively rare in perinatally infected children and is usually only seen in older children or those with end-stage disease. (10, 11).

Polyclonal activation of B cells occurs in most children early in the infection, as evidenced by elevation of IgA, IgM, IgE, and particularly IgG (hypergammaglobulinemia**)**, with high levels of anti–HIV-1 antibody. This response may reflect both dysregulation of T-cell suppression of B-cell antibody synthesis and active CD4 enhancement of B-lymphocyte humoral response. As a result, antibody response to routine childhood vaccinations may be abnormal. Despite the increased levels of immunoglobulins, some children lack specific antibodies or protective antibodies(10).

Vaccination against vaccine preventable disease is one of the supportive measures which can decrease mortality in HIV infected children. Hepatitis B and HIV have common mode of transmission and co-infection with these two infected agents are well documented.

Seroconversion among HIV infected children to Hepatitis B vaccine has been studied before such as in Tanzania was 59 %, andin Moraccowas 29%(12,13).In a study done in India seroconversion after double dose of Hepatitis B vaccine was 94 % (14).In a study after a 3-dose HBV revaccination among HIV-infected children with immune recovery (CD4 cell ≥15%) while on HAART,seroconversion3 years after the revaccination was 71.0% (15).

Response to vaccination depends on CD4 count at the time of vaccination(14). Poor response to vaccine may be due to destruction of CD4 cells by HIV. These cells provide a critical help to B cells in the production of antibody against T cell dependent antigens and the differentiation of B cells into memory cells. Impaired generation of HBV specific memory B cells following vaccination may result in acceleration of decline of protective antibody. With further increase in dose and number of vaccines have added on to better immunization response (14). Several studies have demonstrated that the immune response to HBV vaccine among HIV infected children and adults may be improved by using high vaccine doses, giving additional doses, or using immunomodulatory agent(14,16,17) .

Since immune responses to vaccines are influenced by nutritional status, socioeconomic groups and genetic factors, these may vary in different geographical areas in different population groups. Thus, data obtained from the western/other countries cannot be reliably extrapolated to Indian population. Also as there are not much study in Indian literature on

seroconversion after vaccination among HIV infected children, this study would be useful for assessing the immune response for the Indian scenario. This may help in taking some policy decision on number of doses and schedules of HBV vaccination in children like is being currently done in adults.

The introduction of HAART has resulted in immune recovery and reduction of morbidity and mortality in HIV infected children. So the immune response to HBV vaccine will be different from those who are not on HAART treatment (14). So this study also looked into the effects of HAART treatment on the immune memory cell response to Hepatitis B vaccine in HIV-infected children.

In this study we are evaluating the immune response in HIV positive children against Hepatitis B Vaccine (HBV). As HBV is a subunit vaccine, it is administered safely as a part of routine immunization schedule even in immune compromised children. We will evaluate the cellular immunity by checking the T cell response through IFNγ flowcytometric intracellular cytoplasmic staining assay. The humoral immunity by assessing anti-HBs antibody titre and memory B cells through flow cytometry surface staining. In case where antibodies are not formed, memory B cell detection would give add on to the humoral immune response.

2. Hypothesis and Objectives

Hypothesis: Hepatitis B vaccine immune response is lower in HIV infected children.

Objectives:

1) To assess the CD4+ T cell response against Hepatitis B vaccine in HIV-infected children and healthy children
2) To assess the B cell memory response against Hepatitis B vaccine in HIV-infected children and healthy children.
3) To quantify the difference in the anti HBs antibody titre in HIV-infected children and healthy children.
4) To determine the impact of ART in anti-HBs kinetics among HIV-infected Children.

3. Review of Literature

3.1 History

In 1981, healthy homosexual men were reported with pneumonia associated with rare organism like pneumocystis jirovecii in United States. It was in 1982 that the term "acquired immunodeficiency syndrome," or AIDS was first used by public health officials. In 1983,this virus was isolated from a patient with generalized lymphadenopathy by Dr. Luc Montagnier in Pasteur Institute, Paris and was named as lymphadenopathy-associated virus (LAV) (18,19). Around the same time, this virus was named as HTLV-III (human T-cell lymphotropic virus-type III) by Dr. Robert Gallo with his scientists in National Institute of health (NIH), Maryland isolated and, because it shared some features with HTLV I and HTLV II (20,21). In 1984, Dr. Jay Levy and his team from University of California isolated the virus and named it as AIDS associated Retroviruses (ARV)(22). In 1986, According to the statements of the International Committee on Taxonomy of Viruses in 1986, the retrovirus which was recognized to be the etiologic agent of AIDS was to be renamed as Human immunodeficiency virus (HIV). This was done with an intension to remove the multiple names that were in circulation for the virus then (23).

3.3 Epidemiology

3.31 Global scenario

Since the start of the epidemic77.3 million people have become infected, and 35.4 million people have died from AIDS-related illnesses. By the end of the year 2017, there were 36.9 million people living with HIV with 35.1 million adults and 1.8 million children

(<15 years) (24). In 2017, 21.7 million People were accessing antiretroviral therapy. In the same year, 59% of adults aged 15 years and older living with HIV had access to treatment, as did 52% of children aged 0–14 years. And mortality due to HIV-related illness was 940000 people. 1.6 million adults and 1,80,000 children became newly infected with HIV in 2017 (24–26).

The worst affected region is the sub-Saharan Africa where 1 in every 25 (4.1%) adults is living with HIV which accounts for approximately 70% of people living with HIV worldwide.

In Asia and the pacific- people living with HIV accounted for 5.2 million. Among the new HIV infection which was 280000 ,only 10000 were children of 0-14 years of age and the rest of them were >15 years .2.7 million people had access to treatment and there were 170000 AIDS related deaths in 2017.

In 2013, United Nations set 90–90–90 goals in view of ending the AIDS epidemic by 2030. The goals are by2020, 90% of all people living with HIV will know their HIV status, 90% of all people with diagnosed HIV infection will receive sustained antiretroviral therapy and 90% of all people receiving antiretroviral therapy will have viral suppression.

In 2017, seventy five percent people living with HIV (75%) knew their seropositive status. Among people who knew their seropositive status, 79% were accessing ART. And among people accessing ART, 81% were virally suppressed.(24)

Children living with HIV are mostly from sub-Saharan Africa. Majority of them acquire HIV from their HIV-infected mothers during pregnancy, birth or breastfeeding. In 2013 7,40000 children were on ART.(27).

3.2.2 Indian scenario

The first case of AIDS in India was detected in the year 1986 among commercial sex workers living in Tamil Nadu(1).In India, the estimated prevalence of HIV in adults between 15 and 49 years of age is 0.26%, according to the India HIV Estimation Report for the year 2016-2017.

The highest prevalence of 1.15% is seen in the state of Manipur which is followed by Mizoram with 0.80%, Nagaland with 0.78%, Andhra Pradesh and Telangana with 0.66%, Karnataka having 0.45%, Gujarat with 0.42% and Goa having 0.40%. Also Maharashtra, Chandigarh, Tripura and Tamil Nadu have an adult HIV prevalence rate more than the national prevalence. The national prevalence of HIV among adults has gradually been decreasing from a peak of 0.38% in 2001-2003 through 0.34% in 2007 and 0.28% in 2012 to 0.26% in 2015 (26)The number of people living with HIV (PLHIV) was estimated to be 22.26 lakhs in 2007 and 20.9 lakhs in the end 2017 and among them children (< 15 years) account for 6.54% (1.38 lakhs)(6). The prevalence among high risk groups like female sex workers (2.2%), men who have sex with men (4.3%), transgender (7.5%) and intravenous drug users (9.9%) is assessed by the National Integrated Behavioral and Biological Surveillance (8). However nor the virus or the disease is limited to the high risk groups alone. HIV-1 is transmitted to the general low risk

population through a bridging population which includes long distance truck drivers and migrant labourers who maybe clients or partners of commercial sex workers(28)

3.3 Origin of HIV:

Ancestral date of common ancestor of HIV-1 (group M) based on the molecular clock analysis calculated was estimated to be the year 1930(29). It was a sailor in Manchester who was the earliest known case of AIDS(30).HIV-1 and HIV-2 transmission to the human population was found to be by the non-human primate *lentiviruses a*cross species transmission from the chimpanzee (*Pan troglodytes*) species, HIV-1 entered the human population and that of HIV-2 was from sooty mangabey (*Cercocebusatys*) species. (31,32)

3.4 Structure of HIV

HIV belongs to Lentivirus family of retroviruses. It is an RNA virus with a capsid and a lipid envelope. (33) The structure of HIV is roughly spherical and measures approximately 100 nm in diameter. Host cell membrane forms the outer envelope formed of lipid bilayer. It contains host cell proteins and the viral envelope glycoproteins gp160 consists of a mushroom shaped glycoprotein which has a head of molecular weight 120 KD and trans membrane stalk which is also known as gp41.The electron dense conical component forms the core in HIV 1which contains viral RNA and enzymes like, reverse transcriptase, protease and integrase. These are necessary for the viral replication. The genetic material of HIV-1 virus is two single stranded copies of RNA which is approximately 9 kb in size and are bound with ribonucleoproteins(34,35). Between the

core and the envelope forms the matrix region. **Figure 3.1** shows the structure of the HIV-1 virus and its components

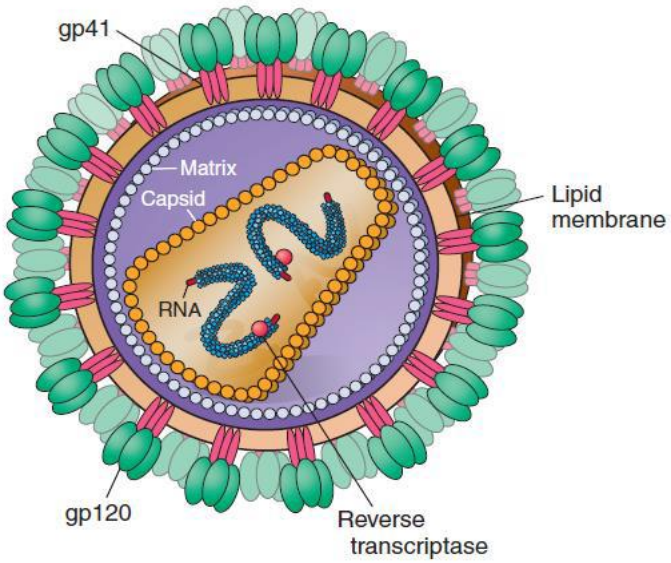


Figure 3.1: Structure of HIV (Adapted from Harrison's Principles of Internal Medicine, 19 th edition)

3.5 Genome

The virus has 2 copies of 9.2 kilo bases of RNA that is single stranded. The long terminal repeat (LTR) flanks the 5' and 3' region of the HIV genome. The HIV genome is classified broadly as genes encoding for the structural proteins ie*gag, pol, and env* and genes encoding for nonstructural proteins proteins that are *tat and rev, vpu, vpr, vif, and nef , vpx , LTR*.The gag precursor protein (55 kDa) protein which is further cleaved down into other proteinsp24 capsid, p17 matrix, p7 nucleo capsid by the protease enzyme.The pol precursor protein is cleaved into various enzyme products such as RT (reverse

transcriptase), PR (protease) and IN (integrase) proteins.*Env*gene encodes for the gp120 and gp41 subunits found in the outer membranes of HIV- 1(33).

These individual proteins is responsible for all the crucial activities for HIV in terms of its infectivity, replication, maintain latency etc. **Figure3.2 shows** the genome organization of the HIV-1 virus.

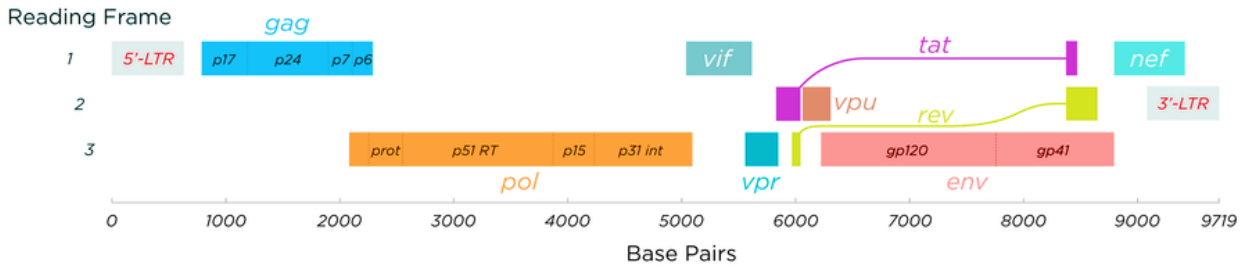


Figure 3.2: HIV-1 RNA genomic structure (Adapted from Scientific Illustration, www.scistyle.com)

3.6 HIV classification:

The Human Immunodeficiency Virus is broadly classified as HIV-1 and HIV-2. The HIV-1 is primarily involved globally in epidemics while HIV-2 is less common and isrestricted geographically (32) to certain regions in Africa and in the Indian subcontinent(36). In comparison with HIV-2, HIV-1 is highly pathogenic and leads to faster disease progression however some HIV-2–infected patients develop advanced immunodeficiency and AIDS-related conditions, which can even lead into death (37). Till now 4 groups/clades of HIV-1 isolates are designated which are M (Major/Main), N (novel or Non-M, Non-O) O (Outlier) and the recently identified P group(38,39).These

are based on nucleotide sequence variation between individual groups by whole genome sequencing of *gag, pol* and *env* gene. The most prevalent group is M group compared to N O and P which are restricted to Central Africa . The M group is further subdivided into 10 non recombinant subtypes (A, B, C, D, F, G, H, J, K) (41,42). Subtype C is prevalent worldwide. The genetic variation seen among different clades ranges from 15 to 20% while 25 to 35% nucleotide variation is seen in case of subtypes. (43).

Table3.1 The geographical distribution of HIV-1 subtypes varies worldwide.

Subtype	Global Prevalence	Regions
Subtype C	45 %	South Africa, Brazil, India
Subtype A	12.3%	Central Africa, Central Asia and Eastern Europe
Subtype B	10.2 %	United states, East Asia and Western Europe
Subtype D	2.5 %	East and West Africa
Subtype G	6.3 %	East and West Africa
Subtype F, H, J and K	< 1 %	Central Africa

Apart from the subtypes the HIV-1 strains also undergo recombination between the subtypes due to the "template switching" ability of the HIV-1 reverse transcriptase

enzyme and so far two types of recombinant forms have been identified 1.) Circulating recombinant forms (CRF) 2.) Unique recombinant forms (URF) (44,45).

In India, subtype C shows a high prevalence. In North India 78.4% and western India 96% of HIV-1 strains were subtype C and the majority of the strain circulating were C3. South Indian data was much similar to this too (46).

HIV-2 subtypes recognized in the world were A-H (35–37). The HIV-2 strains identified in India till date are subtype A (38). The subtype A strain is the predominant in West African countries. Subtype A is estimated to cause 0.11% of all HIV infections in humans(42,46)

3.7 Replication

The primary targets of the HIV virus are the immune cells namely CD4+ T lymphocytes and macrophages. Replication starts when gp120 protein of virus attaches to the CD4 receptors and co-receptors present on the surface of target cells resulting in conformational changes in gp120. The chemokine receptors, CCR5 and CXCR4 serves as the co-receptors for the macrophage trophic and non- macrophage trophic strains respectively. This results in exposure of the transmembrane envelope glycoprotein gp41 leading to fusion of the virion and target cells(47). The HIV enzyme reverse transcriptase uses the host nucleotides and converts the viral RNA into single-stranded DNA. The double stranded DNA is synthesized from single stranded DNA by reverse transcriptase. The enzyme integrase integrates the double stranded DNA into the host DNA. Thus the virus replicates along with the DNA of the host cell. Following this, transcription occurs to produce viral mRNA which is translated into viral proteins in the cytoplasm. The final

steps of HIV replication include assembly of the immature virion to the cell surface which leaves the host cell by budding. The mature virion that is released affects other immune cells thus continuing the process of replication (35,47)

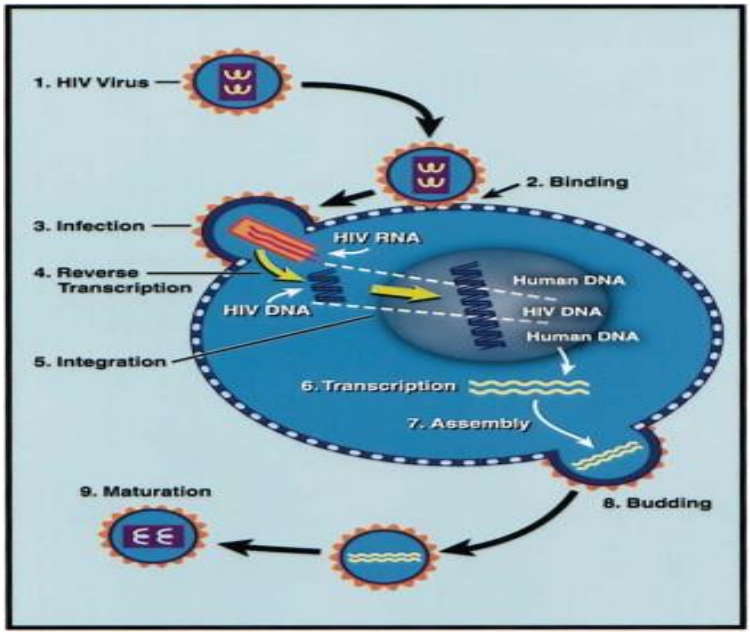


Figure 3.3 Replication of HIV (Adapted from Molecular virology and immunology of HIV infection. Javier Chinen, Shearer. The Journal of allergy and clinical immunology) 2002

3.8 Transmission

HIV-1 is predominantly transmitted through sexual contact (both heterosexual and homosexual), by blood and blood products and from mother to child – intrapartum, perinatal and through breast milk. Transmission largely depends upon the viral load and the duration of exposure to that particular body fluid (48).

The common route of HIV transmission worldwide is through sexual contact and accounts for 80% of adult HIV infection. The prevalence of HIV infection in our country is 9.9% in intravenous drug users(6). The relative proportions of mother to child transmission of HIV are 23-30% before delivery, 50-60% during delivery and 12-20% during breast feeding(34)

Elective cesarean section along with zidovudine therapy in the mother and infant decreases transmission by 87%.The benefit of cesarean section is probably negligible if the mother's viral load is <1,000 copies/mL (8).

WHO recommends that in developing countries where other diseases (diarrhea, pneumonia, malnutrition) substantially contribute to a high infant mortality rate, the benefit of breast-feeding outweighs the risk for HIV transmission, and HIV-infected women in developing countries should breast-feed their infants for at least the 1st 6 months of life (49).

3.9 Pathogenesis

3.91 Immunopathogenesis

HIV infection affects the immune system, dendritic cells being the first cells to be affected. These cells collect and process antigens and transport them to the lymphoid tissue. In the lymphatic tissue, the binds to cells expressing CD4 molecules on their surface, primarily helper T lymphocytes ($CD4^+$ T cells) and cells of the monocyte-macrophage lineage.(8) Follicular dendritic cells produce complement or anti-HIV

antibodies in the lymph node to trap and hold the virus in the germinal centers resulting in generation of effective memory immune response (50,51).

CXCR4, CCR5, CCR1 and CCR3 are chemokines necessary for HIV fusion and entry into cells. CCR5 gene deletion that is protective against HIV infection (CCR5Δ32) is relatively common in whites but is rare in blacks(8). In HIV infected patients, cellular activation is proved by the development of lymphadenopathy by the cell mediated immune response in the lymphoid tissue(52).

HIV infection of thymus affects the development of mature T-lymphocytes that are required for the specific cellular immunity of infection that occurs in early childhood (53) In acute stage of HIV infection HIV infected cells in high titres are present and seen in the lymphoid tissue followed by reduction of circulating $CD4^+$ T cells and eventual spread of HIV in tissues. The cellular deficiency and dysfunction of CD4+ cells are due to various mechanisms (34), as listed in **Table 3.2**

Table 3.2: Mechanisms of CD4 depletion (Adapted from Harrison's Principles of Internal Medicine, 19th edition)

Direct mechanisms	Indirect mechanisms
• Syncytia formation • Accumulation of unintegrated viral DNA • Alteration of plasma membrane permeability due to viral budding • Interference with cellular RNA processing	• Apoptosis and autoimmunity • Infected cells killed by HIV-specific immune response • Inhibition of T cell production by thymus • Bystander killing of viral antigen-coated cell

In the lymphoid organs there is an effective immune response against the virus that in turn lowers the efficiency of entrapment of the virus in the germinal centers by coating the virus particles with complement and antibodies which result in the less attachment of infected viral particles to follicular dendritic cells(5).

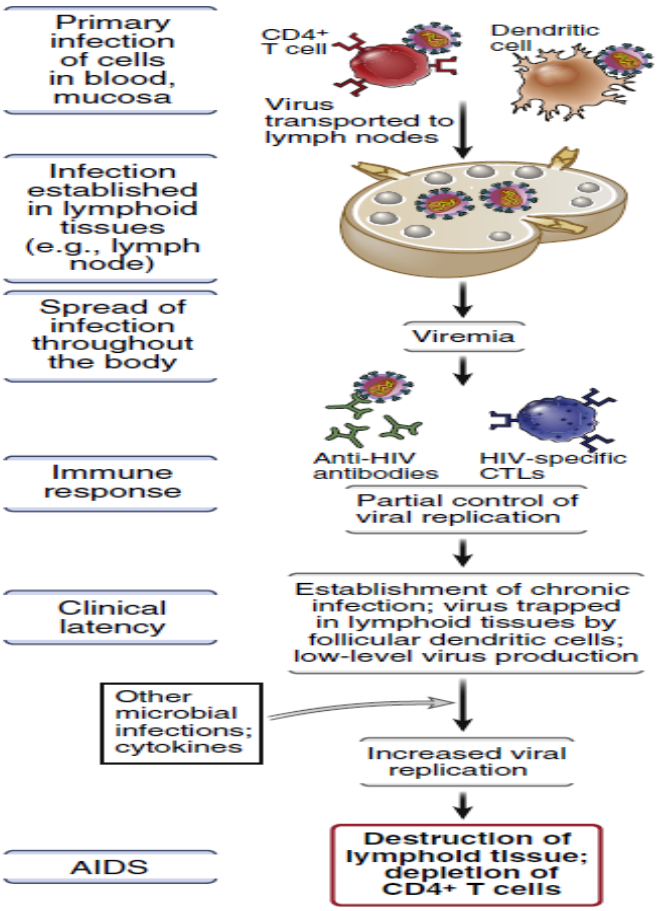


Figure 3.4 Pathogenesis of HIV (Adapted from Abbas AK, Lichtman AH, Pillai S. Basic Immunology. 2015. 3)

HIV infection progresses in the following way. An acute stage characterized by marked viral replication and dissemination, then a chronic asymptomatic phase of continued immune activation and viral replication and finally the advanced stage of AIDS(55).

Multiplication in the target cells following entry is indicated by non-specific symptoms of a viral illness like pyrexia, tiredness, lymphadenopathy, rash and muscle pain, the high viral RNA and p24 antigen in circulation and a transient fall in CD4+ cell counts. The

viremia leads to dissemination of the virus to all the lymphoid tissue (8,34,55). Once the infection is established it persists lifelong.

Slowly the immune system shows response resulting in fall of both viral RNA and p24 antigen the latter becomes undetectable and viral load gets fixed at a low level called the set-point. HIV evades immune-mediated elimination and instead flourishes and develops into a chronic persistent infection which may last a decade. Development of post-integration latency in infected CD4+ cells where the integrated HIV provirus remains latent until further activation is the reason for latency(8,35).

The ratio of infected CD4+ cells and viral RNA level rises and the disease continues to progress till the individual has AIDS. Once the CD4+ cell counts falls further, seemingly innocuous, self-limiting infections becomes fulminant and there increased risk of various malignancies (56). The course of events in an untreated HIV-infected person is shown in **Figure 3.5**.

