

**DETERMINENTS OF EARLY INFANT HIV DIAGNOSIS AND
TREATMENT SUCCESS IN PUNJAB, PAKISTAN**



SUPERIOR UNIVERSITY

**Faculty of Allied Health Sciences
The Superior University, Lahore**

**Determinants of Early infant HIV diagnosis and treatment success in Punjab,
Pakistan**



SUPERIOR UNIVERSITY

Thesis Submitted to

The Superior University Lahore

**In Partial Fulfillment of the Requirement for the Degree of
Master of Science in Allied Health Sciences**

By

AZKA MUBEEN

Roll No. SU91-MSAHW-S23-136

Session: 2023-2025

Faculty of Allied Health Sciences

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DEDICATION

This research is dedicated to my parents, Teachers and siblings. People who never stop believing in me and who along **ALLAH ALMIGHTY** have been my “*FOOTPRINTS IN THE SAND*”

That is

MY PARENTS

Whose prayers have been a force behind all my inspirations and made me successful in all fields of life. They gave me everything but asked for nothing.

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LIST OF ABBREVIATIONS

EID	Early Infant Diagnosis
HIV	Human Immunodeficiency virus
AIDS	Acquired Immunodeficiency Syndrome
ART	Anti-Retroviral Therapy
MTCT	Mother to child transmission
HAART	Highly Active Antiretroviral Therapy
NRTIs	Nucleoside reverse transcriptase inhibitors
IVDU _s	Intravenous drug users
cART	Combined Anti-Retroviral Therapy
HAND	HIV-associated neurocognitive disorders
IL	Interleukin
TNF- α	Tumor necrosis factor-alpha
HTLV-1	Human T-cell lymphotropic virus type 1
PCR	Polymerase Chain Reaction
WHO	World Health Organization
GBD	Global Burden of Disease
IDU	Injection Drug Users
MSM	Men having sex with men
FSW	Female Sex workers

ABSTRACT

Introduction:

Early Infant Diagnosis (EID) is crucial in detecting HIV infection among exposed infants and initiating timely treatment to reduce infant morbidity and mortality. In Pakistan, data on EID and associated outcomes remains limited, especially in regional contexts like Punjab. This study aimed to evaluate the prevalence and outcomes of HIV among exposed infants, while identifying demographic, clinical, and parental factors influencing vertical HIV transmission.

Methodology: A retrospective cross-sectional study was conducted using data from the Punjab AIDS Control Program (2023–2024). Clinical and demographic data of 214 HIV-exposed infants were analyzed, including age, gender, feeding practices, parental viral load and CD4 count, occupations, and risk group classifications. Statistical analysis was done using SPSS v26.0.

Results: Among 214 infants, HIV positivity was highest in the 11–15-month group (16%), with breast-fed infants exhibiting a higher positivity rate (26 cases) than formula-fed ones (9 cases). Male infants showed a slightly lower infection rate compared to females. Parental age group 30–34 years had the highest number of positive cases (16). High parental viral loads (>2000 CP/mL) and low maternal CD4 counts (0–400) were strongly associated with infant HIV positivity. Risk factors included injecting drug use (46 cases), MSM contact (28), blood transfusion (30), and spousal transmission (28).

Conclusion: The study underscores the influence of maternal health, feeding practices, and parental risk behaviors on HIV transmission. Strengthening antenatal care, routine EID, and community awareness are pivotal to reducing the infant HIV burden in Punjab.

Key words: Early infant diagnosis, HIV, Vertical transmission

CHAPTER 1

INTRODUCTION

Human Immunodeficiency Virus (HIV) is an increasing global health concern. HIV, also known as Human Immunodeficiency Virus, leads to acquired immunodeficiency syndrome (AIDS). In 1981, AIDS was first recognized as a new disease, predominantly affecting male homosexuals, intravenous drug users, and haemophiliacs in the United States, as well as sexually active heterosexuals in certain tropical African regions. In 1983, HIV was identified, and by 1984, it was linked to AIDS and associated with populations at high risk of contracting the infection. Additionally, serological testing for HIV was introduced [1]. The earliest infected cohorts were homosexual men and haemophiliacs; however, by the end of the 1990s, HIV primarily affected heterosexual populations in Western countries [2].

