Introduction
1. INTRODUCTION

Acquired immunodeficiency syndrome (AIDS) is a disease caused by a virus called Human Immunodeficiency Virus (HIV) which belongs to the family of Retroviruses and subfamily of Lentiviruses. AIDS was first recognized as a new and distinct clinical entity in young homosexual men who were previously healthy. They had an unusual clustering diseases such as Kaposi’s Sarcoma and Pneumocystis Carinii pneumonia as reported by the Morbidity and Mortality Weekly Report (MMWR), the bulletin of the Centers for Disease Control (CDC), on 5 June 1981 (CDC, 1981). Approximately 70% of patients with HIV infection develop symptoms during the acute infection period (Pilcher et al., 2004).

The most likely explanation for the origin is that HIV was introduced to humans from monkeys. HIV-1 has long been suspected to be of chimpanzee origin and identified a subspecies of chimpanzees native to west equatorial Africa as the original source of HIV-1 (Gao et al., 1999). The researchers believed that the virus crossed over from monkeys to humans when hunters became exposed to infected blood. Monkeys can carry a virus similar to HIV, known as SIV (Simian Immunodeficiency Virus), and there is strong evidence that HIV and SIV are closely related (Simon et al., 1998; Zhu et al., 1998).
In India, the first reported cases of HIV infection were among commercial sex workers in Chennai in May 1986 (Simoes et al., 1987) and the first report of AIDS was in Mumbai (Godbole and Mehendale, 2005).

HIV is a highly variable virus which mutates very readily. This means there are many different strains of HIV, even within the body of a single infected person. Based on genetic similarities, the numerous virus strains may be classified into types, groups and subtypes. Human immunodeficiency virus is categorized into two types, HIV-1 and HIV-2. Worldwide, most HIV infections are HIV-1, whereas HIV-2 largely has been confined to persons in or from West Africa. HIV-1 and HIV-2 have the same routes of transmission, and both can cause AIDS (O’Brien et al., 1992, De cock et al., 1991).

HIV-1 is not just one virus, but comprises four distinct lineages, termed as: M, N, O, and P, each of which resulted from an independent cross-species transmission event. Group M was the first to be discovered and it has infected millions of people worldwide. Group O is much less prevalent than group M (De Leys et al., 1990; Gurtler et al., 1994). Group N is even less prevalent than group O. Finally, group P was discovered in 2009 in a Cameroonian woman living in France (Plantier et al., 2009).

HIV-2 has remained largely restricted to West Africa (Thushan et al., 2008). Viral loads tend to be lower in HIV-2 than HIV-1 infected individuals,
which may explain the lower transmission rates of HIV-2 and the complete absence of mother-to-infant transmissions (Popper et al., 2000; Berry et al., 2002). Most people infected with HIV-2 do not progress to disease, even though the minority who do cannot be distinguished clinically from HIV-1-infected patients (Sarah et al., 2007).

HIV-1 spreads by sexual, percutaneous, and perinatal routes. However, 80% of adults acquire HIV-1 following exposure at mucosal surfaces, and AIDS is thus primarily a sexually transmitted disease (Hladik et al., 2008; Cohen et al., 2011). Since its first identification almost three decades ago, the pandemic form of HIV-1, also called the main (M) group, has infected at least 60 million people and caused more than 25 million deaths (Merson et al., 2008).

Globally, 34 million people were living with HIV at the end of 2011. An estimated 0.8% of adults aged 15-49 years worldwide are living with HIV. In 2011, 1.7 million people died from AIDS-related causes worldwide. This represents a 24% decline in AIDS-related mortality compared with 2005 (when 2.3 million deaths occurred) (www.unaids.org).

In India 23.9 lakh people were infected with HIV, of whom 39% were females and 4.4% were children and an adult prevalence of 0.31 percent in 2009. India is estimated to have had an adult (15-49 years) HIV prevalence of 0.27% in
2011. Adult HIV prevalence among males and females is estimated at 0.32% and 0.22% respectively (www.nacoonline.org).