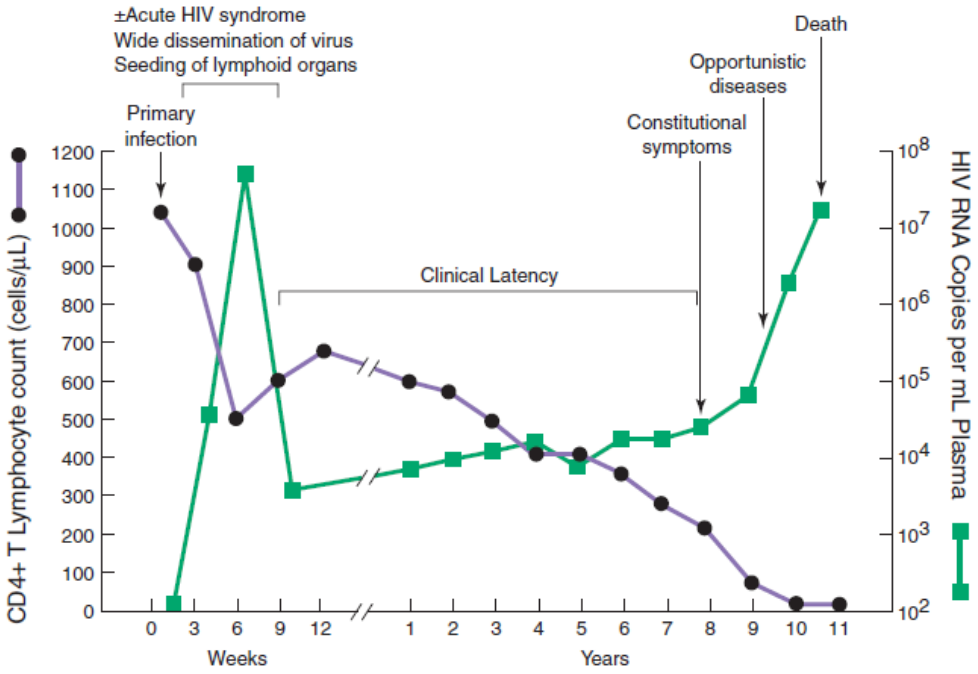


Figure 3.5 Viral load and CD4+ cell count during the course of disease in an untreated individual. (Adapted from Harrison's Principles of Internal Medicine, based on an original from Pantaleo et al, N Engl J Med 328:327, 1993)

3.92 Pathogenesis of Mother to child transmission of HIV

Before HAART became the mainstay of treatment, 3 patterns of disease were described in children. Approximately 15-25% of HIV-infected newborns in developed countries had a rapid disease course**,** with a median survival of 6-9 months if they were not treated. In underdeveloped countries, the majority of HIV-infected newborns will have this rapidly progressing disease. If intrauterine infection and the period of rapid expansion of CD4 cells in the fetus coincided, the virus could effectively infect the majority of the

body's immunocompetent cells, upon migration of these cells to the marrow, spleen, and thymus systemic dissemination of HIV would take place, as the immature immune system of the fetus wouldn’t be able to keep it in check. Thus, infection would be established before the normal ontogenic development of the immune system, resulting in severely impaired immunity. In these children during the first 48 hr of life presents with a positive HIV-1 culture and/or detectable virus in the plasma. This early viral presence indicates that the infection occured in utero. The viral load rapidly increases, peaking by two to three months of age and staying high for two years of life.

The newborns infected perinatally (60-80%) in developed countries have lower progression, with a median survival time of 6 years. Many patients have a negative viral culture or PCR in the 1st wk of life and are therefore considered to be infected intrapartum. In a typical patient, the viral load rapidly increases, peaking by two to three months of age and then slowly declines over 24 months. The slow decline in viral load contrary to that seen in adults may be partially explained by the immaturity of the immune system in newborns and infants.

The 3rd pattern of disease seen in a small percentage (<5%) of perinatally infected children referred to as long-term survivors (LTS), who have minimal or no progression of disease with relatively normal CD4 counts and very low viral loads for longer than 8 yr. Effective humoral immunity and/or CTL responses, host genetic factors (e.g., HLA profile), and attenuated virus may explain the delay in progression. A subgroup of the

LTS called “elite survivors” has undetectable viruses and probably reflects different or greater mechanisms of protection from disease progression.(8)

3.10 Immune response

3.10.1 Innate immune response to HIV

The first line of defense and consisting of innate immune cells which recognize and respond to infections quickly by recognizing pathogens by pattern recognition receptors (PRRs) is the innate immune system (57)

Target cells for HIV are the tissue macrophages. The infected macrophages lose their ability to ingest and kill foreign microbes and present antigen to T cells.

Dendritic cells (DCs**)** present processed antigens to T lymphocytes in lymph nodes. Epidermal DCs are first immune cells to tackle HIV at the mucosal surfaces. DCs provide signals and are responsible for activation of B lymphocytes (58).

Natural killer (NK) *cells* lyse cells that have decreased expression of major histocompatibility complex **(**MHC**)** I antigens. NK cells assume greater importance when HIV escapes the cellular immune response. Type 1 interferon **s**ecreted by DCs result in proliferation of NK cells. NK cells that are stimulated release cytokines such as interferon γ **(**IFN-γ**),** tumour necrosis factor α (TNF-α**),** and chemokines to activate T-cell proliferation (cellular immune response). NK cells by releasing IFN-γ help in viral killing(57,58).

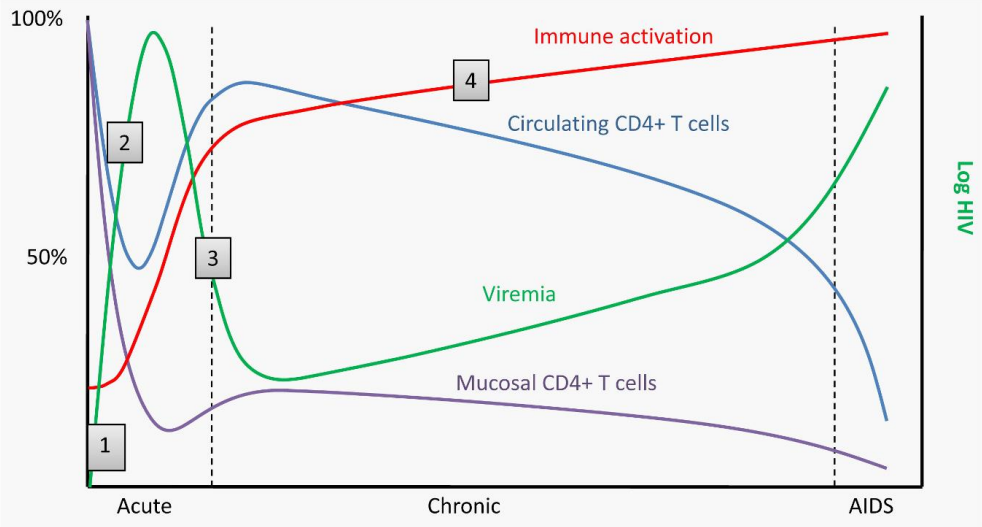


Figure 3.6 Innate immune recognition and activation during HIV infection. (Adapted from Retrovirology. 2010 Jun 22; 7(1):54)

Potential roles of the innate immune system during HIV infection involves

(1) Prevents viral transmission.

(2) Immune mediated T cell death.

(3) Defence against infection already established.

(4) Chronic immune activation .(59)

3.10.2 Adaptive immune response

HIV-infected children have changes in the immune system similar to those in HIV-infected adults after primary viremia, the HIV infected individuals normally mount an effective immune response that can delay the onset of disease by a decade Both humoral and cell mediated immune response have a role to play.

3.10.2.1 Cellular immune response to HIV.

The cellular immune response comes into play upon the entry of HIV into the target cells (e.g., T cells) and synthesis of viral proteins**.** MHC class I on the cell surface displays the HIV peptide fragments for recognition by T-cell receptors (TCR) on CD8+ T cells**.** HIV infected cells are lysed by CD8+ T cells and secrete cytokines, that is tumor necrosis factor α, interferon-γ, and also chemokines, that inhibit virus replication and block viral entry into CD4+ T cells such as i.e. MIP-1 α**,** MIP β and RANTES. CD8+ T cells development is crucial for control of HIV replication. This results in low detectable virus in blood after primary infection. In the early stages of infection, CD4+ T cells lose their capacity to proliferate and therefore their contribution to viral control is minor. But, during chronic infection CD4+T cells are present and they secrete **i**nterleukin-2 **(**IL-2**)** or cytokines, such as IFN-γ, to control viraemia.

Cytokine dysregulation in HIV

Cytokines play an important role in the cell mediated immunity. The cytokines that are secreted by T- lymphocytes are broadly classified into two types: Th1 -type and Th2 – type.(60)

The principal cytokines of the Th-1 group are interleukin-2 (IL-2) and interferon -γ (IFN-γ) and TNF-alpha. The main function of Th2-type cytokines is antibody production, class switching. The principle Th2 cytokines are Interleukin-4 (IL-4), Interleukin-10 (IL-10) and pro-inflammatory cytokines Interleukin-1 (IL-1), Interleukin-6 (IL-6) and Interleukin-8 (IL-8). When the disease in HIV progresses, there is a cytokine switch from Th1 to Th2.(60,61). This marked increase in the Th2 cytokines is not very useful in controlling the HIV virus since it is mostly intracellular (61).

During the HIV-1 disease progression, interferon gamma production decreases but their receptor expression increases, this dysregulation causes impairment in cellular immunity(62).

The interleukin 10 is a pleiotropic cytokine capable of suppressing cytokine production from macrophages and T cells, this is usually increased which has a role in the suppression of T cell and monocyte/macrophage function, crucial in HIV disease control (62). The IL-21 usually improves the HIV-specific cytotoxic T cell responses in HIV infected patient, this is also the best biomarker in the monitoring of the disease progression of HIV (63).

3.10.2.2 Humoral immune response

Within 3-6 weeks of primary infection and without fail within 12 weeks, HIV specific antibodies appear (34). Non-neutralising antibodies to structural proteins (i.e. P17 and P24) appear first. There after later neutralising antibodies which are specific to proteins, involved in the entry of the virus into the cells, will be generated. These antibodies are specific to: (1) the variable region of gp120 (V3); (2) CD4 binding sites and chemokine receptors (i.e., CXCR4 and CCR5); (3) the transmembrane protein gp41. Potent neutralizing antibodies have been shown to play a major role in controlling HIV infection.(34,58).

The Polyclonal activation of B cells occurs in most children early in the infection, as evidenced by elevation of IgA, IgM, IgE, particularly IgG(hypergammaglobulinemia), with high levels of anti–HIV-1 antibody. (8).

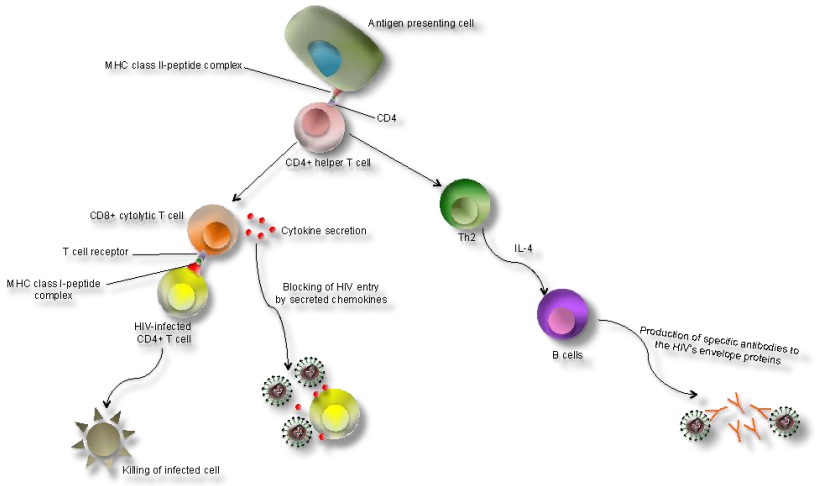


Figure 3.7 Cellular & humoral immune responses to HIV. (Adapted from Human Immunodeficiency Virus (HIV) | British Society for Immunology Available from: https://www.immunology.org/public-information/bitesized-immunology/pathogens-and-disease/human-immunodeficiency-virus-hiv.)

3.11 Clinical manifestations of HIV infection in infants and children

Clinical manifestations of HIV can vary from non-specific symptoms to life threatening opportunistic infections.(64–67). WHO Clinical Staging of HIV/ AIDS in children is given in **Table 3.3.**

3.12 Diagnosis

Algorithm of HIV diagnosis in children less than 18 months by NACO is given in **Figure 3.8** below. In older infants, 2 or more negative HIV antibody tests performed at least 1 month apart past 6 month of age in the absence of hypogammaglobulinemia or clinical evidence of HIV disease can reasonably exclude HIV infection. (68).

3.13 Treatment

3.13 .1 Natural outcome of the disease

Without treatment, HIV infection causes progressive immunosuppression, due to HIV virus-mediated depletion of CD4+ lymphocytes, leaving patients at risk of developing opportunistic infections (OI) and other HIV-related disorders. Most HIV-infected children show progression of HIV infection to AIDS and die before age five years (69,70)

3.13 .2 Treatment

The WHO recommends initiating ART for all infants, children, and adolescents living with HIV, regardless of WHO clinical stage and/or CD4 count. HIV-infected children in the following categories are considered to be at the highest priority for initiating ART: WHO stage 3 or 4, age <2 years, age 2 to 5 years with CD4 count ≤750 cells/mm^3 or CD4 percentage <25 percent and age ≥5 years with CD4 count ≤350 cells/mm^3 (71). Increased thymic function in HIV-infected children and adolescents enhances their immunologic recovery (72).Summary of first line and second line ART drugs for adults and children is given in **Figure 3.9.**

TABLE 1. SUMMARY OF SEQUENCING OPTIONS FOR FIRST-, SECOND- AND THIRD-LINE ART REGIMENS FOR ADULTS (INCLUDING PREGNANT WOMEN AND ADOLESCENTS) AND CHILDREN

Population	First-line regimens	Second-line regimens	Third-line regimens
Adults and adolescents (including women and adolescent girls who are of childbearing potential or are pregnant)[a]	Two NRTIs + DTG[b]	Two NRTIs + (ATV/r or lopinavir/ritonavir (LPV/r))	Darunavir/ritonavir (DRV/r)[g,h] + DTG[i] + 1–2 NRTIs (if possible, consider optimization using genotyping)
	Two NRTIs + EFV[c]	Two NRTIs + DTG[b]	
Children	Two NRTIs + DTG	Two NRTIs + (ATV/r[d] or LPV/r)	
	Two NRTIs + LPV/r	Two NRTIs + DTG[e]	
	Two NRTIs + NNRTI	Two NRTIs + DTG[f]	

[a] An optimized NRTI backbone should be used such as zidovudine (AZT) following TDF or abacavir (ABC) failure and vice versa.
[b] Women and adolescent girls of childbearing potential with consistent and reliable contraception and who are fully informed of the benefits and risks can use DTG.
[c] If population-level pretreatment resistance to EFV or NVP is ≥10%, the choice of alternative options to EFV needs to be made weighing the drug availability and toxicity profile. DTG (with consistent and reliable contraception among adolescent girls and women of childbearing potential) or ATV/r are the drug options to be considered.
[d] ATV/r can be used as an alternative to LPV/r among children older than three months, but the limited availability of suitable formulations for children younger than six years, the lack of a fixed-dose formulation and the need for separate administration of a ritonavir booster should be considered when choosing this regimen.
[e] This applies to children for whom approved DTG dosing is available. RAL should remain the preferred second-line regimen for the children for whom approved DTG dosing is not available.
[f] ATV/r or LPV/r should remain the preferred second-line treatment for the children for whom approved DTG dosing is not available. This applies to children for whom approved DTG dosing is available.
[g] For PI-experienced people, the recommended DRV/r dose should be 600 mg/100 mg twice daily.
[h] Children younger than three years should not use DRV/r.
[i] DTG-based third-line ART following the use of integrase inhibitors must be administered with DTG twice daily.

Figure 3.9 Summaryof first line and second line ART drugs for adults and children. (Adapted from WHO | Updated recommendations on first-line and second-line antiretroviral regimens and post-exposure prophylaxis and recommendations on early infant diagnosis of HIV: http://www.who.int/hiv/pub/guidelines/ARV2018update/en/)(71, 73).

3.14 Vaccine response

3.14.1 Immune response to a vaccine

A vaccine contains killed or weakened form of an antigen that stimulates body's immune response. Antigens are phagocytosed by antigen presenting cell and processed into small peptides, and displayed at the cell surface in the groove of major histocompatibility complex (MHC) class I and/or class II molecules (74).

It is the CD4+ T cells that recognize antigenic peptides which is displayed by class II MHC molecules. Activated B cell differentiate into plasma cells and produce antibodies. Activated CD8+ cytotoxic T cells bind to and destroy any cell containing the antigen. MHC-peptide specificity are activated, provide activation signals to antigen presenting cells and differentiate in effector cells that produce preferentially T helper (Th)1 or Th2 cytokines. Th1 CD4+ T cells support CD8 + T-cell differentiation, which is in contrast inhibited by Th2-like cytokines (74,75).

A small but important fraction of the B and T cell transform into memory helper and killer T cell and memory B cells that react quickly, when they encounter the same antigen next time. The response to first exposure to the antigen is mediated by naïve lymphocytes is called primary response (75,76).

3.14.2 Immune response after vaccination

An exposure to the same antigen after vaccination stimulates the memory B cell and T cell which recognize the antigen and respond quickly and effectively to prevent the disease. Memory B cell differentiate into plasma cells and produce antibodies. Antibodies bind to the antigen and neutralize it, as well as to the cells infected with them for phagocytosis. Memory killer T cell differentiate into active killer T cell and bind to and destroy any cell containing the antigen.

This kind of subsequent response to the same antigen due to the activation of memory lymphocytes is called secondary response(74,75). Immune response to vaccination is represented in **Figure 3.10** and **Figure 3.11**.

3.14.4 Immunization in HIV-infected children

Immune responses to vaccination vary in HIV infected patients, depending on the nature of the vaccine and the individual's immune status (77). The immune system dysfunction that occurs with advanced HIV infections can result in a blunted immune response to immunization, but this response does depend on how affected the immune system is at the time of vaccine receipt. Therefore, it is important to immunize HIV-infected children as quickly as possible so that they can mount protective before the immune system fails(78).

The Expanded Program on Immunizations (EPI) of the World Health Organization (WHO), in collaboration with UNICEF, recommends a narrow and accelerated immunization schedule for HIV-infected children. The WHO has also made recommendations that serve to guide whether particular vaccines should be used in the asymptomatic or symptomatic HIV-infected child (79). **Table 3.4** Immunizations for HIV-infected patients Adapted from Calles NR, Schutze GE. Immunizations for Children with HIV/AIDS.

All standard childhood immunizations in the United States can be given to HIV-infected or exposed children. All inactivated vaccines—whether killed whole organism or recombinant, subunit, toxoid, polysaccharide, or polysaccharide protein-conjugate are generally safe and acceptable in HIV-infected individuals. Certain live vaccines have sufficient safety data and are thus recommended in HIV-infected patients who have CD4 cell percentage ≥15 percent (if <5 years old) or cell counts ≥200 cells/μL (if ≥5 years old) such as varicella vaccine and measles, mumps, and rubella [MMR] vaccines (80). Although vaccine efficacy is usually compromised in advanced disease, adequate responses can be achieved when vaccines are administered early after HIV infection. However, if there was suboptimal antibody response to the initial vaccine, revaccination once immune reconstitution and virologic suppression has been achieved is recommended for certain vaccines (78).

Overall, vaccines tend to be less immunogenic and antibody responses shorter-lived in the setting of HIV-infection. It is recommended to consider serology after vaccination and if found seronegative, then to administer booster dose by the UK immunization schedule, 2015. It also recommends annual serology for vaccines at annual reviews (81). In India HIV infected children follow the same immunization as mentioned in **Table 3.4.** But there are no recommendations for serological test to look for the protective response or the need of revaccination (82,83).

3.14.5 Hepatitis B Virus vaccination in HIV-infected children

The risk factors and modes of transmission among Hepatitis B virus (HBV) and human immunodeficiency virus (HIV) are common. HIV/HBV co-infected individuals have a higher level of HBV replication, with higher rates of chronicity, reactivation, occult infection, and HCC than individuals with HBV only (84), coinfection results in eight times more death.(85)Hepatitis B virus (HBV) infection is a worldwide problem, and the high prevalence in endemic areas is attributed largely to perinatal transmission(86).

Measurement of the humoral immune response (Ab against HBV surface Ag [anti-HBs] Ab levels) is currently the most commonly employed immune marker that correlates with protection from HBVinfection. It is generally accepted that anti-HBs levels 10 IU/l are considered protective against HBV infection (75,87). In the vaccinated population, up to 10% of individuals do not achieve anti-HBs levels of 10 IU/l despite full vaccination (termed “nonresponders”)(86)

However, some data suggest that many individuals whose levels are 10 IU/l are still protected against HBV infection(88,89). On the other hand, there is no absolute protection against HBV infection at any anti-HBs titres(90)

Waning of Anti-HBs titer with advancing age

A number of studies with follow-up of the vaccinees for up to 20 years or more revealed that anti-HBs level in responders can decline fairly rapidly with time. Anti-HBs seropositive rate could decline from 100% at age 2 years to 75% at as early as age 6 years among complete vaccines. In vaccinated children, as many as 22.9% had undetected

antibody levels, which was not influenced by gestational age, birth weight or parental origin.

The decline in anti-HBs is correlated with age (91). In a study on 640 children born to HBsAg positive mothers and with completed vaccination, protective anti-HBs was 93% afterwards, then falling to 70%, 40% and 25% at 5, 10, and 15 years of age respectively (92).Another study on 1095 subjects with early childhood vaccination and assessed at age 1–24 years found that while overall efficacy against infection and carriage were 83.4% and 96.5% respectively, population vaccine efficacy against infection decreased from 92.5% among 1–4 years old to 70.9% among 20–24 years old, and efficacy against carriage decreased from 100% to 91.1% (93)When tested at specific ages, a significant waning in anti-HBs titer can be found at age 15 to 18 years(94).

The situation was even more striking among Micronesians vaccinated at birth in that only 7.3% of uninfected individuals had positive antibody (95).Thus even among complete vaccinees, vaccine-induced immunity should not be taken for granted from the time of adolescence onwards.

Anamnestic response in vaccinees

It has been reported that even though protective antibody concentration was found in only 64% of the children more than 10 years after vaccination in infancy, an anamnestic response was demonstrated in 97% of the remaining children. Indeed, by age 15 years, as many as half of the children vaccinated at birth had absent anamnestic responses to booster vaccination. Therefore without the challenge of a routine booster, the presence of

an adequate anamnestic response in most vaccinees remains an unsubstantiated opinion (95,96)

Vaccine response to hepatitis-B vaccination (standard course) is markedly less in HIV-infected children. This is due to depletion of CD4+ T-cells, and altered distribution of T-cell and B-cell subsets. The production of HBV-specific antibodies by B lymphocytes needs the activation of thoseCD4+ T helper lymphocytes that will elicit B cell proliferation and their differentiation into Ab-secreting plasma cells. There is a decline in the total memory B cells and immature B cells increases. Hence these children are susceptible to a high rate of reinfection and waning vaccination-induced immunity (97)

The response rates to the 'classic' hepatitis-B vaccination schedule (10 μg at months 0-1-6) are much lower in children living with HIV compared to HIV non-infected children. Seroconversion after HBV vaccination in HIV infected children using standard regimen is as low as 35 % (98). Children from several SSA countries have been experiencing suboptimal protection after anti-HBV vaccination (42.9–68.0% in Cameroon) (99). In a study done in Tanzania seroconversion was found in 70.8% of HAART-treated and 44.4% of treatment naïve children (12) . In study done in India on with double dose of HBV vaccination, seroconversion was 94 % (100)

Protective antibody titres elicited using ELISA (Enzyme Linked Immunosorbant Assay) is the common to look for vaccine responses. Currently studies are looking at T cell and B cell responses. Quantification of the antigen-specific CD4+ and CD8+ T cells induced in response to the proteins encoded in a vaccine is an important measure of cellular immunogenicity. Studies on T cells both CD4 + and CD8

+ T cells expression of various cytokines or functions and phenotyping markers after short-term ex vivo stimulation with specific antigen are there(101) .Two common methods, ELISpot (Enzyme Linked Immunospot Assay) and flow cytometry. ELISpot is a modification of ELISA that allows the quantitative detection of cells producing antibodies (plasma) or cytokines (macrophages). ELISPOT is used commonly for quantitating the cytokine producing cells. The advantage of flow cytometry ICS (intracellular staining) is that multiple parameters (8 to 14) can be assessed at the same time. IFN-γ, IL-2 and TNF-α are the cytokines commonly measured for both CD4+ and CD8+ T cells responses(102). Other methods like FLOUROSpot, cytokine bioplex assays are also used(101). Quantification of the antigen-specific B cell which include looking for plasmablast, memory B cell is a measurement of humoral immunity .In flow cytometry B cells are stained by surface staining with specific markers . Other methods like ELISpot, FLOUROSpot, Limiting dilution assay are also used for determining B cells(103,104)

Studies are required on evaluating immune response to hepatitis B vaccine in HIV-infected children who are on receiving ART (as per current guidelines) so that a proper vaccination schedule is established for these children. Studies must also be done to look into the changing the vaccination dose and schedule in accordance with present immune status of the patient like using CD4 +T cell count as one of the parameters.