Mother-to-child transmission of HIV is far more prevalent in low-income countries compared to developed nations, with heterosexual transmission being the predominant route of infection in most of these regions. In Southeast Asia and Central and South America, homosexual transmission occurs more frequently than in Africa. Transmission through injection drug use remains prevalent in parts of Central and South America as well as South and Southeast Asia. Transmission through contaminated blood continues to be a risk, particularly in regions of South Asia and Sub-Saharan Africa. In some countries, commercial blood donation practices heighten the risk of HIV transmission not only to recipients through transfusions but also to donors through exposure to contaminated equipment [3]. In the early years of the epidemic, there was significant concern about so-called casual or non-sexual transmission of HIV, much of which focused on contact with saliva. However, various epidemiological studies and AIDS surveillance data demonstrated that HIV is primarily transmitted through contact with fluids containing the virus, chiefly semen [4].

HIV can be transmitted through contaminated blood and blood products, from an infected mother to her child during pregnancy or childbirth, or from an infected father to a woman during sexual intercourse. Vertical and blood-borne transmission of HIV is highly efficient. While the transmission rate through large-bore needles is approximately 0.3%, a transfusion with a unit of infected blood almost invariably results in infection. In general, the concentration of infected cells in vertically transmitted cases leads to infection in about 25% of newborns, though this rate is highly variable and depends on numerous

factors influencing HIV transmission [5].

Several mechanisms contribute to mother-to-child transmission of HIV. The virus can be transmitted before birth, during delivery, or after birth through breastfeeding. However, determining the relative importance of these transmission routes remains challenging. Clinical and analytical data suggest multiple factors influence the risk of transmission, including the mother's stage of illness and viral load, the fetus's exposure to contaminated maternal bodily fluids during pregnancy and childbirth, and breastfeeding practices. More information is needed to precisely identify the timing of transmission from mother to child. Such data would be crucial for developing effective strategies to prevent HIV transmission and for guiding recommendations on when to initiate treatment. Interruption of transmission is particularly difficult if infection primarily occurs during the early stages of pregnancy; however, intervention may be more feasible if transmission occurs later in pregnancy or after birth [6].

Early Infant Diagnosis (EID) supports healthcare providers in identifying HIV-exposed infants shortly after birth, thereby allowing for the prompt initiation of Antiretroviral Therapy (ART) and other necessary treatments [7]. Early detection significantly reduces the risk of mother-to-child transmission and improves health outcomes for HIV-infected infants. This contributes greatly to the achievement of international targets for HIV prevention and treatment, as well as reductions in vertical transmission rates [8]. It is estimated that approximately 1.4 million pregnant women globally are living with HIV each year, placing their unborn children at risk of contracting the virus in utero, during Labor and delivery, and through breastfeeding. Due to the success of Prevention of Mother-to-Child Transmission (PMTCT) programs, the number of new pediatric HIV infections has significantly declined from 490,000 (430,000–560,000) in 2000 to 160,000 (100,000–220,000) in 2016 [9,10,11].

After highlighting the global significance of Early Infant Diagnosis of HIV, a brief explanation of HIV and its pathogenesis is provided. Currently, HIV infection affects approximately 37 million people worldwide, with more than 75 million individuals having been infected historically. Failure to control HIV replication leads to increased susceptibility to infections and cancers and results in progressive depletion of CD4+ T lymphocytes along with multiple immunological impairments. HIV infection is further associated with serious health complications, including bone disease, cardiovascular dysfunction, as well as renal and hepatic disorders [12].

Several host and viral factors contribute to the variability in Human Immunodeficiency

Virus type 1 (HIV-1) infection outcomes and the progression rate of disease in HIV-positive individuals. Co-receptors, along with cellular tropism, are considered the primary determinants influencing HIV infection by facilitating viral entry and defining viral characteristics. However, despite over two and a half decades of research, the exact mechanisms through which these factors contribute to CD4⁺ T cell destruction and the persistence of R5 and X4 strains during acquired immunodeficiency syndrome (AIDS) progression remain unclear [13].

The absence of overt emotional symptoms in HIV infection is typically associated with mild immune alterations. Following infection, the acute phase extends from one to three months before the individual develops virus-specific antibodies during seroconversion. Numerous factors influence the advancement of infection throughout the body and determine the period before disease symptoms manifest in individual cases [14].