The most common route of transmission is unprotected sexual contact. Different forms of sexual practices carry a variable risk of acquiring HIV. Homosexual men have shown consistently that the receptive partner in oral intercourse is at the highest risk of HIV infection (Winkelstein et al., 1987; Kingsley et al., 1987). Vaginal intercourse can transmit HIV to either the male or the female partner, but studies have shown that the risk is higher to the female partner (Peterman et al., 1988). The multiple sexual partners may be considered as high risk factor in female to male infectivity of HIV-1 among uncircumcised men (Baeten et al., 2005). The amount of virus in genital fluids is important for sexual transmission; generally 10 to 30% of seminal and vaginal fluid specimens have shown the presence of HIV virus and virus infected cells (Anderson et al., 1992).

HIV can be isolated from other bodily fluids, such as saliva, sweat, and tears, but the viral concentration is so low that the transmission risk is negligible. HIV cannot be transmitted from coughing or sneezing, sharing household items, or swimming in a pool with someone who is infected. Mother-to-child transmission (MTCT) of HIV through pregnancy, labour, delivery, or breastfeeding is responsible for over 90 per cent of HIV infections in infants and children under the age of 15 (www.unicef.org).
The more advanced HIV-1 disease in the mother (higher levels of HIV in mother's blood), lower levels of immunity (lower levels of CD4 cells in the blood), and more advanced clinical disease (progression to AIDS) increase the risk of HIV-1 transmission to the infants (Mayaux et al., 1995; Dickover et al., 1996). Perinatal transmission could occur during antepartum, intrapartum and after delivery through breast milk (Rouzioux et al., 1995).

One of the simplest of all drug regimens was tested in the HIVNET 012 trial and it was found that a single dose of nevirapine given to the mother at the onset of labour and to the baby after delivery roughly halved the rate of HIV transmission. This simple, inexpensive, well-tolerated regimen has the potential to significantly decrease HIV-1 perinatal transmission in less-developed countries (Guay et al., 1999). The overall risk of mother-to-child HIV transmission in non-breastfeeding populations is 15-25% and in breastfeeding populations 20-45% (Dunn et al., 1992).

The Department of Experimental Medicine of the Tamil Nadu, Dr MGR Medical University, initiated the country's first rural prevention of mother-to-child HIV transmission (PMTCT) center at Namakkal District Head Quarter Hospital in 2000. Since then single dose Nevirapine is offered to HIV positive pregnant women at the onset of labor and to their infants (Jacob et al., 2011). Single-dose nevirapine prophylaxis to mother and infant is widely used in resource-constrained
settings for PMTCT programs. The simplicity and low cost of nevirapine’s single
dose regimen suggest that this highly efficacious drug might be very useful in
rural settings. As the tablet does not require refrigeration, it can be offered to the
mother in the last week of trimester. The strategies for PMTCT would involve
HIV education, voluntary counseling and testing (VCT) for pregnant women and
providing antiretroviral prophylaxis to them and their infants (Srijanyanth et al.,
2009).

The following methods are used for HIV detection:

- ELISA, Western Blotting (Antibody based)
- Ultra sensitive p24 antigen assay (Viral protein based)
- Viral culture (In vitro culture based)
- Nucleic Acid Testing (DNA, RNA based)

Virologic tests such as Virus cultures, RNA and DNA Polymerase Chain
Reaction (PCR) tests are the most important techniques for earlier diagnosis of
HIV-1 infection in infants. Serological tests are not useful, since maternal
antibodies can be present in children until the age of 18 months. HIV-1 DNA PCR
using whole blood was the optimal test for diagnosing HIV-1 infection in infants
(Rakusan et al., 1991) and which is found to be a gold standard for diagnostic
testing of infants and children younger than 18 months (Jennifer, 2007).
Detection of HIV-1 DNA by PCR is an established method for determining infection status in infants born to HIV seropositive mothers, but some disadvantages were identified using whole blood samples:

- The difficulties encountered during venipuncture in infants less than 18 months old and the need to minimize the risk of anemia.
- The time between specimen collection and testing are important factors.
- The specimen should be transported in cold chain from the rural area to the testing centers.

HIV-1 DNA PCR testing can also be reliably performed on blood coated onto the filter papers and there are many advantages of the use of filter papers in HIV-1 infant diagnosis than the whole blood specimens.

- Lesser quantity of blood is required for HIV-1 DNA PCR testing.
- Once dried, blood samples on filter paper are no longer infectious and to reduce the biohazard risk to health care workers (Ingrid et al., 2001).
- HIV-1 DNA has a remarkable stability in the filter papers (Cassol et al., 1992).
- There is no need of cold chain during the transportation of specimens and it can be transported at room temperature in a sealed cover with a desiccant.