4. MATERIALS AND METHODS

This study was done in the Departments of Clinical Virology, Department of Paediatric Surgery and Department of Paediatric Infectious Diseases, Christian Medical College and Hospital, Vellore. It was approved by the Institutional Review Board (Reference IRB Min. No. 9832 dated 07.01.2016).

4.1 Materials

4.11 Study subjects

In this study, individuals were prospectively recruited using inclusion and exclusion criteria. This study was conducted from February 2016 to September 2018.

The 2 groups recruited are:

Group 1:

Consist of 42 cases of HIV infected children.

Subgroup 1

Consists of HIV infected children who are below 15 years of age (2-15 years of age) who have taken Hepatitis B vaccination, are undergoing follow up at CMC, Vellore and coming regularly for CD4+ T cell estimation. Hepatitis B vaccination history of all children were taken, from immunization records if available, otherwise from recall, including the number of doses received and the date of last dose taken.

Inclusion criteria

1) HIV diagnosed children, with positive HIV antibody.
2) Less than 15 years of age (2-15 years of age).
3) Has taken at least 1 dose of Hepatitis B vaccine.

4) Individuals who have given informed consent / assent for the study.

Group 2:

Consist of 38 children who are not HIV infected, not having any other infections and going for elective surgeries in the same age groups as controls. An additional 5 ml of blood sample was collected from these children while undergoing other blood investigations for the study. Hepatitis B vaccination history of all children were taken, from immunization records if available, otherwise from recall, including the number of doses received and the date of last dose taken.

Inclusion criteria

1) HIV negative children.
2) Not having any other infections.
3) Less than 15 years of age (2-14 years of age).
4) Has taken at least 1 dose of Hepatitis B vaccine.
5) Individuals who have given informed consent / assent for the study.

Exclusion criteria for all the groups

1) Individuals who have not given consent for the study.
2) More than 15 years of age.
3) Children who had received a blood transfusion last 6 months
4) Has not taken at least 1 dose of Hepatitis B vaccine.
5) Severely ill children.
6) HBs Ag positive and HBc Ab Reactive

4.1.2 Sample size

The sample size is calculated using the following formula mentioned below.

Formula

$$H_o : P_1 = P_2; \qquad H_a : P_1 \neq P_2$$

$$n = \frac{\left\{ Z_{1-\frac{\alpha}{2}} \sqrt{2\,\bar{P}\,(1-\bar{P})} + Z_{1-\beta} \sqrt{P_1\,(1-P_1) + P_2\,(1-P_2)} \right\}^2}{(P_1 - P_2)^2}$$

Where,

$$\bar{P} = \frac{P_1 + P_2}{2}$$

P_1 : Proportion in the first group

P_2 : Proportion in the second group

α : Significance level

$1-\beta$: Power

Hypothesis testing of two large proportions-Equal Allocation

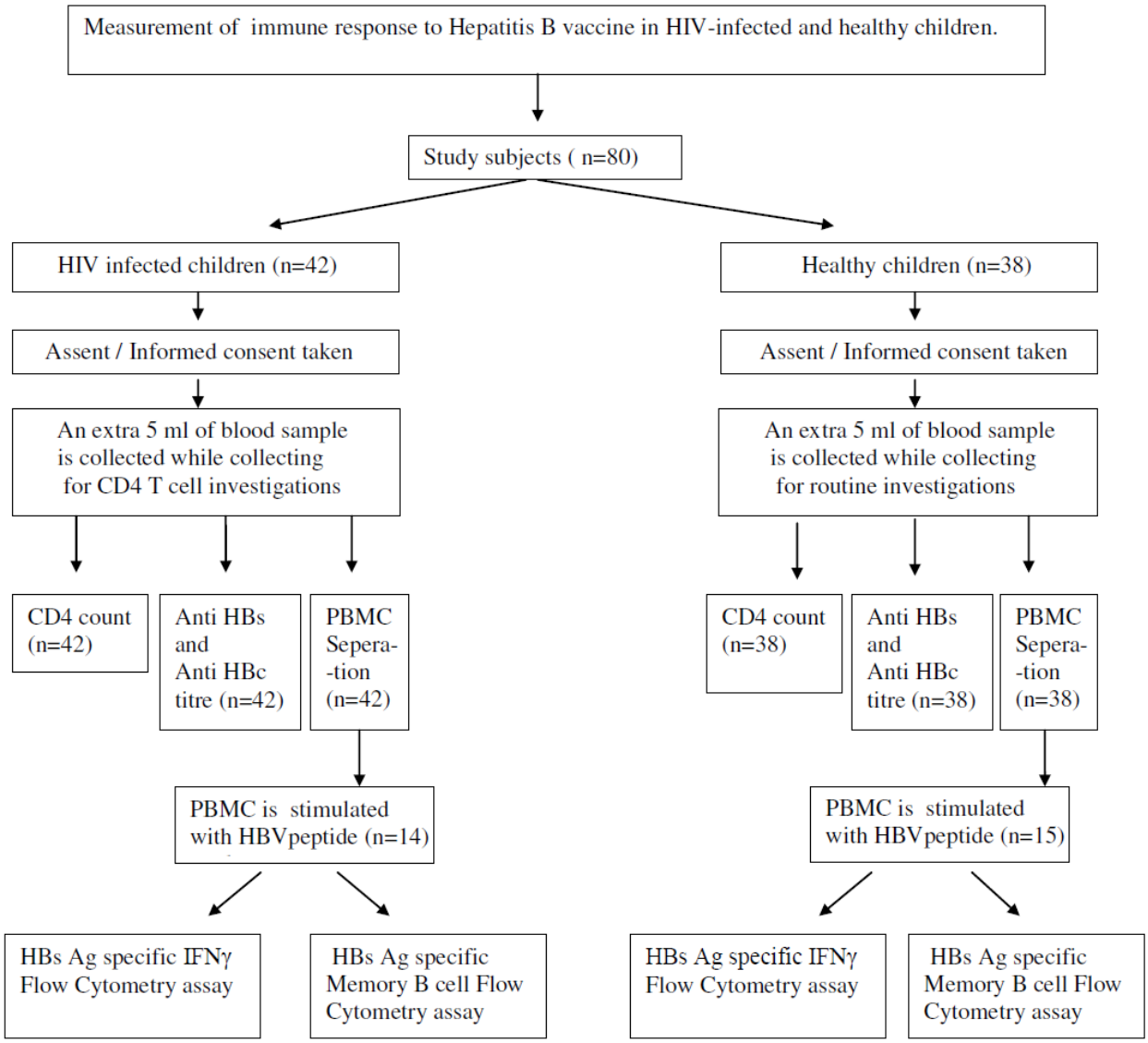
Reference

Sahai H, Kurshid A. **Formulae and tables for the determination of sample size and power in clinical trials for testing differences in proportions for the two sample design: a review.** Statistics in Medicine, 1996; 15: 1-21.

Two Proportion - Hypothesis Testing - Large Proportion - Equal Allocation	
Proportion in group I	0.599
Proportion in group II	0.9
Estimated risk difference	-0.301
Power (1- beta) %	80
Alpha error (%)	5
1 or 2 sided	2
Required sample size for each arm	31

With reference to HIV Medicine (2008), 9, 519–525 the seroconversion to Hepatitis B vaccination in HIV infected children were found to be 59.5% and assuming it to be 90% among the HIV Negative children, with a power at 80% and alpha error at 5% for a two sided test we need to study 31 children with HIV and 31 normal children. The number of children included in the study group 1- 42 HIV children and in study group 2 - 38 healthy children.

4.1.3 Study Algorithm



4.2 Methods

4.2.1 Specimen collection

After obtaining an informed consent and assent, additional 5 ml of blood from participants in 2 groups were collected in a sterile EDTA containing tube while collecting for routine testing. To perform CD4 + T cell estimation, 100 µl of blood was alliquoted. The remaining blood was centrifuged, plasma separated, multiple aliquots made and then stored at -80^{0}C until testing, as part of standard of care. Anti Hbs antibody titre was determined from plasma by CMIA (Chemiluminescent Microparticle Immunoassay) Anti –HBs Assay. PBMC was separated from the remaining blood using Ficoll Paque density gradient centrifugation and stored as multiple aliquots in liquid nitrogen until it was tested.

4.2.2. Sample preparation:

4.2.2.1 Plasma

Plasma was separated from the EDTA tubes after they were centrifuged at 2000 rpm for 10 minutes at 4^{0}C. The plasma was stored as multiple aliquots at -70^{0}C until the time of testing.

4.2.2.2 PBMC

Reconstitution of Reagents

- 1X RBC lysis buffer -10X RBC lysis buffer was diluted in 1: 10 ratio in sterile distilled water and used for the assay.

- 1X phosphate buffered saline (PBS) - 10X phosphate buffered saline was diluted in 1: 10 ratio in sterile distilled water and used for the assay.

PBMC separation was done from the remaining blood after plasma separation, by the following method. Five ml of blood sample was diluted with 5ml of 1X phosphate buffered saline (PBS) in a sterile 15 ml tube. The 10 ml of diluted blood sample was mixed well in a plate mixer. Five ml each of diluted blood samples was overlain into another 2 sterile 15 ml tube containing 5ml of Ficoll-Paque (GE Healthcare, Uppsala, Sweden) carefully. The tubes were centrifuged at 2000 rpm for 30 minutes at 20^0C. After centrifugation the PBMC cell layer which was formed at the center of the tubes was transferred into another sterile15 ml tube carefully. This 15 ml tube was centrifuged at 2000 rpm for 10 minutes at 20^0C in order to pellet the PBMCs. Four ml 1X RBC lysis buffer was added to the PBMC pellet, mixed and incubated at room temperature for 5 minute. There after PBMCs were washed with 6 ml 1X PBS and centrifuged again for 10 min at 2000 rpm. This washing step was again repeated using 6 ml of 1X PBS. After second wash and centrifugation, 1.5 ml FBS was added to the PBMCs cells and were counted using 44 the Beckman coulter UniCel DxH 800 (Miami, USA) in the Department of Clinical Pathology. Initially the PBMCs cells were aliquoted based on their cell counts into sterile multiple tubes in – 20^0C for overnight, -80^0C for 24 hours and there after stored in liquid nitrogen. Flow cytometry assay from stored samples were not giving good result, so all the analysis was done with freshly separated PBMCs.

4.2.3 CD4+ T cell count estimation

The CD4 + T cell enumeration was done using Flow cytometry using the FACS (fluorescence activated cell sorting) Count system (Becton, Dickinson) CD4/CD4 % SW, Version 1.0, San Jose) from freshly collected whole blood. The principle is based on when whole blood is added to the reagents, fluorochrome-labeled antibodies in the reagents bind specifically to lymphocyte surface antigens. After a fixative solution is added to the reagent tubes, the sample is run on the instrument. Here, the cells come in contact with the laser light, which causes the fluorochrome-labelled cells to fluoresce. The information necessary for the instrument to count the cells is provided by this fluorescent light. The linearity of the absolute CD4 + T cell count was between 50 – 5000 cells/ µl and CD4 + T cell % was 5- 60%.

Procedure

The CD4 reagent was brought to the room temperature (20-25^0 C). Each tube was vortexed for 5 seconds. Reagent tube for each patient sample was labeled with patient Id. Three reagent tubes were labeled as low, medium and high for calibrators. Each BD vacutainer tube was inverted 5 to 10 times to adequately mix the blood. 50µl of patient sample was added into the each reagent tube. In to each of three control reagent tubes 50µl of normal whole blood is added. Both sample and control tubes were vortexed for 5 seconds. The tubes were incubated at room temperature in the dark for a period of 30 minutes. To each of tube fifty µl of fixative solution was added followed by vortexing it for 5 seconds. One pair of Zero/Low control beads and one pair of medium/high control beads were removed from the control kit and placed in control area of work station. 50µl

of low, medium, high control were added to the reagent tube before running the tubes on the instrument. The controls were read in BD FACS count machine. Once the control was passed, patient samples were read in the BD FACS count machine and results were printed. In addition to patients samples daily IQC (High Previous day's CD4+ count) was included in each run from Tuesday to Friday except on Mondays.

Quality control for FACS count:

In order to avoid any diurnal variation all, the blood specimens tested for CD4 + T cell estimation were collected only on the day of testing between 8:00 -11:00 AM. The CD4 + T cell count estimation using the FACS Count system (Becton Dickinson) was analyzed using the two daily quality control samples. In our laboratory, the testing was carried out from Monday through Friday and one high internal quality control (HIQC) sample was run every day. The previous day's sample with the highest CD4 + T cell count and CD4% was included as the HIQC. All the samples were stored at room temperature (between 20° and 28°C). The HIQC's CD4 T cell count of the day was compared with the previous day's value and percent variation was calculated as follows: (Observed count/Expected count) -1 ×100. A sample showing more than 10% variation from the previous day's value was considered not acceptable and the clinical samples were retested if necessary. A sample showing not more than difference of 3 from the previous day's value was considered as acceptable. Our daily quality control data from CD4+ T cell count and CD4% showed very good performance. In addition to one internal quality control, we also used commercial stabilized blood (BD Multi-Check control, Becton, Dickinson, San Jose, CA) as external quality control for routine quality control testing.

4.2.4 Anti-HBs antibody titre

Anti-HBs antibody was quantified in plasma by the automated Chemi luminescent Microparticle Immunoassay [CMIA, Architect system, Abbott Laboratories, Sligo, Ireland]. Subjects with anti-HBs >10 mIU/ml were considered as responders to the vaccine. No detectable anti-HBs is defined in the assay as ≤10 mIU/ml, where 10 is the LLOD of the assay.

Principle of the assay- The sample and recombinant HBsAg (rHBsAg) coated paramagnetic microparticles are combined. Anti HBs present in the sample binds to rHBsAg coated paramagnetic microparticles. After washing acridium- labelled rHBsAg conjugate is added in the second step. Following another wash cycle, Pre-Trigger and Trigger solutions are added to the reaction mixture .The Chemiluminescence which is produced is measured in units known as "relative light units (RLU)". A direct relationship occurs between the amount of anti-HBs in the sample and the RLU detected by the ARCHITECH i System optics.

Procedure – Two hundred µl of each sample was dispensed into the sample cups provided by ARCHITECT i2000 SR. The sample cups were placed in the carrier and loaded in the bay as per the test entered in the system. Once the running of the sample has finished, the print out of the report was taken.

Samples with anti HBS concentration less than 10 mIU/ ml were considered Non-Reactive Anti –HBs Assay.

Samples with anti HBS concentration greater than or equal to 10 mIU/ ml were considered Reactive Anti –HBs Assay.

Quality Assurance

In each run one known EQC serum sample – in house (Negative and Positive control) was included, which served as External Quality Control of the assay apart from the controls provided by the manufacturers. The reactivity of EQC was monitored for each run. The controls values must be within +-2 SD range as determined time to time. The coefficient of variation (CV) between the duplicates of each EQC should be less than 20 %. The negative and positive controls provided by the manufacturers were included in each run of the assay where the controls should be within the range provided by the manufacturer.

4.2.5 Anti-HBc antibody Titre

Anti-HBc antibody was quantified in plasma by the automated Chemiluminescent Microparticle Immunoassay [CMIA, Architect system, Abbott Laboratories, Wiesbaden, Germany] according to the manufacturer's instructions.

Principle of the assay- The Anti-HBc assay is a two-step immunoassay for the qualitative determination of anti-HBc titre in human serum and plasma using CMIA technology with flexible assay protocols, referred to as Chemiflex. Sample, assay diluent, specimen diluents, and rHBcAg (recombinant HBcAg) coated paramagnetic microparticles are combined. Anti-HBc present in the sample binds to the rHBcAg coated microparticles. The reaction mixture is washed and anti-human acridinium-labeled conjugate is added. Following another wash cycle, Pre-Trigger and Trigger solutions are added to the mixture. The Chemiluminescence which is produced is measured in units known as "relative light units (RLU)". If the chemiluminescent signal in the reaction is

greater than or equal to cut off signal (determined after an active calibration), the specimen is considered reactive for anti-HBc.

Procedure- Two hundred µl of each sample was dispensed into the sample cups provided by ARCHITECT i2000 SR. The sample cups were placed in the carrier and loaded in the bay as per the test entered in the system. Once the running of the samples was finished, the print out of the report was taken.

Samples with S/CO value <1.00 were reported as Negative. Samples with S/CO value >1.00 to <5.00 were reported as low Positive. Samples with S/CO value >5.00 were reported as Positive.

Quality Assurance

In each run one known EQC serum sample – in house (Negative and Positive control) was included, which served as External Quality Control of the assay. The reactivity of EQC was monitored for each run. The control values must be within +-2 SD range as determined time to time. The coefficient of variation (CV) between the duplicates of each EQC should be less than 20 %.

Negative and positive controls provided by the manufacturers were included in each run of the assay where the controls should be within the range provided by the manufacturer. Negative control value should be within the range of 0-0.8. Positive control value should be within the range of 1.50-3.96.

4.2.6 HBs Antigen level

HBs antigen was quantified in plasma by the automated Chemiluminescent Microparticle Immunoassay [CMIA, Architect system, Abbott Laboratories, Wiesbaden, Germany] according to the manufacturer's instructions.

Principle of the assay: The HBsAg assay is a one -step immunoassay for the qualitative determination of anti-HBc in human serum and plasma using CMIA technology with flexible assay protocols, referred to as Chemiflex. Sample, anti- HBs Ag coated paramagnetic microparticles, anti-HBs acridinium-labeled conjugate are combined to create a reaction mixture. HBs Ag if present in the sample binds to the anti- HBs Ag coated paramagnetic micro particles and to the anti-HBs acridinium-labeled conjugate. After washing ancillary wash solutions are added to the mixture. The Chemiluminescence which is produced is measured in units known as "relative light units (RLU)". If the chemiluminescent signal in the reaction is greater than or equal to cut off signal (determined after an active calibration), the specimen is considered reactive for HBsAg.

Procedure – Two hundred µl of each sample was dispensed into the sample cups provided by ARCHITECT i2000 SR. The sample cups were placed in the carrier and loaded in the bay as per the test entered in the system. Once the running of the sample was finished, the print out of the report was taken.

Samples with S/CO values <1.00 were considered non-reactive and >1.00 were considered reactive for HBsAg.

Quality Assurance

In each run one known EQC serum sample – in house (Negative and Positive control) was included, which served as External Quality Control of the assay. The reactivity of EQC was monitored for each run. The controls values must be within +-2 SD range as determined time to time. The coefficient of variation (CV) between the duplicates of each EQC should be less than 20 %.

Negative and positive controls provided by the manufacturers were included in each run of the assay where the controls should be within the range provided by the manufacturer. Negative control value should be within the range of 0-0.85. Positive control value should be within the range of 1.75- 5.25.

HBs antigen level was checked for 25 samples in which it was not checked previously. Other samples had it already done as a part of routine investigations.

4.2.7 Flow Cytometry assay - HBsAg specific IFN gamma by CD4 T cells - Intracellular cytokine staining

Freshly separated peripheral blood mononuclear cells were stimulated with specific HBs Ag peptide for specific time. For studies of secreted proteins cells are treated with a protein transporter inhibitor to allow accumulation of the target protein inside the cell. Then the cells were permeabilised by permeabilising buffer (commercially available permeabilising solution was diluted to 1:10 ration in distilled water). Permeabilised cells were stained with florescent antibody cocktail consisting of antibodies against CD4, IFN gamma and CD 69 which is the activation marker. Antibody to CD3 were also used for staining.

Reconstitution of Reagents

- AntiCD2849d- Commercially available co-stimulatory molecules from BD BIOSCIENCES. 1 ul of the same was added to each canto tube according to the manufactures instructions.
- Synthetic peptides

  Pepmix^{TM} HBV (large envelope protein) [JPT, Innovative Peptide Technologies GmbH, Berlin, Germany] (protein ID: P17101) consisting of 98 peptides approximate amount of 25 µg per peptide [source – Hepatitis B virus genotype A2 subtype adw2, length 400 aa]. Peptides were dissolved in 40 µl of sterile DMSO and then diluted with 960 µl of sterile PBS. This was aliquoted as 20 µl into fifty 600 µl eppendorf tubes and stored at – 20^{0}C. Final concentration of peptide was 2.5 µg/ µl. So 4 µl (10 µg) of the reconstituted peptide was used for 1 canto tube (300 µl of cell suspension).
- Wash buffer - 10X phosphate buffered saline was diluted in 1: 10 ratio in sterile distilled water. 0.5 mg of bovine serum albumin was added in 500 ml of 10X PBS
- 10% RPMI- Fetal bovine serum was added to commercially obtained RPMI to make the final concentration to 10 %. RPMI 1640, supplemented with 10% FCS, 2 mmol L-glutamine, 100 g/mL streptomycin, and 100 U/mL penicillin
- Phorbol12-myristate13acetate (PMA)- 1 mg/mL in concentration (stored in aliquots at -20C)
- Brefeldin A- commercially obtained from BD Bioscience – 100 µl. This was

reconstituted in 1 in 10 sterile distilled water according to the manufacturer's instruction and used.

All the work performed was carried out in a laminar flow at the appropriate biosafety level.

Procedure

Antigenic stimulation

- Freshly separated PBMC, 3-4 X 10^5 cells were suspended in 300 µl of RPMI 1640 with 10 % FBS into 4 sterile canto tubes.
- First tube was labeled as unstained and contained PBMC cells alone. Second tube was labeled as unstimulated / negative control and it contained PBMC cells and co-stimulatory anti-CD2849d. Third tube was labelled as test and it contained PBMC cells, co-stimulatory anti-CD2849d and hepatitis B surface antigen peptides. Fourth tube was labelled as positive control and it contained PBMC cells, co-stimulatory anti-CD2849d and PMA. Details of the volume and concentrations of the reagents added are provided in the given **Table 4.1.**

Table 4.1 showing details on cells and reagents used in Hepatitis B specific IFN gamma by CD4 T cells assay.

Unstained	Negative Control/ Unstimulated	Test	Positive Control
• PBMC cells in 300 µl of RPMI 1640 with 10 % FBS	• PBMC cells in 300 µl of RPMI 1640 with 10 % FBS • Costimulatory –antiCD28 49d -1 µl	• PBMC cells in 300 µl of RPMI 1640 with 10 % FBS • Costimulatory –antiCD28 49d - 1 µl • Hepatitis B surface antigen (peptides)-5 µl	• PBMC cells in 300 µl of RPMI 1640 with 10 % FBS • Costimulatory –antiCD28 49d 1 µl • PMA – 1 µl

- Antigenic stimulation -All the tubes were incubated at 37 ^{0}C 5 % CO2 incubator for 2 hours , followed by addition of 10 µl reconstituted Brefeldin A to all the tubes followed by 4 more hours of incubation at 37 ^{0}C 5 %CO2 incubator.
- Cells were taken out after the mentioned time of incubation and washed with 2 ml washing buffer (0.5 % BSA in 10 X PBS) at 500 g RCF for 7 min at room temperature.
- Supernatant was discarded and 20 µl cocktail antibodies consisting of antibodies to CD4, IFN gamma and CD 69, 5 µl of anti CD3 conjugated to specific dyes were added to the sediment, vortexed and incubated in dark for 30 min. Details about the antibodies is provided below in **Table.4.2 .** All these

monoclonal antibodies were obtained from BD Diagnostics (BD Research and diagnostics San Jose, USA).

Table.4.2 Details of Antibodies and fluorescent stains used for Hepatitis B specific IFN gamma by CD4 T cells assay.

Antibody panel	**Stain**
CD3	APC
CD4	PerCPCy5.5
IFN gamma	FITC
CD69	PE

- After 30 minutes of incubation cells were washed again with 2 ml washing buffer (0.5 % BSA in 10 X PBS) at 500 g RCF for 7 min at room temperature.
- Supernatent was discarded, sediment reconstituted with 300 µl of wash buffer and analysed in flow cytometry immediately.

FMO (Fluorescent minus One) control for IFN gamma and cd69 were used to gate the positive events. The difference between the percentage of IFN Gamma production in the unstimulated and the one stimulated with Hepatitis B surface antigen is taken as Hepatitis B surface antigen specific IFN Gamma response (105).

Quality Control

Before analysing the samples, a 7 colour Bead Set up was run as a quality check for colour compensation. The 7-colour bead set up covers compensation for 7 dyes which are used for the flow cytometer. All the dyes which we use for our assay is compensated using this 7-colour bead set up. Along with this a cytometer setting and tracking beads were also run before each assay. Positive control was used in each sample as quality control to confirm the performance of stimulation assay. The samples were analysed used BD FACS Diva software (version 8.0.1).