Although an individual may appear physically healthy during the early stages of infection, the virus continues replicating in the bloodstream and lymphatic tissues. Over time, elevated viral loads progressively compromise immune function [15]. In advanced stages of HIV disease (AIDS), opportunistic infections caused by pathogens such as *Mycobacterium avium*, *Mycobacterium tuberculosis*, *Pneumocystis carinii*, *Cytomegalovirus* (CMV), *Toxoplasma gondii*, and *Candida* species become increasingly common [16]. Healthcare providers typically diagnose AIDS when the CD4⁺ T cell count falls below 200 cells/mm³, accompanied by elevated plasma HIV loads [17].

HIV damages the immune system by infecting and destroying CD4⁺ T lymphocytes, leading to immunodeficiency. The virus binds to the CD4⁺ protein on the surface of these cells to gain entry. However, CD4⁺ molecules alone cannot facilitate viral entry into dendritic or monocyte cells, despite these cells being critical for viral dissemination. A co-receptor is necessary to facilitate viral entry, as CD4 binding alone presents a barrier [17]. During the acute phase of infection, rapid HIV-mediated destruction of memory CD4⁺ T cells, particularly in mucosal tissues, severely compromises immune function. The resolution of this acute phase, lasting several weeks, is not fully understood but may result from either the depletion of target cells or the development of an HIV-specific immune response. The chronic phase follows when the immune system cannot fully control the initial damage, typically persisting for several years. The success of HIV in evading immune detection compels ongoing medical efforts to manage the disease and its associated opportunistic infections. Continuous viral replication, coupled with immune-mediated destruction of both naïve and memory T cells, leads to persistent immune

activation, further damaging the immune system. This two-phase model of HIV/AIDS pathogenesis has significant implications for treatment and vaccine development [18].

The success of Highly Active Antiretroviral Therapy (HAART), comprising nucleoside reverse transcriptase inhibitors (NRTIs), protease inhibitors (PIs), and/or non-nucleoside reverse transcriptase inhibitors (NNRTIs), has substantially improved morbidity and mortality among HIV-infected patients. Among haemophiliacs and intravenous drug users (IVDUs), liver-related complications associated with chronic Hepatitis C Virus (HCV) or Hepatitis B Virus (HBV) infections—often acquired prior to HIV—have become a significant health concern due to high mortality rates. Several non-exclusive pathogenic mechanisms contribute to the rising incidence of liver disease in this population, including drug-induced hepatotoxicity, chronic hepatitis B or C infections, steatosis, non-alcoholic steatohepatitis, and other conditions associated with alcohol or substance use. Although PIs have been shown to reduce liver disease incidence and hepatic mortality, liver-related morbidity and mortality persist at approximately 9% and 5%, respectively, among patients co-infected with HIV and HCV, rates comparable to those observed in the general population [19].

Chloroquine (CQ) has been shown to inhibit HIV-1 replication in vitro and in vivo and has been suggested to modify the glycosylation pattern of the gp120 envelope glycoprotein. These findings indicate that CQ could modulate the gp120 envelope structure to enhance the production of broadly neutralizing antibody responses. In addition to its potential as an HIV-1 therapeutic, CQ might serve as a valuable adjunct in modulating gp120 for vaccine development.

Host factors play a crucial role in determining disease progression rates in HIV-infected individuals. HIV targets CD4⁺ T lymphocytes—key orchestrators of immune responses—and induces the release of pro-inflammatory cytokines to facilitate its replication, thereby weakening the host immune system. The recognition of chemokine receptors as critical co-factors for HIV entry has expanded our understanding of host factors that influence HIV pathophysiology. Chemokine receptor ligands can modulate infection severity, highlighting the importance of host biology in disease progression. Long-term non-progressors offer valuable models for investigating host variables in HIV pathogenesis. Genetic factors significantly shape these host responses; for example, mutations in the CCR5 co-receptor gene confer strong resistance to HIV infection in homozygous individuals and partial protection against disease progression in heterozygotes. Moreover, robust HIV-specific immune responses, such as neutralizing

antibodies and cytotoxic T lymphocyte (CTL) activity, contribute to halting disease progression [20].