The most frequently reported adverse events related to nevirapine are rash, fever, nausea, headache, and abnormal liver function tests (Jackson et al., 2003a). High
levels of the enzyme ALT are suggestive of acute liver damage due to exposure to hepatotoxic agents (Taha et al., 2002).

Patients in resource-poor settings often experience poor nutritional status, inadequate housing, and limited access to clean water, all of which increase the HIV-positive person’s susceptibility to opportunistic infections. Therefore, all HIV positive patients’ baseline white blood cell count (WBC), hemoglobin (Hb) and hematocrit (HCT) are the important tool in the evaluation of the patient’s nutritional status and will provide the baseline information that is necessary before the initiation of therapeutic agents that may have myelosuppressive or hepatotoxic effects (Judith et al., 2004).

The CD4 cell count of HIV-infected individuals is an important predictor of HIV disease progression and AIDS associated mortality. CD4 count, along with HIV viral load and clinical symptoms, is used to determine when to start highly active anti-retroviral therapy (HAART) (Mair et al., 2008).

World Health Organization’s (WHO) recommendations for initiating anti-retroviral therapy in HIV-infected individuals include WHO clinical stage IV (clinical AIDS) regardless of CD4 count, clinical stage III with CD4 count < 350 cells/mm³ (www.who.int).

In resource limited areas, there is scarcity of CD4 cell counters to initiate of highly active antiretroviral therapy. The determination of CD4 count has become a
standard measure of immunodeficiency in adults infected with HIV in resource rich areas and this may not be feasible in resource limited areas. To overcome this problem, the guidelines from World Health Organization acknowledged that total lymphocyte count (TLC) may be used to make treatment decision in resource poor settings when CD4 count is not available and patients are mildly symptomatic (Deresse et al., 2008).

Virus load testing is used to assess prognosis, determine the need for antiretroviral therapy and the type of antiretroviral therapy required, define a baseline laboratory value so that the response to therapy can be measured. (Judith et al., 2004).

Antiretroviral agents are drugs that are used to act at various stages of the life cycle of HIV in the body and work by interrupting the process of viral replication (www.nacoonline.org). Advances in antiretroviral therapy (ART) have markedly suppressed viral activity, improved health, and increased longevity among patients infected with HIV (Wanke et al., 2002).

A significant concern about the use of single dose nevirapine is drug resistance. There is also some evidence to suggest that if a mother develops nevirapine-resistant HIV, then this may be passed through breast milk to her infant (Jacob et al., 2011). Oligo nucleotide ligation assays are rapid, specific and sensitive reactions for the detection of known point mutations of K103N and Y181C of non nucleoside reverse transcriptase (NNRTI) based regimen such as
nevirapine. The drug resistance resulting from single dose nevirapine is usually short lived, if the mother can wait for six months before beginning antiretroviral treatment, then it is unlikely to fail (Lockman et al., 2007).

Use of whole blood spotted on filter paper in HIV infant testing is a feasible alternative to collection of whole blood for, less blood is needed and immediate processing is not required. Storage and transportation of blood spotted filter papers is easy since it can be stored at room temperature and DNA has a good stability in blood coated filter papers (Fischer et al., 2004).

A rapid, simple and efficient method of DNA extraction from the whole blood spotted onto filter papers has to be developed for the diagnosis of HIV-1 infection. Spotting of whole blood on filter paper offers technical and economical advantages over conventional venipuncture methods since it simplifies sample collection and transport to reference laboratories.

Therefore this study will explore to identify the suitable filter paper in which the whole blood is spotted and to develop a rapid and simple DNA extraction method for the diagnosis of HIV-1 infection using molecular methods in infants.
The present research work is based on the following objectives:

- To identify the HIV-1 infection among HIV exposed infants by DNA nested PCR using whole blood spotted filter papers.
- To calculate the sensitivity, specificity, negative predictive value (NPV) and positive predictive value (PPV) for each whole blood spotted filter paper.
- To determine the liver marker enzyme abnormalities of the infants and their mothers and to find out whether there are any alterations in the liver enzymes.
- To study the hematological and immunological status of the infants and their mothers.
- To determine the correlation between viral load and CD4 cell count of HIV positive infants and their mothers.
- To identify the drug resistance mutations for HIV positive infants and their mothers.