4.2.8 Flow Cytometry assay for HBsAg specific memory B cells – Cell surface staining

Freshly separated peripheral blood mononuclear cells were stimulated with specific HBs Ag peptide for specific time. Cells were stained with three florescent antibodies against the corresponding surface antigens ie, CD19 which is marker for all B cells, CD 20 and CD27 which are markers of memory B cells.

Reconstitution of Reagents

Procedure

Antigenic stimulation

- Freshly separated PBMC ie, 3-4 X 10^5 cells were suspended in 300 µl of RPMI 1640 with 10 % FBS into 4 sterile canto tubes.

- First tube was labelled as unstained-and contained PBMC cells alone. Second tube was labelled as unstimulated / negative control and it contained PBMC cells and costimulatory –antiCD2849d. Third tube was labelled as test and it contained PBMC cells, costimulatory –antiCD2849d and hepatitis B surface antigen peptides. Fourth tube was labelled as positive control and it contained and it contained PBMC cells, costimulatory –antiCD2849d, PMA. Details of the volume of cells and concentrations of the reagents added are provided in the below **Table 4.3.**

  Table 4.3 Shows details on cells and reagents used in Hepatitis B specific memory B cells assay.

Unstained	**Negative Control/ Unstimulated**	**Test**	**Positive Control**
• PBMC cells in 300 µl of RPMI 1640 with 10 % FBS	• PBMC cells in 300 µl of RPMI 1640 with 10 % FBS • Costimulatory –antiCD28 49d -1 µl	• PBMC cells in 300 µl of RPMI 1640 with 10 % FBS • Costimulatory –antiCD28 49d - 1 µl • Hepatitis B surface antigen (peptides)-5 µl	• PBMC cells in 300 µl of RPMI 1640 with 10 % FBS • Costimulatory –antiCD28 49d 1 ul • PMA – 1 µl

- Antigenic stimulation -All the tubes were incubated at 37 ^{0}C 5 %CO2 incubator for 48 hours.

- Cells were taken out and washed with 2 ml washing buffer (0.5 % BSA in 10 X PBS) at 500 g RCF for 7 min at room temperature.
- Supernatant is discarded and 5 µl of CD19, 20 µl of CD20 and CD27 antibodies conjugated to specific dye were added to the sediment, vortexed and incubated in dark for 30 minutes. All these monoclonal antibodies were obtained from BD Diagnostics (BD Research and diagnostics San Jose, USA).

Table.4.4 Details of Antibodies and fluorescent stains used for the Hepatitis B specific memory B cell assay.

Antibody panel	**Stain**	**Titre(ul/ sample)**
CD19	APC	5
CD20	PerCPCy5.5/ APC-H7	20/ 5
CD27	FITC	20

- After 30 minutes of incubation, cells were washed again with 2 ml washing buffer (0.5 % BSA in 10 X PBS) at 500 g RCF for 7 min at room temperature.
- Supernatant was discarded, sediment reconstituted with 300 µl of wash buffer and were analysed in flow cytometry immediately.

FMO (Flourescent Minus One) control for CD20 and CD27 were used to gate the positive events.The difference between the percentage of memory B cell production

in the unstimulated and the one stimulated with Hepatitis B surface antigen is taken as Hepatitis B surface antigen specific memory B cell response.

Quality Control

Before analysing the samples, a 7 colour Bead Set up was run as a quality check for colour compensation. The 7-colour bead set up covers compensation for 7 dyes which are used for the flow cytometer. All the dyes which we use for our assay is compensated using this 7-colour bead set up. Along with this a cytometer setting and tracking beads were also run before each assay. Positive control was used in each sample as quality control to confirm the performance of stimulation assay. The samples were analysed used BD FACS Diva software (version 8.0.1).

Statistical analysis

Microsoft Excel was used for Data entry. MedCalc Statistical Software version 14.8.1 (MedCalc Software bvba, Ostend, Belgium; http://www.medcalc.org; 2014) was used for performing the statistical analysis. The results were represented as mean, median or number and percentages. Comparison of qualitative data between the two groups was performed by Chi-square test. The differences in mean cytokine expression levels and memory B cell among different groups were measured using independent T test and Mann– Whitney U tests. P-value less than 0.05 is considered significant. Relations were calculated by Spearman correlation.

5. Results

The study period was from February 2016 to September 2018.

Study groups

HIV infected children - In this study, 42 HIV infected children who had a vaccination history of Hepatitis B in childhood and who came to the Department of Clinical Virology for routine CD4+ T cell estimation were included.

Healthy children - In this study, 38 healthy children with no history of other infections who had a vaccination history of Hepatitis B in childhood were recruited as the control group. These children came to the Department of Paediatric Surgery for sterile surgical procedures and the blood sample was collected during routine blood examination.

An informed consent was taken from the parents/guardians of children at the time of sample collection. An informed assent was also taken from all children of age 7 years or more at the time of sample collection.

Demography of the study participants

The demographic distribution of HIV infected children is shown in **Figure 5.1.**

All the children were from different parts of the country. Majority of them were from Tamil Nadu (n=29, 69%) followed by Andhra Pradesh (n=4, 9%) and West Bengal (n=4, 9%).

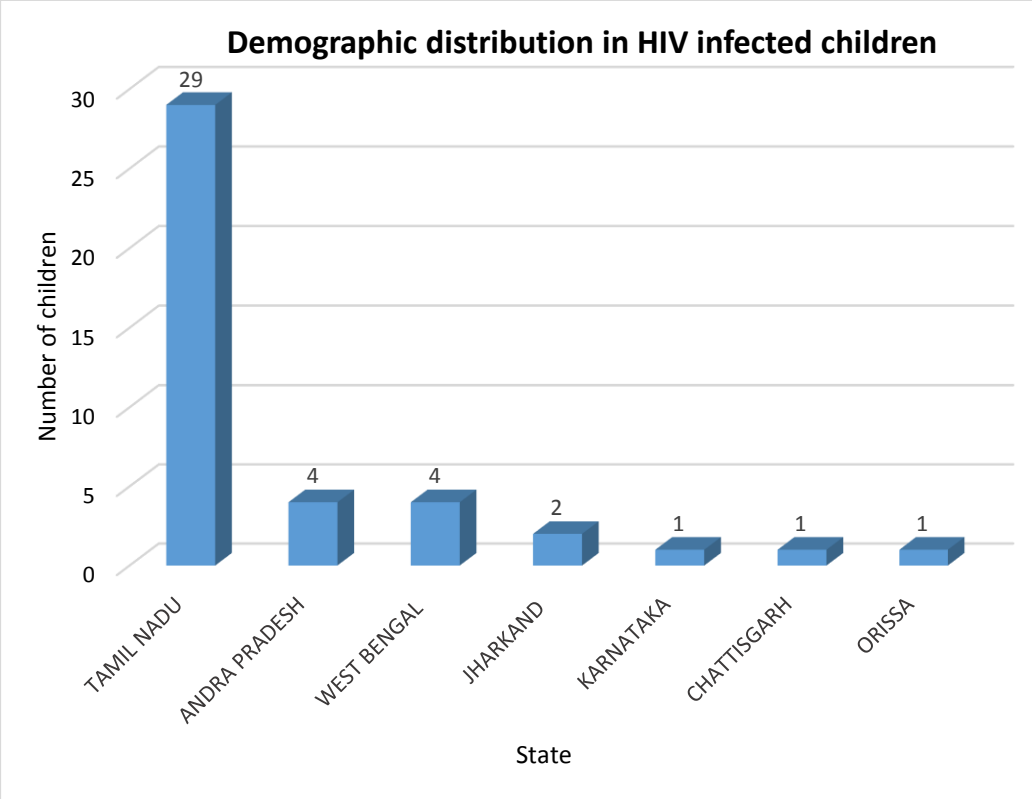


Figure 5.1 The demographic distribution of HIV infected children recruited in the study.

The demographic distribution among the healthy children is shown in **Figure 5.2.**

All the children were from different states. Majority of them were from West Bengal (n=12, 31%) followed by Jharkhand (n=8, 21%) and Tamil Nadu (n=7, 18%).

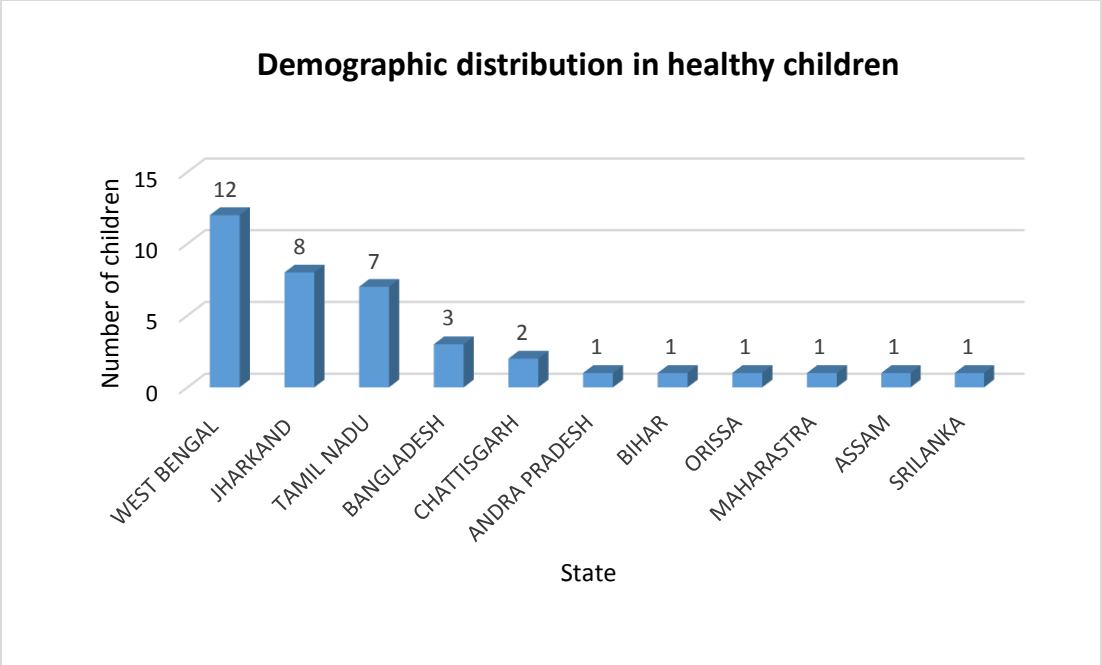


Figure 5.2 The demographic distribution of healthy children recruited in the study.

Gender wise distributions

Among the HIV infected children who were recruited for the study, there were 26 male and 12 female children. The gender distribution is represented in **Figure 5.3.** The majority of the children were male (n=26, 62%).

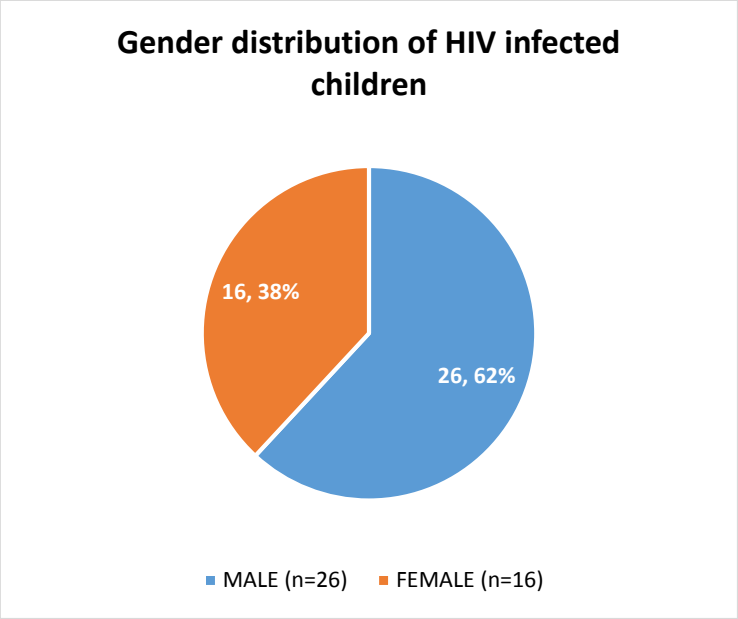


Figure 5.3 Gender-wise distribution in HIV infected children recruited in the study.

Among the healthy children who were recruited for the study, there were 26 male and 12 female children. The gender distribution is represented in **Figure 5.4.** The majority of the children are male (n=26, 68%).

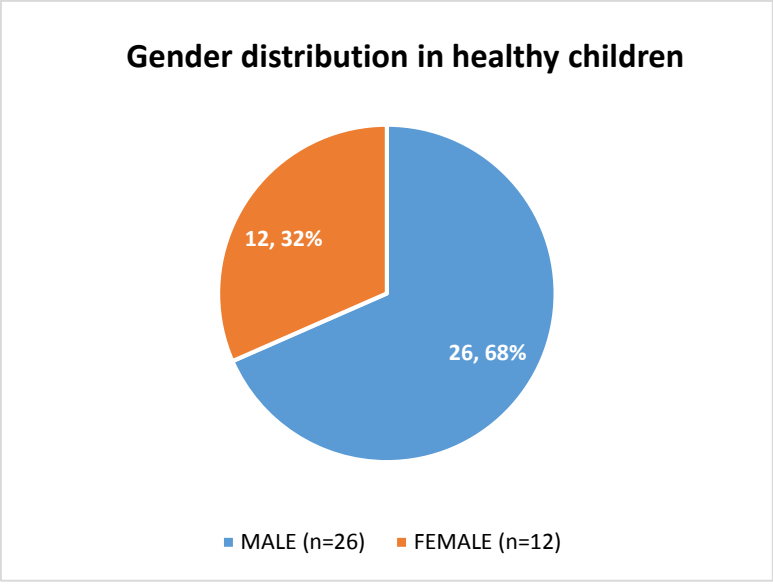


Figure 5.4 Gender-wise distributions in healthy children recruited in the study.

Age group distribution

The age distribution of the HIV infected children recruited for the study ranged from 2 to 15 years, as shown in **Figure 5.5.** The majority (n=26, 62%) belonged to the age group of 11-15 years (n=26, 29%). The mean age was 10 years of age and median was 11 years of age (Interquartile range 9-13 years).

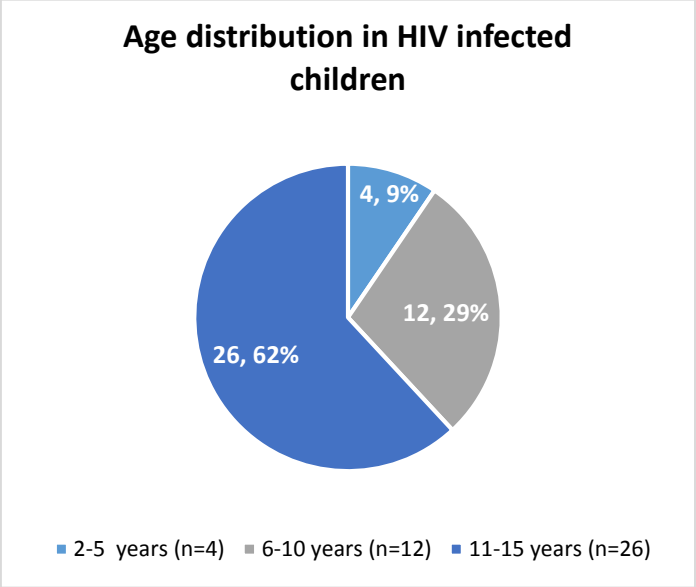


Figure 5.5 Age group distribution in HIV infected children recruited in the study.

The age distribution of healthy children recruited for the study ranged from 2 to 15 years, as shown in **Figure 5.6.** All children are recruited evenly in all 3 age groups. The mean age group was 8 years and the median was 7 years age (Interquartile range 5-11 years).

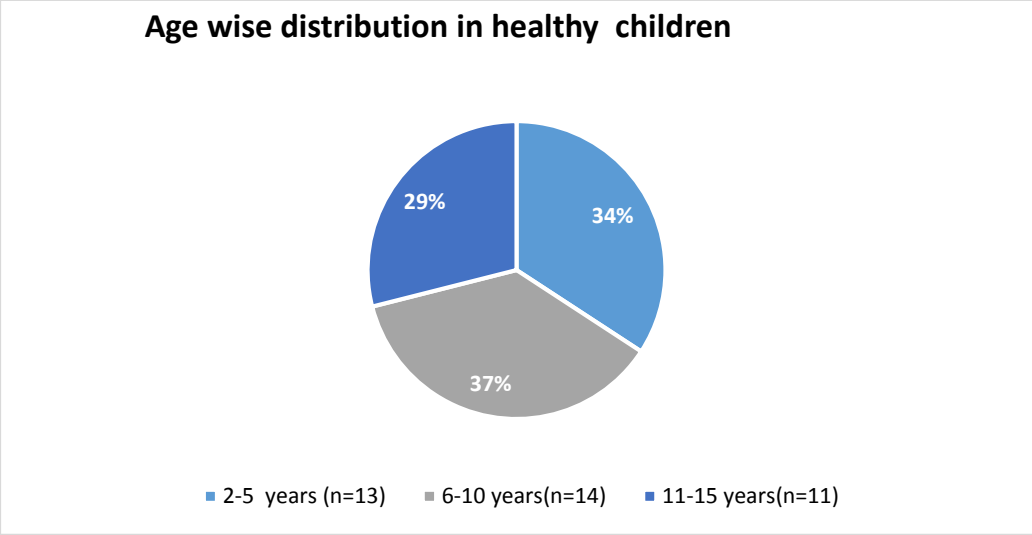


Figure 5.6 Age group distribution in healthy children recruited in the study.

Gender distribution in different age groups

The gender wise distribution of the HIV infected children in the different age group for the study is given in **Figure 5.7**. The majority of the children were males in all age groups.

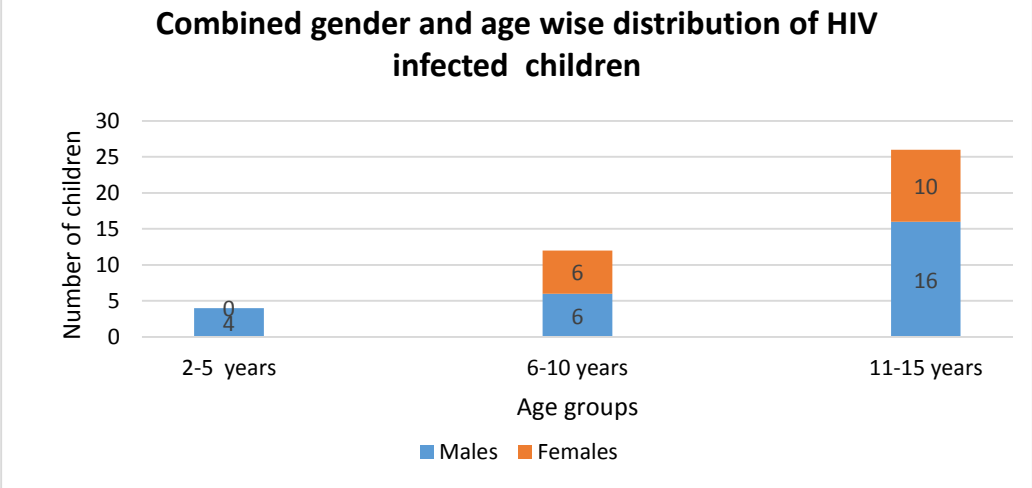


Figure 5.7 Gender distribution of the HIV infected children in different age groups

The gender wise distribution of healthy children recruited along with the different age groups for the study is given in **Figure 5.8**. The majority of the children were males in all age groups.

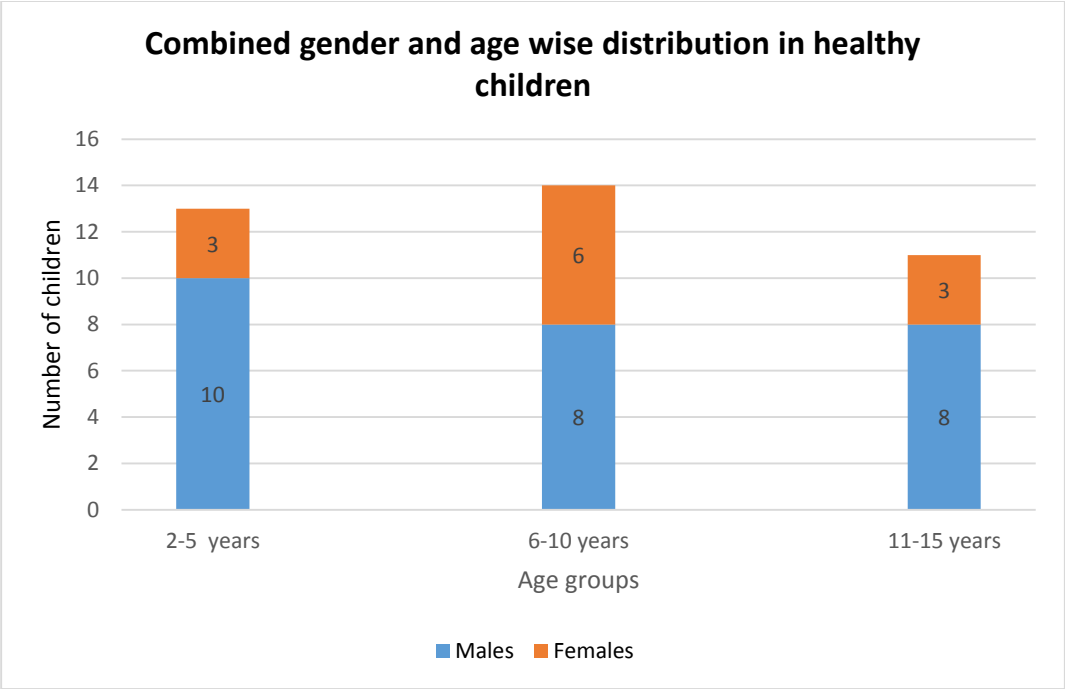


Figure 5.8 Gender distribution of the healthy children in different age groups.

Mode of transmission

The majority of the HIV infected children acquired the infection from mother to child transmission, except two. Among these two children who had not received the infection from an infected mother, the first child was a 13 year old male from Chhattisgarh who was diagnosed to have Thalassemia major and hence would have acquired the infection during multiple transfusions, while the second child was a 15 year old male in whom the mode of transmission is unknown.

WHO Clinical Stage

Most of the HIV infected children were classified as WHO Stage 2 (n=17, 40.4%) and Stage 3 (n=17, 40.4%) and majority of them were males (**Table 5.1**) when they first visited the hospital and prior to ART initiation. Stage 1 had 5 children and Stage 4 had only 3 children as represented in the **Figure 5.9.**

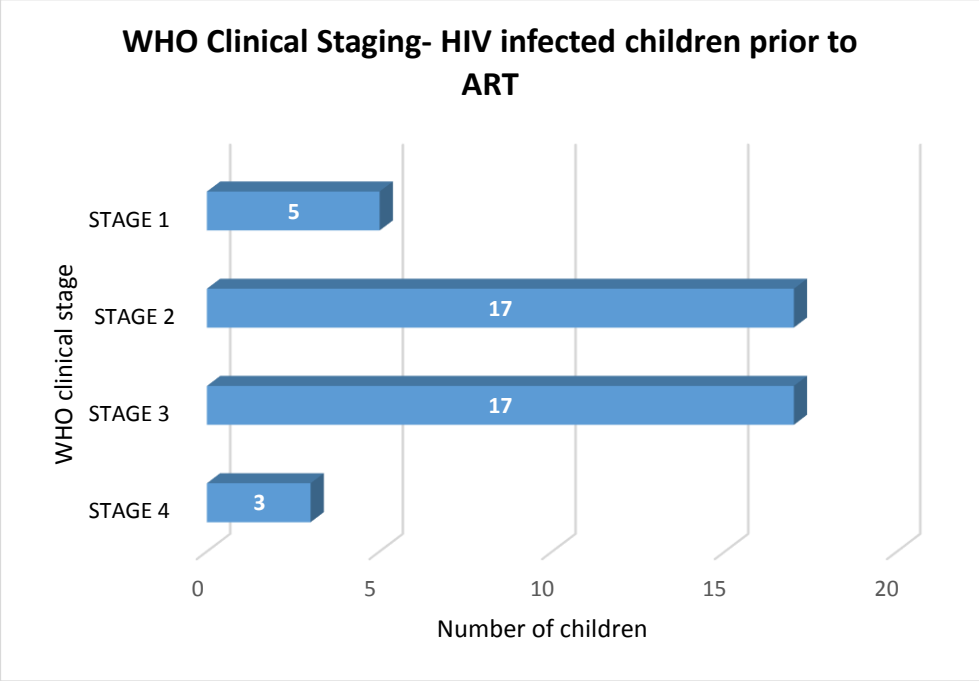


Figure 5.9 Distribution of HIV infected children based on WHO clinical staging prior to ART.