HIV also affects the central nervous system (CNS), causing neurological complications despite combination antiretroviral therapy (cART). HIV-associated neurocognitive disorders (HAND) persist, albeit in milder forms. Contributing factors include insufficient control of viral reservoirs in monocytes and glial cells, suboptimal cART CNS penetration, co-morbid conditions, substance abuse, and adverse effects on antiretrovirals [21]. Gut-associated lymphoid tissue (GALT) is another key site for early HIV replication and severe CD4⁺ T cell depletion. Even with antiretroviral therapy, viral replication in GALT persists, and CD4⁺ T cell restoration remains incomplete compared to peripheral blood. Chronic immune activation in GALT promotes viral reservoirs and enteropathy through heightened inflammation and impaired mucosal regeneration [22]. Following transmission to a new host, HIV targets CCR5⁺ CD4⁺ effector memory T cells, causing rapid depletion in mucosal sites. Although naïve and central memory T cells are initially preserved, their chronic activation during infection replenishes mucosal tissues with short-lived CCR5⁺ effector cells, increasing vulnerability to secondary infections. Persistent immune activation fuels ongoing HIV replication despite targeted T cell depletion [23]. HIV replicates continuously in CD4⁺ T cells and cells of the mononuclear phagocyte lineage, with the host immune response delaying but not preventing disease progression. Subclinical infection progresses to immune dysfunction characterized by selective depletion of CD4⁺ T-helper/inducer lymphocytes, culminating in opportunistic infections, malignancies, and death. Recent studies underscore the role of macrophages as major sites of HIV replication [24].

Most HIV-infected individuals experience neurological effects. HIV encephalitis-induced brain inflammation leads to AIDS dementia complex through neuronal loss in the limbic system and basal ganglia, accompanied by microglial and astrocyte proliferation. The quantity of HIV-1 antigen in brain tissue correlates directly with the extent of damage. Viral proteins such as gp120, Nef, and Tat contribute to neurodegeneration in HIV-1 brain infections [25]. HIV infection typically progresses through distinct stages, beginning with failed transmission, acute viremia, chronic immune activation, and culminating in advanced immune deficiency marked by severe CD4⁺ T cell loss. Studies of human samples, animal models, and in vitro systems have elucidated the mechanisms of HIV dissemination and pathogenesis during these phases [26].

For people living with HIV (PLWH), lung disease remains a major cause of morbidity

and mortality despite ART. In ART-accessible populations, the spectrum of lung diseases has shifted from acute, life-threatening opportunistic infections to chronic pulmonary disorders. Persistent systemic and pulmonary immune activation leads to aberrant cytokine and chemokine responses, progressive T and B cell dysfunction, and compromised innate immunity. HIV significantly disrupts immune cell function and is detectable in the lungs of PLWH [27]. Declines in CD4⁺ T cell counts and increases in HIV-1 viral loads in plasma and lymphoid tissues are directly associated with AIDS progression. Immune activation is a key driver of HIV-1 disease progression, enhancing viral replication through increased assembly and release, decreased chemokine production, elevated viral entry and integration, and upregulation of co-receptors CCR5 and CXCR4. Immune activation also disrupts cytokine networks, increasing the production of interleukins (IL-2, IL-4, IL-6, IL-10) and tumor necrosis factor-alpha (TNF- α). Moreover, immune activation is linked to apoptosis, immune exhaustion, and functional impairment, accelerating immunodeficiency and reducing survival. Co-infections may exacerbate HIV infection, replication, and disease progression, as all concurrent infections induce varying degrees of immune activation in vivo [28].

Human Immunodeficiency Virus (HIV) infection is characterized by a prolonged period of clinical latency, typically lasting several years following the initial infection. During this period, the number of infected cells (i.e., viral burden) in the blood remains very low, viral replication in these cells is almost imperceptible, and viremia is generally minimal or undetectable. These observations have been interpreted as indicative of an inactive phase of HIV disease, during which viral replication occurs slowly or is effectively controlled by HIV-specific immune responses. Nevertheless, a gradual decline in immune function and a continuous depletion of CD4⁺ T lymphocytes occur during this time, ultimately culminating in clinical disease manifestation [29].

Anaemia is a frequent finding among individuals living with HIV, though its multifactorial etiology complicates both diagnosis and treatment. Deficiencies of iron, folate, and vitamin B12 are commonly reported in HIV patients. However, some studies suggest that these micronutrients are often present in adequate amounts, and while supplementation may benefit certain individuals, anemia is often not reversed by such interventions. Reticulocytopenia is frequently observed in anemic HIV patients. Moreover, there have been numerous reports indicating inappropriately low levels of endogenous erythropoietin. Therefore, it is hypothesized that an impaired erythropoietin feedback mechanism significantly contributes to the pathophysiology of anemia in

individuals living with HIV [29].