Table 5.1 Distribution of children in different WHO clinical stages based on gender.

WHO stage	Number of children	Male	Female
STAGE 4	3	2	1
STAGE 3	17	11	6
STAGE 2	17	10	7
STAGE 1	5	3	2
Total	42	26	16

In the study 81% (n=34) of the children were on Anti-Retroviral Therapy (ART) and eight children were treatment naïve (19%). **Figure 5.10** shows the distribution of children on ART and those not on treatment.

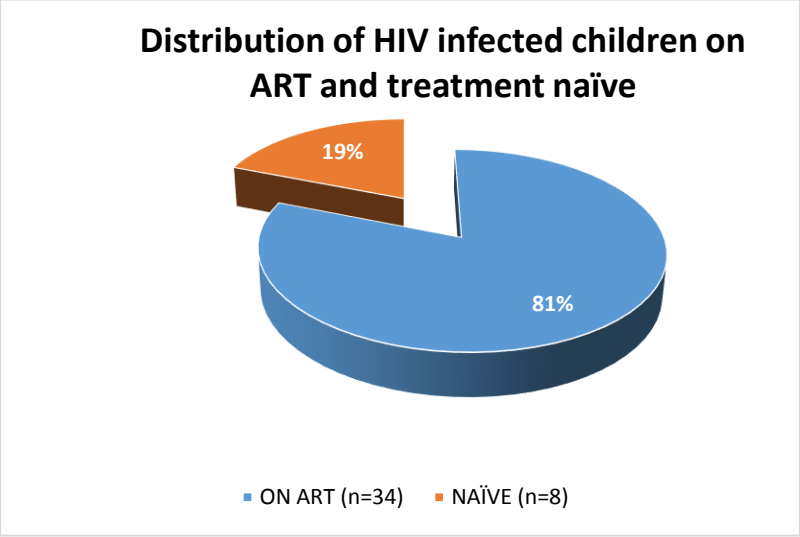


Figure 5.10 Distribution of HIV infected children on ART and treatment naïve

Duration of treatment among the HIV infected children recruited in the study ranged from 1 year to 8 years. Fifty percent of the children were started on treatment within the last 3 years and 21% in the last one year. **Figure 5.11** shows the distribution of duration of ART in HIV infected children.

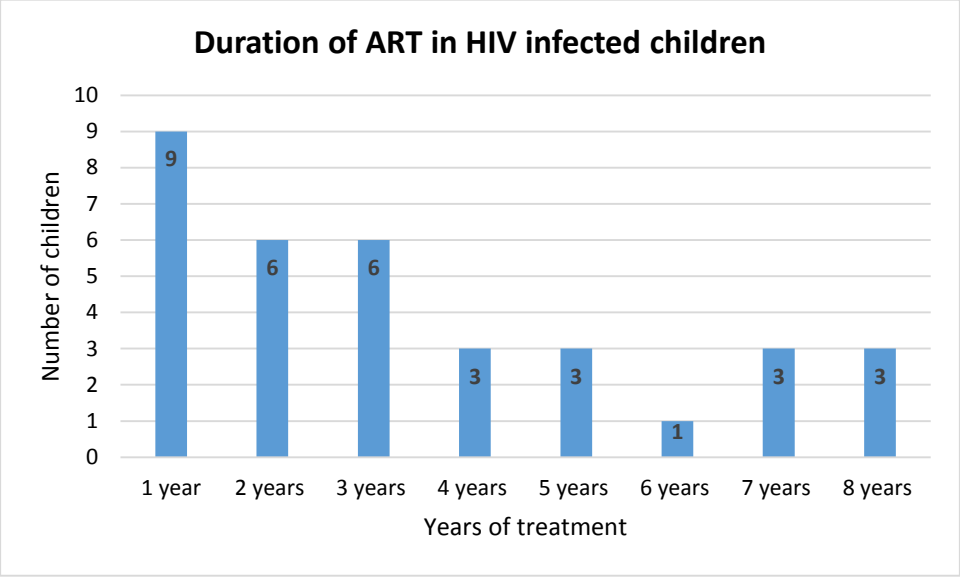


Figure 5.11 Duration of ART among the HIV infected children

Around half of the children on ART followed the regimen AZT + 3TC + NVP (42%, n=18), followed by AZT + 3TC + EFV (16%, n=7). (AZT- Zidovudine, 3TC- Lamivudine, NVP-Nevirapine, EFV- Efavirenz)

CD4 + T cell estimation

The median CD4+ T cell count in HIV infected children was 725.5 (Interquartile range=369.0 to 1053.0) and showed a mean of 708.8. The median of CD4+ count in healthy children was 980 (Interquartile range=684.0 to 1342.0) and showed a

mean of 1040.38. This difference in the count was statistically significant (p=0.0021). A Box and Whisker plot showing the distribution of CD4 +T cell count among HIV infected children and healthy children is shown in **Figure 5.12.**

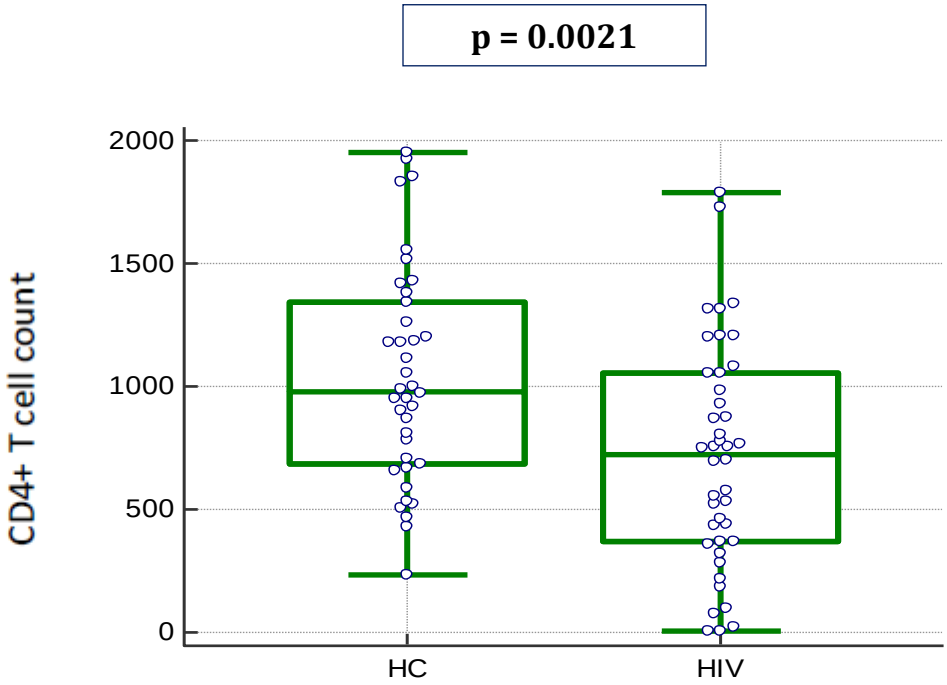


Figure 5.12 Box and Whisker plot showing the distribution of CD4+ T cell count among HIV infected and healthy children.

CD4+T cell %

The median CD4 + T cell % in HIV infected children was 27.29 (Interquartile range=16.6 – 32.6) and showed a mean of 24.8. The median of CD4+ count in healthy children was 29.44 (Interquartile range=26.43-39.49) and showed a mean of 30.85. This difference in percentage was not statistically significant (p=0.067). A Box

and Whisker plot showing the distribution of CD4+ T cell % among HIV infected children and healthy children shown in **Figure 5.13.**

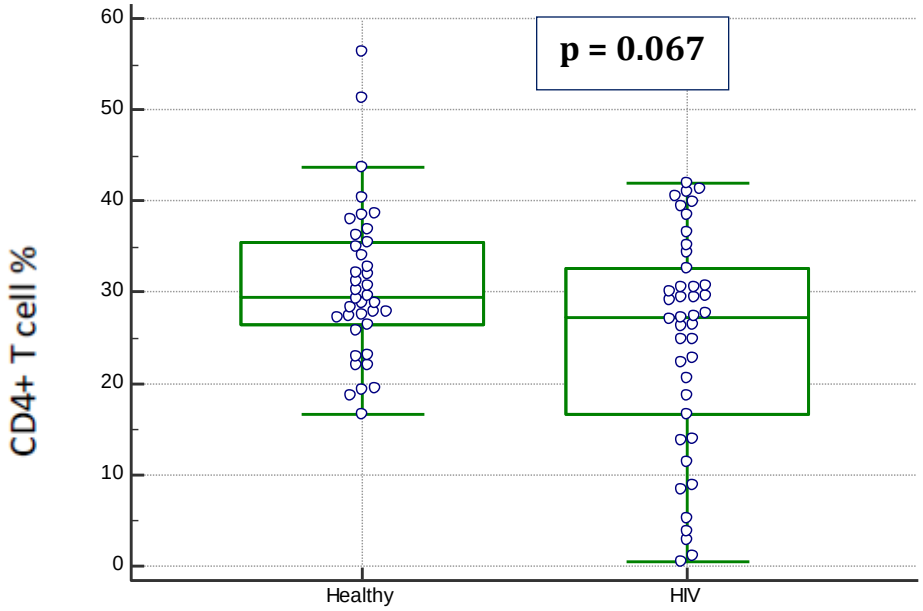


Figure 5.13 Box and Whisker plot showing the distribution of CD4 +T cell % among HIV infected children and healthy children.

All the 80 children recruited in the study were Hepatitis B virus uninfected. All the samples were HBs Ag 'Non-Reactive'. The total core antibody testing was also negative for all the 80 samples which rules out any past infection with HBV.

Anti HBs Titre

Only 4 (10%) out of the 42 HIV infected children had protective antibody levels while the remaining 74% (n=31) had anti HBs titre <10 mIU/ml. No child had more than 100 mIU/ml of anti HBs titre. A pie chart showing the number of children with protective Anti HBs titre is given in **Figure 5.14.** Among the healthy children 47% (n=18) had

protective anti HBs titre while 53% (n=20) were not protected. The number of healthy children with protective and non-protective AntiHBs titre is given in **Figure 5.15.** This difference in the proportion of protected children among the two HIV infected and controls was statistically significant (p=0.0006).

Overall the median of anti HBs titre in HIV infected was 0.34 (Interquartile range=0.07 to 1.15) and the mean was 4.33 mIU/ml. Overall the median of anti HBs titre in healthy children was 9.19 mIU/ml (Interquartile range=2.69 to 37.87) and the mean was 59.23 mIU/ml. This difference was statistically significant (p<0.0001). This data is represented as a Box and Whisker plot showing the distribution of antiHBs titre among HIV infected children and healthy children in **Figure 5.16.**

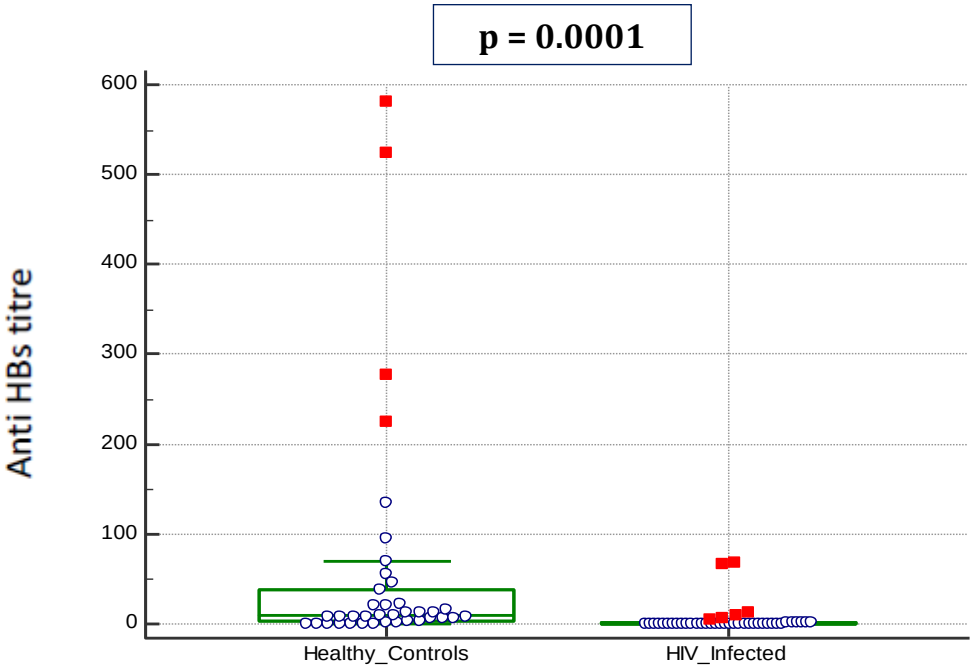


Figure 5.16 Box and Whisker plot showing the distribution of anti HBs titre among HIV infected children and healthy children.

Protective anti HBs titre

Among the protected HIV infected children the highest titre value was 66.09 mIU/ml and the lowest value was 10.19 mIU/ml. Two of the 4 protected were ART naïve. The details of the children with protective antibody titre are given in **Table 5.2.**

Table 5.2 Details of 4 HIV infected children who were having protective anti HBs titre.

Sl. no.	**Age (years)**	**Sex**	**Mode of transmission of HIV**	**State**	**Duration of ART**	**CD4 count**	**CD4 %**	**Anti HBs titre**	**WHO Stage**
1	3	M	MTCT	Tamil Nadu	Naïve	1787	29.51	67.39	2
2	5	M	MTCT	Tamil Nadu	2 years	1314	39.9	10.19	3
3	13	M	MTCT	Tamil Nadu	Naïve	578	29.44	12.44	1
4	13	M	Transfusion associated	Chhattisgarh	8 years	522	35.14	66.09	1

Among the protected 18 healthy children, median of anti HBs titre was 41.55 mIU/ml (Interquartile range=15.28 to 133.95). In this protected group majority 72% (n=13) had anti HBs titre between 10-100 mIU/ml.

Mean protective antibody titre in HIV infected children was 39.02 and that in healthy children was 120.56.

Age wise distribution children having protective Anti HBs titre.

The age wise distribution of HIV infected children having protective Anti HBs titre given in **Figure 5.17**. The majority of children, 54% (n=23) with non-protective antibody titre fall in the 11-15 age group. There are no children with protective antibody titre in 6-10 age group.

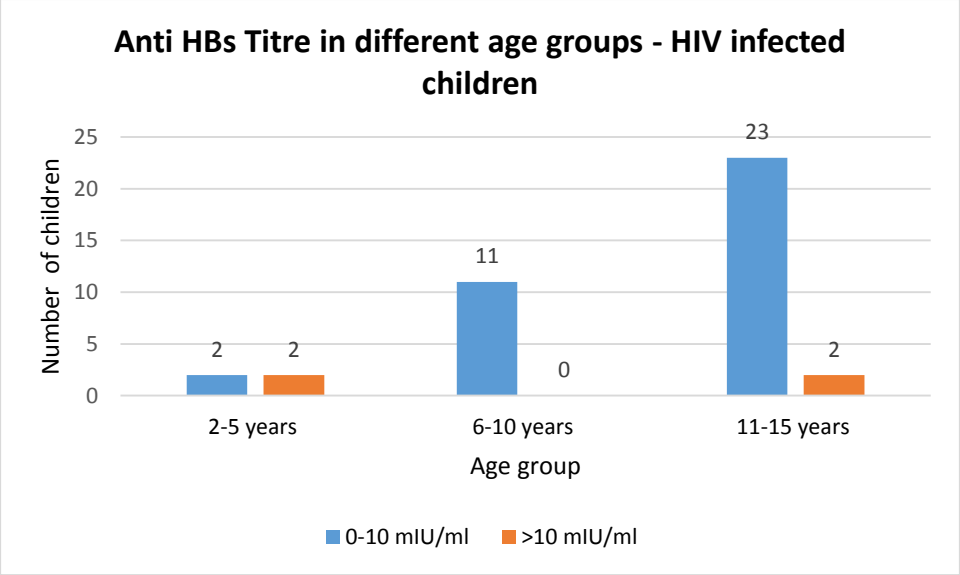


Figure 5.17 Age wise distribution of HIV infected children having protective Anti HBs titre.

Correlation between age and anti HBs titre was -0.202, which is a negative correlation stating as age increases the anti HBs titre decreases.

Age wise distribution of healthy children having protective Anti HBs titre is shown in **Figure 5.18.** The majority of protective anti HBs titre 38.8% (n=7) was between 6-10

years of age. The majority of non-protective anti HBs titre 25% (n=7) was between 2-5 and 11-15 years of age.

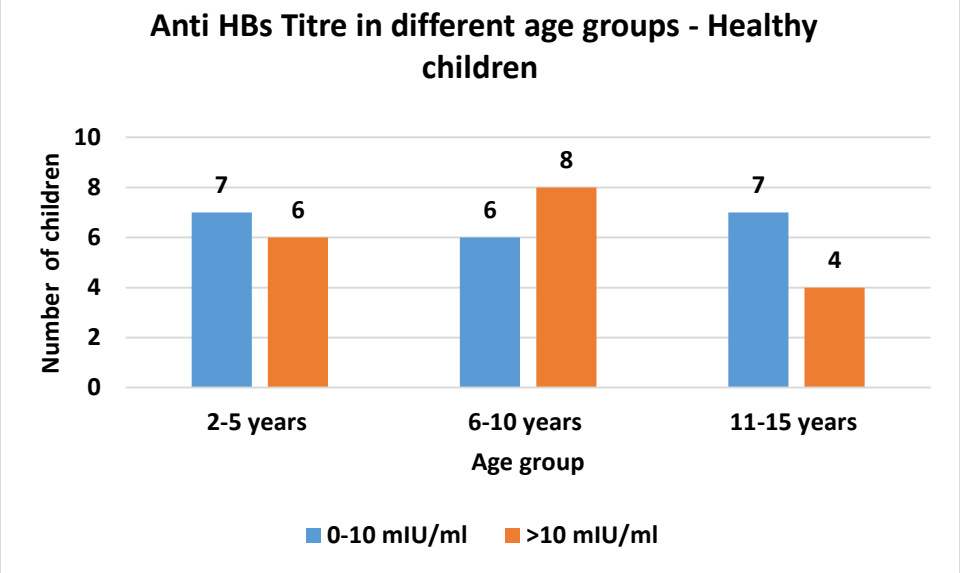


Figure 5.18 Age wise distribution of healthy children having protective Anti HBs titre.

Comparison anti HBs titre in children on ART and treatment naïve

Anti HBs titre showed a median of 0.29 mIU/ml in children on ART (n=34) and a mean of 2.95 mIU/ml. In treatment naïve HIV infected children (n=8) anti HBs titre showed a median of 0.44 mIU/ml and mean of 10.18 mIU/ml. This difference was not statistically significant (p=0.42). This data is represented as a Box and Whisker plot showing the distribution of anti HBs titre among HIV infected children on ART (n=34) and treatment naïve (n=8) in **Figure 5.19.**

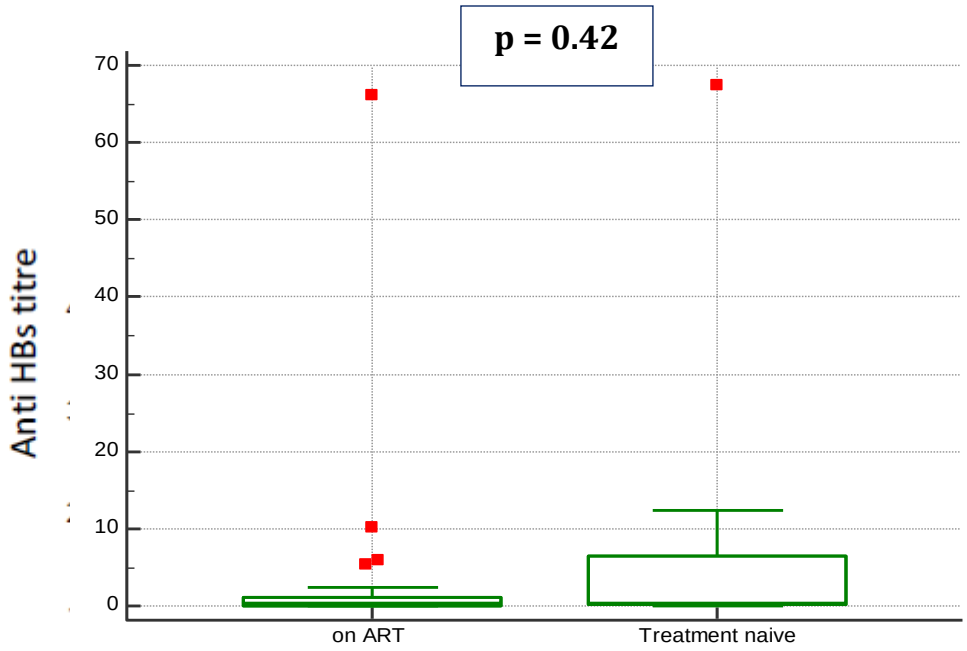


Figure 5.19 Box and Whisker plot showing the distribution of anti HBs titre among HIV infected children on ART (n=34) and treatment naïve (n=8).

There was positive correlation observed in HIV infected children with both CD4+T count and anti HBs titre (0.249), and also with CD4+T % and anti HBs titre (0.176), But both were statistically not significant (p=0.112 and p=0.265 respectively).

There was a negative (-0.312) correlation observed between anti HBs titre and WHO staging (p=0.045) among HIV infected children, ie as the WHO stage increases the anti HBs titre level decreases.

Functional study to look at the memory T and B cell activity by Flow cytometry assay

A proportion of the samples from HIV infected children (n=14) and healthy children (n=15) were subjected to functional assays to look at the presence of Hepatitis B surface antigen specific IFN-γ produced by CD4+ T cell and Hepatitis B surface antigen specific memory B cell .

Percentage of HBsAg specific IFN-γ by CD4+ T cells- Flow cytometry assay

The percentage of CD4+ T cells with Hepatitis B surface antigen specific IFN-γ in HIV infected children is plotted in **Figure 5.20**. The percentage of cells varied from 0 to 2.1%.

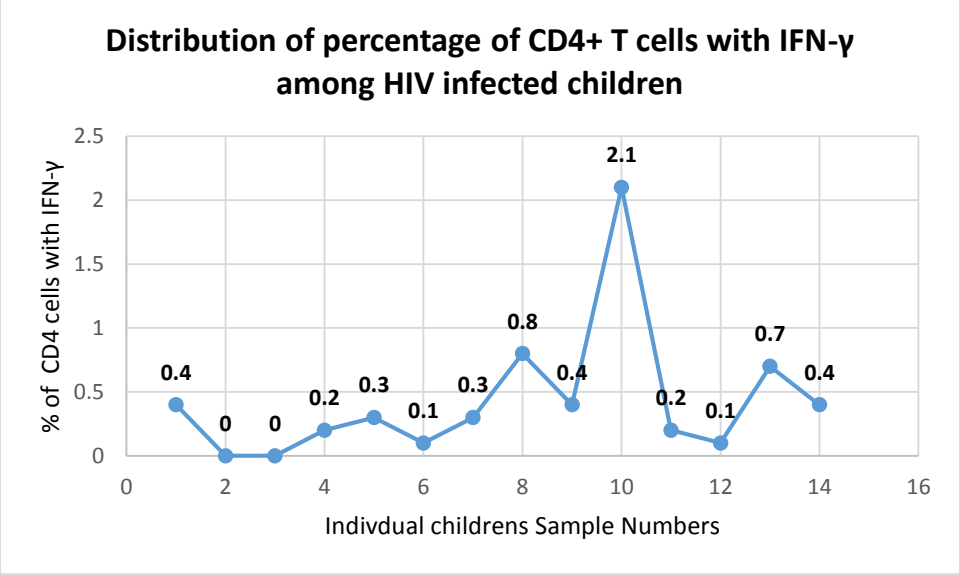


Figure 5.20 Distribution of percentage of CD4+ T cells with Hepatitis B surface antigen specific IFN-γ among HIV infected children.

The percentage of CD4+ T cells with Hepatitis B surface antigen specific IFN-γ in healthy children is plotted below in the **Figure 5.21**. The percentage of IFN-γ positive cells varied from 0.0 to 0.3.

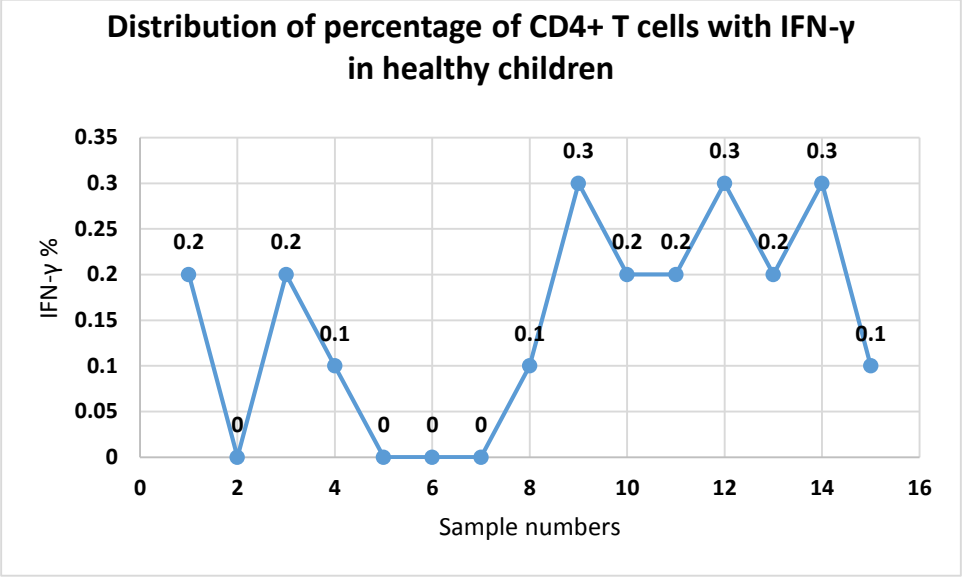


Figure 5.21 Distribution of percentage of CD4+ T cells with Hepatitis B surface antigen specific IFN-γ among healthy children.

A representative image of gating strategy used for estimating % of CD4+ T cells producing Hepatitis B surface antigen specific IFN-γ is shown in **Figure 5.22.a-c.**

Figure 5.22.a shows the representation of unstimulated PBMC/ negative control in which % of CD4 +T cells producing Hepatitis B surface antigen specific IFN-γ is 0.1%. **Figure 5.22.b** shows the representation of PBMC stimulated with Hepatitis B surface antigen in which % of CD4+ T cells producing Hepatitis B surface antigen specific IFN-γ is

increased to 0.3%. **Figure 5.22.c** shows the representation of PBMC stimulated with PMA (positive control) in which % of CD4 +T cells producing Hepatitis B surface antigen specific IFN-γ is increased to 2%.

The median % of CD4+ T cells producing Hepatitis B surface antigen specific IFN-γ in HIV infected children was 0.3 (Interquartile range= 0.10 to 0.40) and the mean was 0.43%. The median of % of CD4+ T cells producing IFN-γ in healthy children was 0.2 (Interquartile range=0.26 to 0.20). The difference in the median % between the two groups was significant (p = 0.0427). This data is represented as a Box and Whisker plot showing the distribution of percentage of CD4+ T cells producing Hepatitis B specific IFN-γ among HIV infected children and healthy children in **Figure 5.23.**

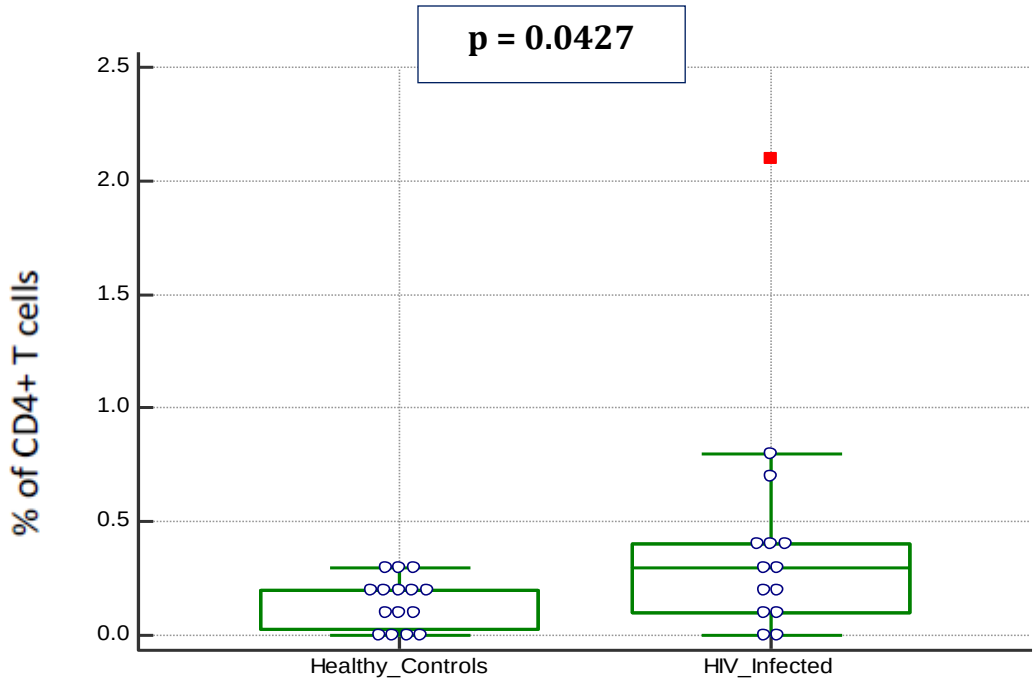


Figure 5.23 Box and Whisker plot showing the distribution of percentage of CD4+ T cells producing Hepatitis B specific IFN-γ among HIV infected children and healthy children.

Percentage of Hepatitis B surface antigen specific memory B cell - Flow cytometry assay

The percentage of Hepatitis B surface antigen specific memory B cell was plotted in HIV infected children in **Figure 5.24**. The highest percentage of Hepatitis B surface antigen specific memory B cell was 5.7% and lowest percentage was 0.

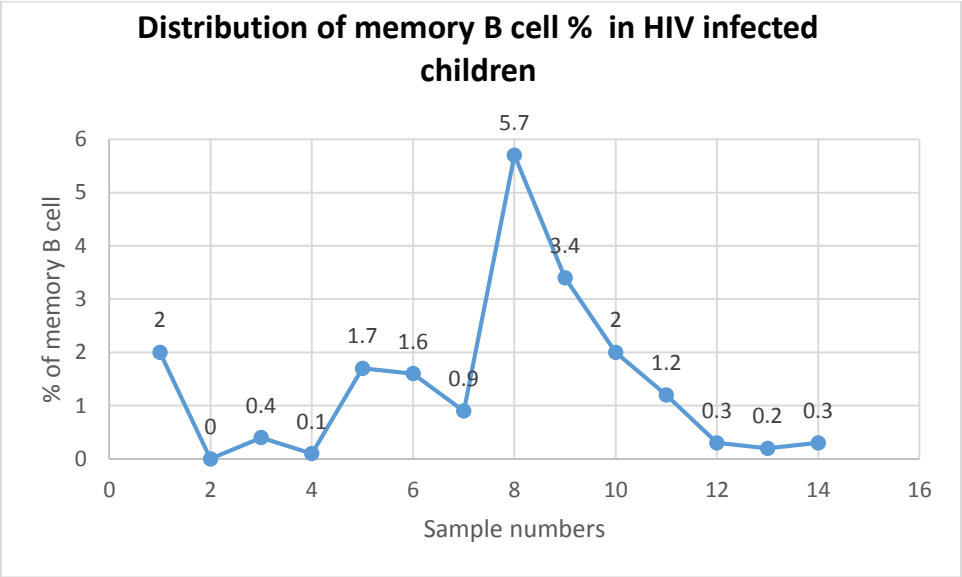


Figure 5.24 Distribution of percentage of Hepatitis B surface antigen specific memory B cell in HIV infected children.

The percentage of Hepatitis B surface antigen specific memory B cell in healthy children is plotted in **Figure 5.25**. The highest percentage of memory B cell was 3.4 and the lowest percentage was 0.2.

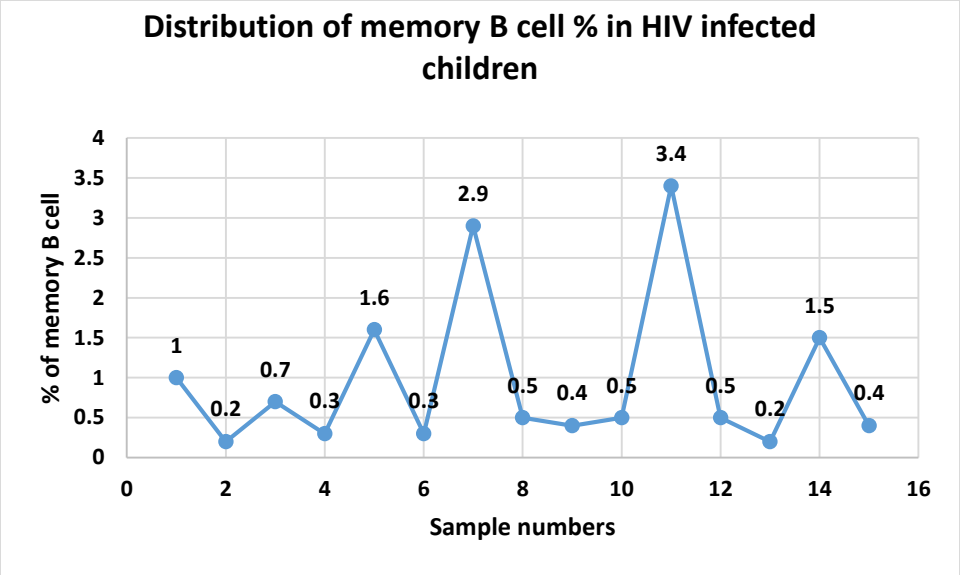


Figure 5.25 Distribution of percentage of Hepatitis B surface antigen specific memory B cell among healthy children.

A representative image of gating strategy used for estimating percentage of Hepatitis B surface antigen specific memory B cell is shown in **Figure 5.26.a-c.**

Figure 5.26.a shows the representation of unstimulated PBMC/ negative control in which % of Hepatitis B specific memory B cell is 3.5%. **Figure 5.26.b** shows the representation of PBMC stimulated with Hepatitis B surface antigen in which % of Hepatitis B specific memory B cell is reduced to 3.1%. **Figure 5.26.c** shows the representation of PBMC stimulated with PMA (positive control) in which % of Hepatitis B specific memory B cell is reduced to 0.3%.

The median % of Hepatitis B surface antigen specific memory B cell in HIV infected children was 1.05 (Interquartile range=0.30 to 2.00) and the mean was 1.41. The median % of memory B cell in healthy children was 0.5 (Interquartile range=0.33 to 1.38) with a mean of 0.96. This difference in the % between the two groups was not significant (p=0.6942). This data is shown as a Box and Whisker plot in **Figure 5.27.**

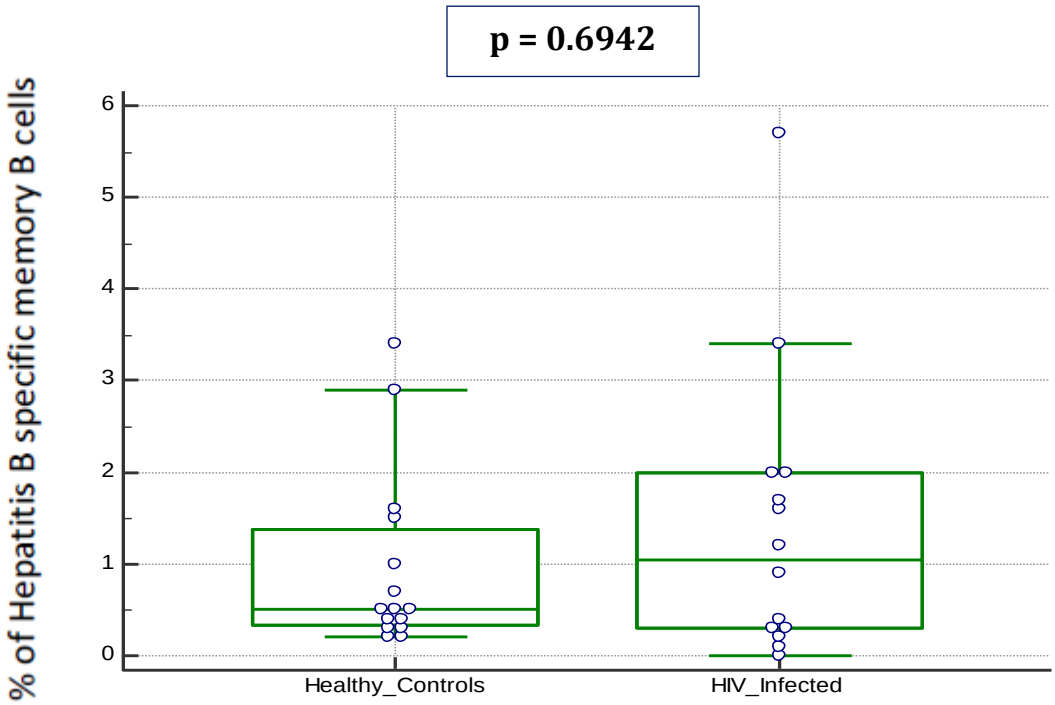


Figure 5.27 Box and Whisker plot showing the distribution of percentage of Hepatitis B specific memory B cell among HIV infected children and healthy children.

6. Discussion

Globally, there were 36.9 million people living with HIV at the end of year 2017. Sub-Saharan Africa had 25.7 million people living with HIV. An estimated 1.8 million children are living with HIV at the end of 2017, mostly (88%) in sub-Saharan Africa (3–5). According to 2017 UNAIDS reports, there were 160,000 new HIV infections in children at the end of 2016 and 120,000 children died of AIDS-related illnesses in the same year. Estimated 43 % of children living with HIV are on ART. (5). As per the recently released, India HIV Estimation 2016 report, the total number of People Living with HIV (PLHIV) in India is estimated at 21.17 lakhs (17.11 lakhs–26.49 lakhs) in 2015. Children (< 15 years) account for 6.54% (1.38 lakhs) of the HIV infected individuals (6). More than 90 % of the children living with HIV acquired the disease through mother to child transmission. The relative proportions of mother to child transmission of HIV are 23-30% before delivery, 50-60% during delivery and 12-20% during breast feeding(34). Currently 3 drug ART with TDF + 3TC + EFV (TDF-Tenofovir, 3TC- Lamivudine, EFV-Efavirenze) in all pregnant and breastfeeding women and infant prophylaxis with AZT +3TC + NVP (AZT-Zidovudine, 3TC- Lamivudine, NVP-Nevirapine) markedly reduced the mother to child transmission (71)

Immunizations can help human immunodeficiency virus (HIV)–infected children to prevent life threatening diseases. Since the antibody response to vaccination is critically dependent upon CD4+ T cell function, a poor vaccine response can be expected in individuals infected with HIV, particularly those with advanced disease. It is very

important to immunize HIV-infected children as soon as possible so that they can mount protective responses prior to the failing of their immune system. However, if there was suboptimal antibody response to the initial vaccine, revaccination once immune reconstitution and virologic suppression has been achieved is recommended for certain vaccines (78).

Risk factors and modes of transmission among Hepatitis B virus (HBV) and human immunodeficiency virus (HIV) are common. The prevalence of chronic HBV infection in HIV-infected individuals is 5%-15% (84) . Co-infection can increase the risk of mortality by up to 8 times (85). Thus it is important to prevent hepatitis B virus infection in HIV infected children.

HBV vaccine is a recombinant yeast derived vaccine. It is given as 1 ml intramuscular injection at 0, 1, 6 months of age according to national immunization schedule. Protective level of immune response – anti HBs titres > 10 IU/L. Overall reported immune seroconversion following the vaccination is 95 % in adults and children after 3 completed dose. It is also available in combination such as Twinrix (Hepatitis A & Hepatitis B Recombinant Vaccine), Pentavalent (DTwP+Hib+ Hep B) and Hexavalent (DTwP+Hib+ Hep B+IPV) vaccine (106,107).

In the study carried out in this thesis was from February 2016 to September 2018, there were 2 groups: the first group consisted of 42 HIV infected children and the second group, consisted of 38 healthy children between 2-15 years of age and all of them had taken primary Hepatitis B vaccine.. Majority of the HIV infected children in our study were from Tamil Nadu 69 % (n=29), while the healthy children were from West Bengal

39% (n=12). The median age group was 11 years (interquartile range 9-13years) in HIV infected while 7 years (interquartile range 5-11years) in healthy controls. Majority of the study subject in our study, both in HIV infected (62%, n=26) and healthy controls (69%, n=26) were males. Median CD4 + T cell count in HIV infected children was 725.5 (Interquartile range=369.0 to 1053.0) and mean was 708.8. The Median of CD4+ T cell count in healthy children was 980 (Interquartile range=684.0 to 1342.0) and mean was 1040.38. This difference in CD4+T cell was statistically significant (p value = 0.0021) as expected and reported earlier in the literature.

Median CD4 + T cell % in HIV infected children was 27.29 (Interquartile range= 16.6 – 32.6) and showed a mean of 24.8. Median of CD4 + T cell % in healthy children was 29.44 (Interquartile range=26.43-39.49) and showed a mean of 30.85. This difference in CD4 + T cell % was not statistically significant (p value = 0.0672).

In our study only 10 % of the 42 HIV infected children had (n=4) protective anti HBs titre which was significantly (p = 0.0006) lower than the 47% observed among 38 healthy children. Overall the median anti HBs titer of 9.19 IU/L observed among the healthy children were significantly (p = < 0.0001) higher than the median of 0.34 IU/L estimated among the HIV infected children.

In a similar study done by Haban et.al (2017) in Morocco among 49 HIV infected children and 112 HIV uninfected healthy children with age between 10 months to 10 years showed 29 % (14/49) of the HIV infected children had protective anti HBs titre and 76 %(85/112) of the control group had protective antibody titer. This frequency of protected children was higher compared to the proportion of seroprotection observed in

our study group but there was significantly higher proportion of healthy children were protected in both the studies. The difference in the median CD4+T cell % of 34.89 in healthy controls and 25.6 in HIV infected children reported in that study was statistically significant ($p < 0.0001$). In our study though the median CD4+T cell % was higher in healthy controls, but was not statistically significant (13).

Similarly Nlend etal. (2016), reported data from 25 HIV infected children, 29 HIV exposed but uninfected children and 28 healthy children. All the children recruited were between the age group of 6 months to 5 years of age in their study. Among the HIV infected children (n=25), 20 % (5\20) were seroprotected. In same study seroprotection among HIV exposed but uninfected children were 51 % (15/29) and healthy controls it was 60% (17/28). These findings were consistent with the data presented in this thesis (108).

Cuilla etal, (2012) enrolled 242 HIV infected children: 128 children from Cameroon and 114 children from Senegal in a similar study. The median age of the children in the study was 15 months. Majority of the children were males 53 % (130/242) like in our study. Seroprotection rates to Hepatitis B vaccine in first group from Cameroon had 92 % (118/128) and from the second group from Senegal had 58% (66/114(109).). Cameroon had a very high seroprotection of 92 % not similar to the already published data (12,13,108,110).

Pessoa et.al (2010) studied 40 HIV infected children and 23 healthy controls from Brazil in the age between 10-20 years. Median age in HIV group was 12.7 years and 13.9 years

in control group. Sex distribution was even for this study. Forty five % (18/40) of HIV infected were seroprotected while the protection was 78% (18/23) in the control group (Chi-square test, p = 0.010). In our study the seroprotection in both groups were lower compared to this study. Mean anti-HBs antibody levels from HIV-infected adolescents were lower than mean antibody levels from control group. This finding is consistent with our study as mean anti-HBs antibody levels from HIV-infected adolescents were lower than mean antibody levels from control group (111). In the study reported in this thesis, there was no significant relation between the gender and anti HBs titers among both HIV infected children (p=0.37) and healthy children (p=0.26).

Pippi et.al (2008) studied 84 HIV infected children from Tanzania aged 1-10 years. The seroprotection to Hepatitis B vaccine in 84 HIV infected children was 59.5 %. This was higher than the seroprotection observed among HIV infected children in our study. ART use and absolute CD4+ T cell count were independently associated with probability of seroconversion and with higher anti-HBs titers (15). Even in our study there was positive correlation between CD4+ T cell counts and anti-HBs titers but it was not significant.

In an Indian study done by Bose et.al (2015) seroprotection in HIV infected children(n=40) less than 15 years of age after double the dose of standard Hepatitis B vaccination was 94 %. This study showed higher seroconversion with previous studies reported globally and also compared one Indian study discussed below (12,13,108,110). The median CD4 +T cell count observed in that study among HIV infected was 738/mm^3(112). Likewise a similar study by Siddique et.al (2017) compared the

seroprotection of double dose Hepatitis B vaccine with standard regimen in HIV infected children (n=55) between 18 month to 18 years. It showed a 74 % seroprotection in double dose regimen compared to 60 % in standard regimen. This study showed not much of difference in seroprotection in both cases. (110).

In our study the seroprotection to Hepatitis B vaccine was 47 % (18/38) among the HIV uninfected control groups. After the full course of Hepatitis B vaccine the seroprotection in otherwise healthy individuals was approximately 90-95 %. There are several studies which showed the protection level against HBV following vaccination can come down as the age increases. In a study reported on 640 healthy children by Roznovsky et.al (2010), with complete 3 dose Hepatitis B vaccination, 93% showed protective anti-HBs titer following 4 weeks of vaccination. However, this fell to 70%, 40% and 25% at 5, 10, and 15 years of age respectively(92). This showed a declining anti HBs titre with age. Hammitt et.al (2007), has demonstrated lack of amnestic response to booster dose following infant Hepatitis B vaccine at 15 years of age with a response of 51%.????. Remaining 49% of the children did not show evidence of immune memory(113). In our study among HIV infected child the correlation between between age and anti HBs titre was -0.202, which showed that as age increases the anti HBs titre decreases. Similar finding was shown in several studies (13, 21)

In our study there was slight increase in anti HBs titres when there is an increase in % CD4+T cell count among both HIV infected group and the control group (rho=0.16) though it was not significnat. Haben etal.(2017) showed lower % CD4 +T cell were observed in HIV infected children with a poor antiHBs response (13). Pessoa et.al (2010)

showed that among the HIV infected group a lower CD4+ T cell % was shown in children having anti HBs titre < 10 IU/ml compared to those with protective antiHBs titre > 10 IU/ml (111).

Among HIV infected children in our study, most of the children were classified in WHO Stage 2 (40 %, n=17) and Stage 3 (40 %, n=17) when they came to the health facility for the HIV management. This might be one of the reason for having a very poor protective antibody response (seroprotection=10 %) in our study group compared to that found by Nlend etal., (2016) with a seroprotection of 20% in which all children were in WHO stages 1 and 2 (108).

In the study reported in this thesis 81% (n=34) of the HIV infected children were on ART, while 19 % (n=8) were treatment naïve. There was no significant (p=0.45). difference between the anti HBs titers in HIV infected children on ART and those who were treatment naïve. In the study by Haben etal (2017) 71.4 % (85/12) on ART and they observed that percent of children on ART with suboptimal anti HBs response was lower compared to children with a protective level (13). In another study seroconversion was found in 70.8 % of children on ART compared to 44.4% in treatment naïve children (12)

In the present study, we have looked at HBV specific T and B memory cell in a proportion of both HIV infected and HIV uninfected healthy children. The percentage of CD4 +T cells producing hepatitis B surface antigen specific IFNγ in HIV infected children showed a significantly (p = 0.043) higher % with a median of 0.3% (Interquartile rang= 0.10 to 0.40) compared to the median of % of CD4+T cells

producing IFNγ in healthy children (0.2%) (Interquartile range=0.26 to 0.20). In a study done by Giacomet et.al (2018) on immune response to Hepatitis B vaccine in HIV infected children, following booster vaccine there was an increase in IFNγ secretion in those with protective anti HBs titre compared to those who do not have protective titre (114). In a study by Gelinas et.al (2017) among healthy children there was a positive correlation between HBs Ag specific IFNγ and anti HBs titre (115). In a study by Paricha et.al (2006) there was an increase in Hepatitis B specific IFNγ level in HIV infected children compared to healthy controls (116). Study done by Weinberg et.al (2018) on persistence of memory B and T cell in HIV infected children between the age of 7 -12 years to HPV vaccination showed that there was no significant difference on both T cell memory and B cell memory among children with 3 doses of vaccine and 4 doses of vaccine (117).

In our study percentage of Hepatitis B surface antigen specific memory B cell in HIV infected children showed a median of 1.05 (Interquartile rang= 0.30 to 2.00) while the median % of memory B cell in healthy children was 0.5 (Interquartile range=0.33 to 1.38. This difference in memory B cell % was not statistically significant (p value =0.6942). Similar findings on B cell memory were not found in any other studies. However, Anti-HBs titre decay and antibody kinetics among HIV infected children needs further detailed investigation to look at the equal or higher T and B cell memory activities compared to healthy children but lower anti HBs titre.

There are several studies on the memory T and B cell activity among HIV infected children following vaccines other than HBV. In a study done by Luo et.al (2015) on B cell activation pattern after influenza vaccine in HIV infected and healthy controls showed that HIV-infected patients tended to have a similar or even higher quantity and quality of Ag-specific Abs post-vaccination. This study also showed vaccine-induced fold change of Ag-specific IgM was higher in HIV infected individuals as compared to controls, but this was not true for IgA or IgG. This suggests a possible defect in class switching perhaps as a result of elevated apoptosis and dysfunctional T follicular helper cells among HIV infected children (118). In the study reported here there was no significant correlation observed between anti HBs titers and both T and B cell memory activity among HIV infected individuals and healthy controls.

One of the other important findings observed in our study is the lower rate of protection against HBV following vaccination in both HIV infected and healthy children. This low level of anti HBS titer may be because of various reasons. One of the reasons may be malnutrition. It is a factor for the attainment of low protective immunity to vaccination. In a study by Cuilla etal, (2012) showed that moderate to severe malnutrition was found in 62 % of the HIV infected children with anti HBs titre <10 IU/L , while it was 31 % with those with antibody titres > 10 IU/L among the same population (109). In our study nutritional status and socio economic status of the study participants were not looked into.

Another reason may be the potency of the vaccine due to inappropriate storage. Evaluation of the vaccination programmes in developing and under developed countries

need supplemental and regular biological monitoring to keep check on storage and transport of the vaccine. Hepatitis B recombinant vaccine has to be stored only at 2-8 ^{0}C. Storage issues can interfere with vaccine efficacy.

In conclusion the levels of CD4 + T cell and anti HBs titre were higher in healthy controls compared to HIV infected children as expected. However, the percentage of HBV specific IFNγ producing CD4 + T cell and the memory B cell response were relatively equal or higher among HIV infected children than the healthy controls. Even though the Hepatitis B specific memory B cells response was more in HIV infected children, the anti HBs antibody titre was found to be low which may be due to functionally defective B/T-cell population in HIV infected children . Similarly the percentage of HBV specific CD4 +T cells is more or equal in HIV infected children than the control group in spite of lower CD4 +T cell count. Another possible reason behind this could be due to chronic activation of immune cells in HIV infected children showed relatively higher immune response compared to healthy controls (116).

Our study had shown that HIV-infected children studied developed a suboptimal response to HBV vaccine. Hence, post-vaccination monitoring of the anti-HBs seroconversion is fundamental, for HIV-infected children. This post-vaccination monitoring will help optimize vaccination against hepatitis B, for these patients, across the country. Since the T cell and B cell memory are higher in HIV infected children compared to healthy children, giving a booster dose of Hepatitis B vaccine should be considered to bring in good immune response in the HIV infected children.

Anti-HBs titre decay and antibody kinetics among HIV infected children needs further detailed investigation to maximize the benefits of HBV vaccination in HIV infected individuals.

Limitations

- The sample size was small to clearly T cell and B cell response, further study is required to confirm the response.
- More number of treatment naïve HIV infected subjects are needed to elicit the difference in anti HBs titre kinetics among those on ART and treatment naïve.
- Lack of data on nutritional status also restricts the breath of findings on protective antibodies both the cases and the controls. Other factors like climate, cold chain supply system may also contribute to the vaccine response which was not looked into.
- CD4+T cell count as well as suppressed viral load at the time of vaccination (base line) was not known.
- In flow cytometry assay, absolute count of HBs antigen specific CD4 +T cell and memory B cell should have been assessed rather than percentage.

7. Summary and Conclusions

Summary

- This study was conducted on 42 HIV infected children and 38 healthy controls
- Majority of the HIV infected children were from Tamil Nadu 69 % (n=29), while the healthy children were from West Bengal 39% (n=12).
- Majority of the study subject in both HIV infected (62%, n=26) and healthy controls (69%, n=26) were males.
- The median age group was 11 years (interquartile range 9-13years) in HIV infected group while 7 years (interquartile range 5-11years) in healthy controls.
- Majority of the HIV infected children acquired the infection from mother to child transmission, except two.
- Among HIV infected children most of the children were classified in WHO Stage 2 (40 %, n=17) and Stage 3 (40 %, n=17) prior to ART
- In this study 81% (n=34) of the HIV infected children were on ART, while 19 % (n=8) were treatment naïve.
- The Median CD4 + T cell count in HIV infected children was 725.5 (Interquartile range=369.0 to 1053.0) while the Median CD4+ T cell count in healthy children was 980 (Interquartile range=684.0 to 1342.0).The difference in the count was statistically significant (P = 0.002).
- Median CD4 + T cell % in HIV infected children was 27.29 (Interquartile range= 16.6 – 32.6) while the Median CD4+ T cell % in healthy children was 29.44

(Interquartile range=26.43-39.49). This difference just fell short of statistical significance (p value = 0.067).

- Among the 42 HIV infected children 4(10%) had protected level of Anti HBs and out of the 38 healthy children 18 (47%) were protected (p=0.0006). The Median anti HBs titre in HIV infected children is 0.34 (Interquartile rang= 0.18 to 0.45). Median of anti HBs titre in healthy children is 9.19 (Interquartile range=2.69 to 37.87). This difference was statistically significant (p = < 0.0001.
- There was no significant difference (p =0.42) between the median anti HBs titers in HIV infected children on ART and those who were treatment naïve.
- Percentage of CD4 +T cells producing Hepatitis B surface antigen specific IFN-γ in HIV infected children showed a median of 0.3 (Interquartile rang= 0.10 to 0.40). Median of % of CD4+T cells producing IFN-γ in healthy children is 0.2 (Interquartile range=0.26 to 0.20). The difference was statistically significant (p = 0.0427).
- Percentage of Hepatitis B surface antigen specific memory B cell in HIV infected children showed a median of 1.05 (Interquartile rang= 0.30 to 2.00) while among healthy children it was 0.5 (Interquartile range=0.33 to 1.38) and the difference was not statistically significant (p =0.694).

The levels of CD4 + T cell and anti HBs titre were higher in healthy controls compared to HIV infected children as expected. However, the percentage of HBV specific IFNγ

producing CD4 + T cell and the memory B cell response were relatively equal or higher among HIV infected children than the healthy controls. Even though the Hepatitis B specific memory B cells response was more in HIV infected children, the anti HBs antibody titre was found to be low.

In our study since the T cell and B cell memory are higher in HIV infected children compared to healthy children, giving a booster dose of Hepatitis B vaccine should be considered to bring in good immune response in the HIV infected children. We have also shown that HIV-infected children studied developed a suboptimal response to HBV vaccine. Therefore, post-vaccination monitoring of the anti-HBs seroconversion is fundamental, for HIV-infected children. This will help optimize vaccination against hepatitis B, for these patients, across the country.

Anti-HBs titer decay and antibody kinetics among HIV infected children needs further detailed investigation to maximize the benefits of HBV vaccination in HIV infected individuals.

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January 31, 2016

Dr. Sonia M,
PG Registrar,
Department of Clinical Microbiology,
Christian Medical College,
Vellore - 632 004.

Sub: **Fluid Research Grant NEW PROPOSAL:**
Measurement of immune response to Hepatitis B vaccine in HIV-infected and healthy children
Dr. Sonia M, Employment Number:21285, Post graduate Registrar, Department of Clinical Microbiology, Dr. Rajesh Kannangai, Employment Number:20093, Professor and Head, Dr. Priya Abraham, Employment no: 11714, Professor, Dr. John G Fletcher, Employment no: 31449, Assistant Professor, Department of Clinical Virology, Dr. Valsan Philip Verghese, Employment no: 13878, Professor and Head, Dr. Winsley Rose, Employment no: 28222, Professor, Dr. Anila Chacko, Employment no: 28283, Associate Professor, Paediatrics Unit-III, Dr. John Mathai, Employment No:13178, Professor and Head, Department of Paediatric Surgery, Dr. Jujju Jacob Kurian, Associate Surgeon., Employment No: 28590, Dr. Tarun Jacob John, Employment No: 20785, Assistant Professor, Mr. John Paul Demosthenes, Employment no: 33324, Associate Research Officer, Mrs. R. Veena Vandhini, Employment no: 32651, Associate Research Officer, Department of Clinical Virology.Mrs. Grace Rebekah, Employment no: 32070, Lecturer, Department of Biostatistics

Ref: IRB Min No: 10410 [OBSERVE] dated 05.12.2016

Dear Dr. Sonia M,

I enclose the following documents:-
1. Institutional Review Board approval 2. Agreement

Could you please sign the agreement and send it to Dr. Biju George, Addl. Vice Principal (Research), so that the grant money can be released.

With best wishes,

Dr. Biju George
Secretary (Ethics Committee)
Institutional Review Board

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Director, Christian Counseling Center,
Chairperson, Ethics Committee.

Dr. Anna Benjamin Pulimood, M.B.B.S., MD., Ph.D.,
Chairperson, Research Committee & Principal

Dr. Biju George, M.B.B.S., MD., DM.,
Deputy Chairperson,
Secretary, Ethics Committee, IRB
Additional Vice-Principal (Research)

January 31, 2016

Dr. Sonia M,
PG Registrar,
Department of Clinical Microbiology,
Christian Medical College,
Vellore - 632 004.

Sub: **Fluid Research Grant NEW PROPOSAL:**
Measurement of immune response to Hepatitis B vaccine in HIV-infected and healthy children
Dr. Sonia M, Employment Number:21285, Post graduate Registrar, Department of Clinical Microbiology, Dr. Rajesh Kannangai, Employment Number:20093, Professor and Head, Dr. Priya Abraham, Employment no: 11714, Professor, Dr. John G Fletcher, Employment no: 31449, Assistant Professor, Department of Clinical Virology, Dr. Valsan Philip Verghese, Employment no: 13878, Professor and Head, Dr. Winsley Rose, Employment no: 28222, Professor, Dr. Anila Chacko, Employment no: 28283, Associate Professor, Paediatrics Unit-III, Dr. John Mathai, Employment No:13178, Professor and Head, Department of Paediatric Surgery, Dr. Jujju Jacob Kurian, Associate Surgeon., Employment No: 28590, Dr. Tarun Jacob John, Employment No: 20785, Assistant Professor, Mr. John Paul Demosthenes, Employment no: 33324, Associate Research Officer, Mrs. R. Veena Vandhini, Employment no: 32651, Associate Research Officer, Department of Clinical Virology.Mrs. Grace Rebekah, Employment no: 32070, Lecturer, Department of Biostatistics

Ref: IRB Min No: 10410 [OBSERVE] dated 05.12.2016

Dear Dr. Sonia M,
The Institutional Review Board (Blue, Research and Ethics Committee) of the Christian Medical College, Vellore, reviewed and discussed your project titled "Measurement of immune response to Hepatitis B vaccine in HIV-infected and healthy children" on December 05th 2016.

The Committee reviewed the following documents:

1. IRB Application format
2. Cvs of Drs. Anila Chacko, Sonia M, Tarun, John Fletcher, John Mathai, Jujju, Priya Abraham, Valsan, Winsley, Mr. John, Mrs. Grace, Rajesh C V and Veena.
3. Consent and Assent forms
4. Patient Proforma
5. No. of documents 1 – 4

...tee Blue, Office of Research, 1st Floor, Carman Block, Christian Medical College, Vellore, Tamil Nadu 632 002
...: 0416 – 2284294, 2284202 Fax: 0416 – 2262788, 2284481 E-mail: research@cmcvellore.ac.in

Dr. B.J. Prashantham, M.A., M.A., Dr. Min (Clinical)
Director, Christian Counseling Center,
Chairperson, Ethics Committee.

Dr. Anna Benjamin Pulimood, M.B.B.S., MD., Ph.D.,
Chairperson, Research Committee & Principal

Dr. Biju George, M.B.B.S., MD., DM.,
Deputy Chairperson,
Secretary, Ethics Committee, IRB
Additional Vice-Principal (Research)

The following Institutional Review Board (Blue, Research & Ethics Committee) members were present at the meeting held on December 05th 2016 in the BRTC Conference Room, Christian Medical College, Bagayam, Vellore 632002.

Name	Qualification	Designation	Affiliation
Dr. Biju George	MBBS, MD, DM	Professor, Haematology, Research), Additional Vice Principal , Deputy Chairperson (Research Committee), Member Secretary (Ethics Committee), IRB, CMC,Vellore	Internal, Clinician
Dr. B. J. Prashantham	MA(Counseling Psychology), MA (Theology), Dr. Min (Clinical Counselling)	Chairperson, Ethics Committee, IRB. Director, Christian Counseling Centre, Vellore	External, Social Scientist
Dr. Ratna Prabha	MBBS, MD (Pharma)	Associate Professor, Clinical Pharmacology, CMC, Vellore	Internal, Pharmacologist
Dr. Rekha Pai	BSc, MSc, PhD	Associate Professor, Pathology, CMC, Vellore	Internal,Basic Medical Scientist
Rev. Joseph Devaraj	BSc, BD	Chaplaincy Department, CMC, Vellore	Internal, Social Scientist
Mr. C. Sampath	BSc, BL	Advocate, Vellore	External, Legal Expert
Dr. Simon Pavamani	MBBS, MD	Professor, Radiotherapy, CMC, Vellore	Internal, Clinician
Dr. Rajesh Kannangai	MD, PhD.	Professor, Clinical Virology, CMC, Vellore	Internal, Clinician
Ms. Grace Rebekha	M.Sc., (Biostatistics)	Lecturer, Biostatistics, CMC, Vellore	Internal, Statistician
Mrs. Pattabiraman	BSc, DSSA	Social Worker, Vellore	External, Lay Person

Ethics Committee Blue, Office of Research, 1st Floor, Carman Block, Christian Medical College, Vellore, Tamil Nadu 632 002
Tel: 0416 – 2284294, 2284202 Fax: 0416 – 2262788, 2284481 E-mail: research@cmcvellore.ac.in

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Chairperson, Research Committee & Principal

Dr. Biju George, M.B.B.S., MD., DM.,
Deputy Chairperson,
Secretary, Ethics Committee, IRB
Additional Vice-Principal (Research)

Dr. Anuradha Rose	MBBS, MD, MHSC (Bioethics)	Associate Professor, Community Health, CMC, Vellore	Internal, Clinician
Dr. Balamugesh	MBBS, MD(Int Med), DM, FCCP (USA)	Professor, Pulmonary Medicine, CMC, Vellore	Internal, Clinician
Dr. Santhanam Sridhar	MBBS, DCH, DNB	Professor, Neonatology, CMC, Vellore	Internal, Clinician
Mrs. Emily Daniel	MSc Nursing	Professor, Medical Surgical Nursing, CMC, Vellore	Internal, Nurse
Dr. Mathew Joseph	MBBS, MCH	Professor, Neurosurgery, CMC, Vellore	Internal, Clinician

We approve the project to be conducted as presented.

Kindly provide the total number of patients enrolled in your study and the total number of withdrawals for the study entitled: "Measurement of immune response to Hepatitis B vaccine in HIV-infected and healthy children" on a monthly basis. Please send copies of this to the Research Office (research@cmcvellore.ac.in).

Fluid Grant Allocation:
A sum of 1,00,000/- INR (Rupees One Lakh Only) will be granted for 2 years. 50,000/- INR (Rupees Fifty Thousand only) will be granted for 12 months as an Ist Installment. The rest of the 50,000/- INR (Rupees Fifty Thousand only) each will be released at the end of the first year as 2 nd Installment..

Yours sincerely,

Dr. Biju George
Secretary (Ethics Committee)
Institutional Review Board

Dr. BIJU GEORGE
MBBS., MD., DM.
SECRETARY - (ETHICS COMMITTEE)
Institutional Review Board,
Christian Medical College, Vellore - 632 002.

IRB Min No: 10410 [OBSERVE] dated 05.12.2016

Ethics Committee Blue, Office of Research, 1st Floor, Carman Block, Christian Medical College, Vellore, Tamil Nadu 632 002
Tel: 0416 – 2284294, 2284202 Fax: 0416 – 2262788, 2284481 E-mail: research@cmcvellore.ac.in

Informed Consent form for the parent / guardian of children participating in a research study (Controls)

Study Title: Immune response to Hepatitis –B vaccine in HIV infected children and healthy children.

Study Number: _____

Subject's Initials: _____ Subject's Name: _____

Date of Birth / Age: _____

(Subject)

(i) I confirm that I have read and understood the information sheet dated _____ for the above study and have had the opportunity to ask questions. [ ]

(ii) I understand that my child's participation in the study is voluntary and that he/she is free to withdraw at any time, without giving any reason, without his/her medical care or legal rights being affected.[ ]

(iii) I understand that the Ethics Committee and the regulatory authorities will not need my permission to look at my child's health records both in respect of the current study and any further research that may be conducted in relation to it, even if I withdraw from the trial. I agree to this access. However, I understand that his /her identity will not be revealed in any information released to third parties or published. [ ]

(iv) I agree not to restrict the use of any data or results that arise from this study provided such a use is only for scientific purpose(s). [ ]

(v) I agree my child to take part in the above study. [ ]

(vi) I hereby give my permission to the investigator(s) to draw an extra 5ml of blood from my child's vein while taking for routine investigations to use for this study

Signature (or Thumb impression) of the parent/ guardien

Date: _____/_____/_____

Signatory's Name: _____

Or

Signature of the Investigator: _____

Date: _____/_____/_____

Study Investigator's Name: _____

Signature or thumb impression of the Witness: _____

Date: _____/_____/_____

Name & Address of the Witness: _____

Informed Consent form for the parent / guardian of children participating in a research study

Study Title: Immune response to Hepatitis –B vaccine in HIV infected children and healthy children.

Study Number: _____

Subject's Initials: _____ Subject's Name: _____

Date of Birth / Age: _____

(Subject)

(i) I confirm that I have read and understood the information sheet dated _____ for the above study and have had the opportunity to ask questions. [ ]

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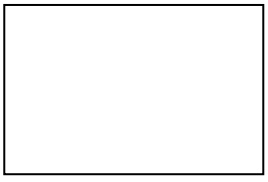
Signature (or Thumb impression) of the parent/ guardien

Date: _____/_____/_____

Signatory's Name: _____

Or 

Signature of the Investigator: _____

Date: _____/_____/_____

Study Investigator's Name: _____

Signature or thumb impression of the Witness: _____

Date: _____/_____/_____

Name & Address of the Witness: _____

Informed Assent Form (Controls)

STUDY TITLE: Immune response to Hepatitis –B vaccine in HIV infected and healthy children.

This document may contain the contents or words you do not understand clearly. If you have any questions, please don't hesitate to ask.

1. Why is this study being done?

In this study we are checking the immune response of HIV positive children and comparing with those of normal healthy children .

2. Do I have to do that?

No. If you don't want, you do not have to join this study. It will not affect you.

3. If I agree to join this study, what would I need to do?

If you join the study, we will collect an extra 5 ml of your blood sample while collecting for other routine investigations . You do not need additional needle prick. You would be questioned about your symptoms.

4. What is the benefit for me if I join this study?

You will not be paid to take part in this study. And if you have not taken or completed Hepatitis B vaccination, it will be given free of cost. You will know about your immune response to Hepatitis B vaccine and whether you are protected against Hepatitis B infection.

5. Who do I contact if I have problems, questions or want more information?

If you have any questions or want more information, please contact Dr.Sonia M

6. If I join the study, can I stop?

Yes. Taking part in this study is always a choice. If you decide to be in the study, you can change your mind at any time.

7. What would my signature on this form mean?

Your signature on this form would mean:

(1) This study has been explained to me.

(2) I understand what will be done to me and what I am being asked to do.

(3) I voluntarily agree to take part in this study.

You will receive a signed copy of this Informed Assent Form.

To joint this study, your parents or legal guardian also have to sign on informed consent form.

Printed name of subject child	Signature / Thumb Impression	Date
Printed name of investigator	Signature / Thumb Impression	Date

.

If I have questions or concerns about this research study, whom can I call?

You can contact the following person for your questions about the study

Dr. Sonia M
PG Registrar
Department of Microbiology,
Christian Medical College, Vellore - 632004
Phone: 0416 2282588, 09496334526

Informed Assent Form

STUDY TITLE: Immune response to Hepatitis –B vaccine in HIV infected and healthy children.

This document may contain the contents or words you do not understand clearly. If you have any questions, please don't hesitate to ask.

1. Why is this study being done?

Children getting infected with HIV , will have a weak immune system . They fall sick badly to infections compared to normal healthy children . In this study we are checking the immune response of HIV positive children and comparing with those of normal healthy children .

2. Do I have to do that?

No. If you don't want, you do not have to join this study. It will not affect you.

3. If I agree to join this study, what would I need to do?

If you join the study, we will collect an extra 5 ml of your blood sample while collecting for other routine investigations . You do not need additional needle prick. You would be questioned about your symptoms.

4. What is the benefit for me if I join this study?

You will not be paid to take part in this study. CD4 test will be done free of cost for you. And if you have not taken or completed Hepatitis B vaccination, it will be given free of cost. You will know about your immune response to Hepatitis B vaccine and whether you are protected against Hepatitis B infection.

5. Who do I contact if I have problems, questions or want more information?

If you have any questions or want more information, please contact Dr.Sonia M

6. If I join the study, can I stop?

Yes. Taking part in this study is always a choice. If you decide to be in the study, you can change your mind at any time.

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Phone: 0416 2282588, 09496334526

INFORMED CONSENT FOR PARENTS / GAURDIANS - Information sheet

CHRISTIAN MEDIAL COLLEGE VELLORE

DEPARTMENT OF MICROBIOLOGY

STUDY TITLE : Immune response to Hepatitis –B vaccine in HIV infected children and healthy children.

Please read this carefully. It tells you important information about the study. A member of the research team will explain you about your participation in this study. If you have any questions about the research or about this form, please ask us. If you decide to take part in this study, you must sign or provide your thumb impression in this form to show your willingness to take part in this study

Why is this study being done?

Human immunodeficiency virus (HIV) weakens your immune system making prone to many infections including those which normally doesn't cause infection. 3.2 million children are infected with HIV . More than 90 percent of HIV infections in children result from mother-to-child-transmission, where the virus is passed from a mother living with HIV to her baby during pregnancy, childbirth, or breastfeeding. Because children's immune system are not fully developed, children living with HIV get sick more severely than adults. They may experience the same common pediatric infections as HIV-negative children, but cannot fight these infections as effectively like them. The purpose of the study is to assess the immune response after Hepatitis B vaccination in HIV positive children and to compare how immune system responds in children those on specific treatment for HIV and not on any specific treatment. If the immune response is low ,there is a chance that they will get Hepatitis B infection and the severity of the disease will be high.

What will happen in this study?

Children in the age group between 4 -13 years of age , who are HIV infected individual will be recruited in this study. If you agree to participate and your child meets all the criteria required for the study as patient, an extra 5 ml of blood will be collected while collecting blood for routine investigations and will be used for the study. Basic information, medical history and details of treatment will be collected from you.

Will my child be paid to take part in this study?

You will not be paid for taking part in this research.

What are the risks and possible discomforts from being in this study?

There is no risk or discomforts. The consent is for collecting an extra 5 ml of the blood sample of your child while collecting for routine investigations .

What are the possible benefits from being in this study?
By being a part of this study, CD4 test will be done free of cost for your child . And if your child has not taken or completed Hepatitis B vaccination, it will be given the vaccine free of cost. As a result of your participation, we will be able to find the immune response in different HIV positive children compared to normal healthy children .And also the difference in immune response in HIV positive children on specific HIV treatment and not on treatment .

If my child takes part in this research study, how will you protect my child's privacy?
Information collected from you for this research study will be stored in the investigator's research files. Your name and other information that might identify you will be recorded with a unique code number, protecting your identity and information from others. The research consent form that you sign may be inspected by the regulatory agencies or the Institutional Review Board in the course of carrying out their duties. If the signed research consent form is inspected or copied, the hospital will use reasonable efforts to protect your privacy. The results obtained from this study will be published in the scientific journals, but no information about your identity will be disclosed.

Can my child withdraw from the study after it starts?

Your child's participation in this study is entirely voluntary and is also free to decide to withdraw permission to participate in this study. If your child does so, it will not affect your child's usual treatment at this hospital in any way.

If I have questions or concerns about this research study, whom can I call?

You can contact the following person for your questions about the study
Dr. Sonia M
PG Registrar
Department of Microbiology,
Christian Medical College, Vellore - 632004
Phone: 0416 2282588, 09496334526

INFORMED CONSENT FOR PARENTS / GAURDIANS - Information sheet

(Controls)

CHRISTIAN MEDIAL COLLEGE VELLORE

DEPARTMENT OF MICROBIOLOGY

STUDY TITLE : Immune response to Hepatitis –B vaccine in HIV infected children and healthy children.

Please read this carefully. It tells you important information about the study. A member of the research team will explain you about your participation in this study. If you have any questions about the research or about this form, please ask us. If you decide to take part in this study, you must sign or provide your thumb impression in this form to show your willingness to take part in this study

Why is this study being done?

The purpose of the study is to assess the immune response after Hepatitis B vaccination in HIV positive children compared to the response in normal healthy children. If the immune response is low , there is a chance that they will get Hepatitis B infection and the severity of the disease will be high.

What will happen in this study?

Children in the age group between 4 -13 years of age , who are HIV infected individual will be recruited in this study. If you agree to participate and your child meets all the criteria required for the study as participant, an extra 5 ml of blood will be collected while collecting blood for routine investigations and will be used for the study. Basic information, medical history and details of any treatment will be collected from you.

Will my child be paid to take part in this study?

You will not be paid for taking part in this research.

What are the risks and possible discomforts from being in this study?

There is no risk or discomforts. The consent is for collecting an extra 5 ml of the blood sample of your child while collecting for routine investigations .

What are the possible benefits from being in this study?

If your child has not taken all 3 doses of Hepatitis B vaccination and the immune response is not protective, he/she will be given the Hepatitis B vaccine free of cost. As a result of your participation, we will be able to find the immune response in different HIV positive children compared to normal healthy children.

If my child takes part in this research study, how will you protect my child's privacy?

Information collected from you for this research study will be stored in the investigator's research files. Your name and other information that might identify you will be recorded with a unique code number, protecting your identity and information from others. The research consent form that you sign may be inspected by the regulatory agencies or the Institutional Review Board in the course of carrying out their duties. If the signed research consent form is inspected or copied, the hospital will use reasonable efforts to protect your privacy. The results obtained from this study will be published in the scientific journals, but no information about your identity will be disclosed.

Can my child withdraw from the study after it starts?

Your child's participation in this study is entirely voluntary and is also free to decide to withdraw permission to participate in this study. If your child does so, it will not affect your child's usual treatment at this hospital in any way.

If I have questions or concerns about this research study, whom can I call?

You can contact the following person for your questions about the study

Dr. Sonia M
PG Registrar
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Christian Medical College, Vellore - 632004
Phone: 0416 2282588, 09496334526

PATIENT PROFORMA

STUDY TITLE : Measurement of immune response to Hepatitis B vaccine in HIV infected children and healthy children.

Study number: Date:

Name:

Hospital number:

Date of birth :

Age: Sex:

Address:

State: Mother tongue:

Date of HIV diagnosis:

Mode of transmission:

History/Diagnosed other infections:

Date of first Hepatitis B vaccination :
Site of vaccination:

Date of second Hepatitis B vaccination :
Site of vaccination:

Date of third Hepatitis B vaccination :
Site of vaccination:

Additional dose of Hepatitis B vaccination if any :
Site of vaccination

Total number of Hepatitis B vaccination taken :

History of Anti Retro Viral treatment :

When started :

Regimen followed :

Last CD4 count :

Current CD4 count :

History of blood transfusion :

Sample collected by

Name:

Signature:

PROFORMA – Controls

STUDY TITLE: Measurement of immune response to Hepatitis B vaccine in HIV infected children and healthy children.

Study number: Date:

Name:

Hospital number:

Date of birth :

Age: Sex:

Address:

State: Mother tongue:

History/Diagnosed other infections:

Date of first Hepatitis B vaccination :

Site of vaccination:

Date of second Hepatitis B vaccination :

Site of vaccination:

Date of third Hepatitis B vaccination :

Site of vaccination:

Additional dose of Hepatitis B vaccination if any :

Site of vaccination

Total number of Hepatitis B vaccination taken :

Current CD4 count :

Sample collected by

Name:

Signature:

Data Sheet

Healthy controls

SL NO	AGE	SEX	CITY	STATE	MOB NO	VACCINATION	CD4 COUNT	CD4 %	Anti HBs	Anti HBsmIU/ml	Anti HBc	Anti HBc S/CO	T cell NC	T CELL TEST	T CELL PC	t cell diff	B CELL NC	B CELL TEST	B CELL PC	b cell diff
C1	2	F	BHARREGAON	CHATTISGARH	7803040429	√	1555	18.75	Reactive	69.07	Non Reactive	0.11								
C2	10	F	PARGANAS	WEST BENGAL	7908963778	√	705	27.59	Reactive	45.24	Non Reactive	0.1								
C3	5	F	KOLKOTA	WEST BENGAL	9051383989	√	904	38.58	Reactive	12.6	Non Reactive	0.09								
C5	10	F	VELLORE	TAMIL NADU	9789402781	√	524	81.48	Reactive	95.1	Non Reactive	0.17								
C6	5	M	TIRUVANAMALAI	TAMIL NADU	9790010997	√	533	27.27	Reactive	580.82	Non Reactive	0.08								
C7	11	M	KAMRUP	ASSAM	9954621652	√	1002	38.04	Non Reactive	0.64	Non Reactive	0.16								
C8	11	M	JAI PAIGURI	WEST BENGAL	9475841877	√	235	29.26	Non Reactive	1.01	Non Reactive	0.06								
C9	6	F	VELLORE	TAMIL NADU	9965302124	√	973	31.94	Non Reactive	0.41	Non Reactive	0.07								
C10	9	F	HOOGLY	WEST BENGAL	9903052378	√	1053	35.49	Reactive	277.82	Non Reactive	0.29								
C11	12	M		WEST BENGAL	7602569020	√	505	30.22	Reactive	523.51	Non Reactive	0.1								
C12	12	M	GOHIRA	BANGLADESH	9007199434	√	1178	28.4	Reactive	22.39	Non Reactive	0.08								
C13	11	M	DHANBAD	JHARKAND	9835348604	√	1853	28.86	Non Reactive	0.67	Non Reactive	0.13								
C15	8	M	VELLORE	TAMIL NADU	9952958607	√	1421	16.62	Non Reactive	1.47	Non Reactive	0.07								
C16	9	F	CHAKDANADIA	WEST BENGAL	8017655783	√	1342	56.31	Non Reactive	3.77	Non Reactive	0.13								
C17	6	M	JAMTARA	JHARKAND	7654961929	√	1179	30.69	Reactive	37.87	Non Reactive	0.11								
C18	3	M	BAGERHAT	BANGLADESH	8100127114	√	1519	31.2	Non Reactive	6.4	Non Reactive	0.13								
C20	6	F	PURBA	WEST BENGAL	7407543837	√	952	19.27	Reactive	19.95	Non Reactive	0.12								
C21	2	M	RANCHI	JHARKAND	8051899990	√	1114	27.32	Non Reactive	6.92	Non Reactive	0.08								
C22	14	F	GIRIDH	JHARKAND	9835195690	√	658	27.92	Non Reactive	8.52	Non Reactive	0.05								
C24	5	M	GAJAPAH	ODISHA	9438155449	√	1186	23.18	Non Reactive	7.82	Non Reactive	0.11								
C25	13	M	DHANBAD	JHARKAND	9430703890	√	432	27.89	Reactive	12	Non Reactive	0.14								
C26	6	M	PARGANAS	WEST BENGAL	9836307999	√	1830	26.43	Reactive	20.35	Non Reactive	0.04								
C27	4	M	MEDNIPUR	WEST BENGAL	9002368794	√	1924	40.32	Reactive	15.28	Non Reactive	0.11								
C28	8	M	VELLORE	TAMIL NADU		√	1380	19.4	Reactive	12.84	Non Reactive	0.06	0.1	0.3	1.3	0.2	1.2	0.2	0	1
C29	15	F	JAMSHEDPUR	JHARKAND	9661884938	√	987	38.45	Reactive	133.95	Non Reactive	0.08	0	0	1.2	0	0.3	0.1	0	0.2
C30	2	M	RAIPUR	CHATTISGARH	9993455536	√	1262	43.71	Reactive	225.25	Non Reactive	0.07	0	0.2	3.4	0.2	1.4	0.7	0.1	0.7
C31	13	F	NELLORE	ANDRA PRADESH	9959127454	√	950	33.97	Non Reactive	2.69	Non Reactive	0.18	0	0.1	9.1	0.1	0.3	0	0	0.3
C32	7	M	ARSHIPURULIA	WEST BENGAL	9735812289	√	670	22.91	Reactive	10.14	Reactive	0.13	0	0	3.3	0	2.8	1.2	5.5	1.6
C33	7	M		SRILANKA	9587561376	√	812	22.01	Non Reactive	7.39	Non Reactive	0.09	0	0	4	0	0.6	0.3	0	0.3
C34	3	M	PURBA	WEST BENGAL	9775017418	√	1953	51.37	Non Reactive	9.81	Non Reactive	0.03	0	0	0.9	0	15.9	13	14.6	2.9
C35	12	M	CHENNAI	TAMIL NADU	9884453502	√	784	28.77	Non Reactive	0.05	Non Reactive	0.19	0	0.1	7.3	0.1	3.6	3.1	0.3	0.5
C36	6	M	JAMUI	BIHAR	9631388547	√	684	21.99	Non Reactive	7.97	Non Reactive	0.12	0	0.3	4.2	0.3	1.7	1.3	0.4	0.4
C37	4	F	PALLABI	BANGLADESH	8420214034	√	919.54	36.18	Non Reactive	5.67	Non Reactive	0.46	0	0.2	9.2	0.2	2.6	2.1	0.7	0.5
C 39	5	M	DHANBAD	JHARKAND	9334359105	√	872	36.85	Non Reactive	0.22	Non Reactive	0.2	0	0.2	0.1	0.2	8.9	5.5	1.3	3.4
C40	3	M	VELLORE	TAMIL NADU	9994309072	√	587	25.82	Non Reactive	0.38	Non Reactive	0.03	0.1	0.4	0.2	0.3	0.9	0.4	0.4	0.5
C 41	14	M		MAHARASTRA	9822206067	√	470	34.91	Non Reactive	8.57	Non Reactive	0.17	0	0.2	0.1	0.2	0.2	0	0	0.2
C 42	3	M	JAMTARA	JHARKAND	7542952966	√	1427	32.71	Reactive	55.98	Non Reactive	0.1	0	0.3	11.4	0.3	2.8	1.3	6.5	1.5
C43	7	M	WEST BENGAL	WEST BENGAL	9004344666	√	1200	29.62	Non Reactive	0.41	non Reactive	0.09	0	0.1	4.5	0.1	3	2.6	2.2	0.4

Data Sheet HIV Infected

SL NO	AGE	SEX	CITY	state	date of HIV Diagnosis	mode of transmission	history / diagnosed other infections	Number of hepatitis B vaccination	MOB NO	ART	TREATMENT	DATE OF CD4
P2	12	M	west bengal	WEST BENGAL	23/1/2018	MTCT	Hep B , HCV -NEGATIVE ORAL CANDIDIASIS	√	8158879930	3TC NVP AZT	NAÏVE	29/1/18
P7	13	M	CHATTISGARH	CHATTISGARH	2010	TRANSFUSION	THALESSEMIA MAJOR HCV - POSITIVE ,Hep B - NEGATIVE	√	9406076043	ABC 3TC NVP	8 YEARS	7/2/2018
P9	7	M	CHENNAI	TAMIL NADU	2011,9.5M	MTCT	Hep B , HCV -	√	9940569966	AZT 3TC NVP	6 YEARS	13/2/18
P10	12	M	VELLORE	TAMIL NADU	2012	MTCT	Hep B , HCV -NEGATIVE PULMONARY TB PARENTS DEAD	neighbour ph	7373552426/ 9659891044	AZT 3TC NVP	3 YEARS	15/2/18
P11	3	M	VELLORE	TAMIL NADU	2016	MTCT	Hep B , HCV -NEGATIVE	√		AZT 3TC NVP	NAÏVE	22/2/18
P14	9	M	VELLORE	TAMIL NADU	2012	MTCT	STAGE 3 , ANAEMIA CCF,Hep B , HCV -	√		ABC NVP 3TC	7 YEARS	28/2/18
P16	11	M	VELLORE	TAMIL NADU	2014	MTCT	Hep B , HCV -NEGATIVE TB LYMPH NODE PARENTS DEAD	√	7826960745/9 655688571	AZT 3TC EFV	2.5 YEARS	2/3/2018
P17	10	F	ERODE	TAMIL NADU	2009	MTCT	Hep B , HCV -	√		AZT 3TC NVP	5 YEARS	22/3/18
P18	13	M	NELLORE	ANDRA PRADESH	2007	MTCT	Hep B , HCV -	√	9959694450	AZT 3TC NVP	7 YEARS	
P20	13	M	VELLORE	TAMIL NADU	2010	MTCT	Hep B , HCV	√	8012471300	EFV 3TC TDF	NAÏVE	2/4/2018
P23	8	F	VELLORE	TAMIL NADU	7 YEARS	MTCT	Hep B , HCV -NEGATIVE	√		AZT 3TC EFV	1 YEAR	30/4/18
P24	12	F	VILLUPURAM	TAMIL NADU	2008	MTCT	Hep B , HCV -	√	9842366288	AZT 3TC EFV	4 YEARS 2013	30/4/18
P25	13	M	GIRIDIH , TAMBA	TAMIL NADU	2011	MTCT	Hep B , HCV -NEGATIVE	NOT cmc pt	8271890422	3TC TDF EFV	5 YEARS	20/4/18
P26	8	M	NILGIRIS	TAMIL NADU	Jun-17	MTCT	Hep B , HCV -	√	9443426740	AZT 3TC EFV	6 MONTHS	9/5/2018
P28	9	F	BANGALORE	KARNATAKA	2012	MTCT	Hep B , HCV	√	9880798252		NAÏVE	18/5/18
P29	12	M	VELLORE	TAMIL NADU	2014	MTCT	Hep B , HCV -NEGATIVE	√		AZT 3TC NVP	2YEARS	22/5/18
P30	16	M	VELLORE	TAMIL NADU	2017	MTCT	Hep B , HCV -NEGATIVE, VDRL NEGATIVE PARENTS BOTH NEGATIVE	√		ABC 3TC EBV	1 YEAR	25/5/18
P31	12	F	VELLORE	TAMIL NADU	2010	MTCT	Hep B , HCV -	√		AZT 3TC NVP	8 YEARS	30/5/18
p33	5	M	VELLORE	TAMIL NADU	2016	MTCT	Hep B , HCV -NEGATIVE MEDIASTINAL LN	√	9655601564	AZT ?ABC 3TC EFV/LPV	2 YEARS	4/6/2018
p34	11	M	VELLORE	TAMIL NADU	2010	MTCT	Hep B , HCV -	√			4 YEARS	5/6/2018
p35	12	F	VELLORE	TAMIL NADU	2013	MTCT	Hep B , HCV -NEGATIVE	CNP	8870818632	AZT 3TC NVP/ABC LEFV	1 YEAR	5/6/2018
p36	10	F	SUNDARGARH	ORISSA	2012	MTCT	Hep B , HCV - PULMONARY TB	√	9776409876	AZT 3TC EFV	2 YEARS	7/6/2018
p37	13	F	PURBA , MEDINIPUR, WEST BENGAL	WEST BENGAL	2018	UNKNOWN	Hep B , HCV -NEGATIVE PARENTS NEGATIVE	√	7797363232		NAÏVE	19/6/18
p38	13	F	VELLORE	TAMIL NADU	2014	MTCT	Hep B , HCV	√		AZT 3TC NVP	2 YEARS	19/6/18
p39	12	M	VELLORE	TAMIL NADU	2009	MTCT	Hep B , HCV -NEGATIVE	√			NAÏVE	25/6/18
p40	9	M	HAZARIBAG	JHARKAND	2014	MTCT	Hep B , HCV -NEGATIVE	√		ABC 3TC NVP	4 YEARS	26/6/18
p41	4	M	PURBA , MEDINIPUR, WEST BENGAL	WEST BENGAL	2018	MTCT	Hep B , HCV -NEGATIVE	√	9593035416		NAÏVE	29/6/18
p 42 R	10	F	NELLORE	ANDRA PRADESH	2014	MTCT	Hep B , HCV -	√	9441867799	AZT 3TC NVP	2 YEARS	28/2/18
P43	14	F	VELLORE	TAMIL NADU	2016	MTCT	Hep B , HCV -NEGATIVE	√	9789238471	TDF AZT 3TC	1 YEAR	3/7/2018
P 44 R	14	F	VELLORE	TAMIL NADU	2010	MTCT	Hep B , HCV -	√	9790150249	TDF LPV EFV	7 YEARS	9/2/2018
P 45 R	12	F	VELLORE	TAMIL NADU	14/05/2012	MTCT	Hep B , HCV -NEGATIVE ITP	wrong no		3TC TDF EFV	1 YEARS	30/1/18
P 46 R	8	F	VELLORE	TAMIL NADU	26/06/2014	MTCT	Hep B , HCV -	√	7373122649	TDF 3TC EFV	3 YEARS	30/1/18
P47	6	M	UTTIKULI , TRIPUR	TAMIL NADU	2012	MTCT	Hep B , HCV -	√	9047057673	ABC 3TC EFB	3 YEARS	27/7/18
P48 R	11	M	VELLORE	TAMIL NADU	Oct-17	MTCT	Hep B , HCV -, TB +, PARENTS SIBLING HIV NEGATIVE	√	9894141679	AZT 3TC NVP	6 MONTHS	7/2/2018
P49 R	2	M	VELLORE	TAMIL NADU	Sep-17	MTCT	Hep B , HCV -NEGATIVE ABONDED BY FATHER	wrong no	9788780288	AZT 3TC NVP	1 YEARS	23/2/18
P 50 R	11	M	VELLORE	TAMIL NADU	2010	MTCT	Hep B , HCV -NEGATIVE	√	9366112321	AZT 3TC NVP	8 YEARS	14/5/18
P51	9	F	CHITOOR	ANDRA PRADESH	2016	MTCT	Hep B , HCV -NEGATIVE PCP/TB	√	9878245899	AZT 3TC EFV	3 YEARS	20/08/18
P52	11	M	NADIA , GAGHNAPUR	WEST BENGAL	20/09/2018	MTCT	17/9/18 HIV 1 +	√	7365991889		NAÏVE	19/9/18
P 53 R	13	M	VELLORE	TAMIL NADU	2011	MTCT	Hep B , HCV -	√	9940800506	ABC 3TC EFV	3 YEARS	23/1/18
P54 R	8	M	VELLORE	TAMIL NADU	2010	MTCT	Hep B , HCV	√	9751895520	AZT 3TC NVP	5 YEARS	1/6/2018
P55	11	F	BISRAMPUR	JHARKAND	2014	MTCT	Hep B , HCV	√	9934802202	AZT 3TC NVP	3 YEARS	3/10/2018
P56 R	13	M	CHITTOR	ANDHRAPRADESH	2007	MTCT	Hep B , HCV ????	√	9000343761	EFV 3TC TDF	4 MONTHS 2018	19/4/2018

who STAGE	CDC stage	TREATMENT	CD4 COUNT	CD4 %	PREVIOUS CD4	Anti HBs	Anti HBs mIU/ml	protective /low/No	Anti HBc	Anti HBc S/CO	T CELL NC	T CELL TEST	T CELL PC	t cell diff	B CELL NC	B CELL TEST	B CELL PC	b cell diff
III	III	NAÏVE	20	2.87		Non Reative	0.5		Non Reative	0.35								
I	I	8 YEARS	522	35.14		Reactive 66.09	66.09		Non Reative	0.23								
II	I	6 YEARS	755	27.7	686/29.63	Non Reative	0		Non Reative	0.12								
II	II	3 YEARS	319	8.37	429/10.18	Non Reative	1.25		Non Reative	0.19								
II	I	NAÏVE	1787	29.51	1859/39.44	Reactive 67.39	67.39		Non Reative	0.12								
III	I	7 YEARS	1205	40.56	1279/3587	Non Reative	1.92		Non Reative	0.33								
III	I	2.5 YEARS	874	29.62	488/28.79	Non Reative	0		Non Reative	0.22								
I	I	5 YEARS	284	13.77	463/16.39	Non Reative	1.15		Non Reative	0.13								
II	I	7 YEARS	697	30.58	6/1/17 -775/30.20	Non Reative	0.37		Non Reative	0.09								
I	I	NAÏVE	578	29.44	30/6/16-1149/38.11	Reactive 12.44	12.44		Non Reative	0.11								
III	II	1 YEAR	464	24.78	475/21.55	Non Reative	0.4		Non Reative	0.05								
II	I	4 YEARS 2013	1053	34.28	1187/34.51	Non Reative	0.24		Non Reative	0.09								
III	I	5 YEARS	1729	41.3	1729/41.30	Non Reative	1.18		Non Reative	0.07								
II	I	6 MONTHS	748	27.13	694/21.12	Non Reative	0.18		Non Reative	0.1								
II	I	NAÏVE	1335	30.11	1216/27.67	Non Reative	0		Non Reative	0.1								
II	II	2YEARS	357	11.35	429/16.30	Non Reative	0.27		Non Reative	0.11								
IV	III	1 YEAR	3	0.52		Non Reative	0.07		Non Reative	0.1								
II	I	8 YEARS	532	38.46	895/44.87	Non Reative	0		Non Reative	0.12								
III	I	2 YEARS	1314	39.9	1638/33.14	Reactive 10.19	10.19		Non Reative	0.09								
III	II	4 YEARS	370	22.8	422/21.31	Non Reative	0.05		Non Reative	0.17								
II	II	1 YEAR	440	30.71	436/26.25	Non Reative	0.21		Non Reative	0.13								
III	I	2 YEARS	1208	39.38	1237/39.72	Non Reative	0.31		Non Reative	0.16								
III	III	NAÏVE	6	1.03		Non Reative	0.07		Non Reative	0.16								
III	I	2 YEARS	1314	41	1285/42.13	Non Reative	0.4		Non Reative	0.06								
III	III	NAÏVE	98	5.29	281/11.23	Non Reative	0.41		Non Reative	0.3					2.7	2.4	3	0.3
III	II	4 YEARS	435	16.62		Non Reative	0.08		Non Reative	0.14					0.9	0.7	2	0.2
II	III	NAÏVE	183	8.84	225/6.6	Non Reative	0.18		Non Reative	0.1	3.3	3.7	13.9	0.4	14.6	12.6	6.5	2
II	I	2 YEARS	703	26.25	709/25.17	Non Reative	0.01		Non Reative	0.1	0	0	0.5	0				
IV	I	1 YEAR	1079	27.4	352/22.62	Non Reative	0.03		Non Reative	0.08	0	0	1.2	0				
II	II	7 YEARS	217	18.74	247/16.22	Non Reative	0.06		Non Reative	0.1	0.1	0.1	2.5	0	0	0	0	0
III	I	1 YEARS	766	36.6	627/32.75	Non Reative	0.91		Non Reative	0.07	0	0	1.7	0	0.8	0.4	0	0.4
II	II	3 YEARS	556	29.2	471/28.19	Non Reative	5.82		Non Reative	0.1	0.4	0.6	4.5	0.2	1	0.9	0.2	0.1
II	I	3 YEARS	806	26.46	1046/28.16	Non Reative	0.46		Non Reative	0.06	0.1	0.4	2.9	0.3	2.7	1	0.8	1.7
IV	II	6 MONTHS	369	14	6/.56	Non Reative	0.38		Non Reative	0.12	0.1	0.2	1.3	0.1	1.6	0	0.2	1.6
I	I	1 YEARS	1200	22.3	1106/10.88	Non Reative	2.35		Non Reative	0.13	0.1	0.4	0.8	0.3	1.4	0.5	0	0.9
III	I	8 YEARS	985	20.53	32/2.41	Non Reative	0.14		Non Reative	0.18	0.5	1.3	2.5	0.8	7.5	1.8	2.2	5.7
III	I	3 YEARS	776	24.86	610/23.74	Non Reative	0.39		Non Reative	0.12	0.1	0.5	0.3	0.4	3.4	0	1.1	3.4
III	III	NAÏVE	74	3.8		Non Reative	0.47		Non Reative	0.13	0.5	2.6	3.1	2.1	2	0	0	2
II	I	3 YEARS	932	32.63	1159/35.96	Non Reative	0		Non Reative	0.16	0	0.2	9.3	0.2	1.2	0	0	1.2
II	I	5 YEARS	1054	41.95	1408/35.89	Non Reative	0.2		Non Reative	0.06	0.1	0.2	1.5	0.1	0.3	0	0	0.3
I	I	3 YEARS	869	27.17	557/19	Non Reative	5.37		Non Reative	0.22	0.1	0.8	2.3	0.7	1.7	1.5	0	0.2
III	I	4 MONTHS 2018	757	30.55		Non Reative	0.11		Non Reative	0.09	0.1	0.5	1.1	0.4	0.8	0.5	0.7	0.3