The development and progression of HIV infection are influenced by a complex interplay of multiphasic and multifactorial immunopathogenic pathways. Following primary infection, HIV disseminates widely, resulting in high-level viremia. During this acute viremic phase, the virus becomes sequestered within follicular dendritic cell processes in the germinal centers of lymphoid tissues. Additionally, some individuals exhibit significant expansions of specific subsets of CD8⁺ T lymphocytes during this phase, characterized by expression of variable regions of the T-cell receptor β -chain. These expansions likely reflect HIV-specific immune responses that may play a crucial role in limiting viral spread. Furthermore, HIV infection is associated with increased production of certain pro-inflammatory cytokines and dysregulated immune activation, both of which influence HIV expression in tissues. Infection of progenitor cells in the thymus and bone marrow impairs the regeneration of immunocompetent cells. Dendritic cells facilitate the initiation and propagation of HIV infection in CD4⁺ T lymphocytes. Studies of long-term non-progressors—individuals who maintain stable CD4⁺ T lymphocyte counts and remain free from clinical disease for years—have demonstrated low viral burdens, restricted viral expression, and preserved lymph node architecture [30].

Interferons (IFNs) play a significant role in the pathophysiology and treatment of HIV infection. Recent studies indicate that interferon-alpha (IFN- α) may be beneficial in the management of early HIV infection and HIV-associated Kaposi's sarcoma. These therapeutic effects are corroborated by in vitro studies demonstrating that IFN- α treatment at the time of viral challenge can prevent HIV infection of monocytes. In IFN- α -treated cells, there is no evidence of productive HIV infection; viral gene expression returns to baseline levels post-treatment. While high levels of proviral DNA persist in these cells, evidence of viral transcription is minimal, suggesting true virological latency. Unlike most viruses that induce IFN production upon infection, HIV inhibits IFN- α production following poly (I)·poly (C) stimulation and does not induce monocytes to secrete IFN- α . This represents a key viral mechanism to evade the host's innate antiviral response. Paradoxically, elevated serum IFN activity in HIV-infected individuals has been associated with disease progression rather than resolution. This contradiction may be explained by recent findings indicating that interactions between peripheral blood mononuclear cells (PBMCs) and HIV-infected monocytes yield IFN- α with diminished antiviral efficacy. Consequently, IFN- α occupies a complex yet pivotal role in HIV pathogenesis. Identifying cellular mechanisms governing IFN- α 's antiviral effects could

inform novel therapeutic strategies for HIV [31].

Despite advancements in combination antiretroviral therapy (cART), neurocognitive impairment remains a significant health concern for over half of individuals living with HIV. The underlying pathophysiology and risk factors for HIV-associated neurocognitive disorders (HAND) are not yet fully elucidated. The complexity of diagnosing HAND contributes to this challenge, as its clinical manifestations are heterogeneous and fluctuate over time. HIV infiltrates the central nervous system (CNS) during infection, where it persists and replicates within resident immune and supportive cells. The resulting immune response and inflammation are believed to contribute to HAND development. Host genetic variations influence immune responses and susceptibility to HAND. This overview discusses research examining the role of human genetics in HAND pathogenesis, including potential genetic risk factors. Similarities and distinctions between HAND and Alzheimer's disease are also considered. Although numerous host genes regulating immune and neurotransmission functions have been linked to HAND susceptibility or protection, their effects are often modest and inconsistently replicated. However, certain specific genetic polymorphisms appear to influence HAND risk and advance our understanding of its pathogenesis [32].

Type I interferons (IFN-Is), primarily produced by plasmacytoid dendritic cells (pDCs), activate natural killer (NK) cells and enhance the expression of restriction factors targeting various stages of the HIV life cycle during acute infection. IFN-Is may also contribute to immune exhaustion and CD4⁺ T cell depletion during chronic infection. During long-term infection, circulating pDCs decrease after infiltrating lymphoid and mucosal tissues, although other cell types may compensate by producing IFN-I. Nonhuman primate studies have emphasized the necessity of timely IFN-I signalling in acute infection. While some studies have reported therapeutic benefits of IFN-I in the absence of cART, others indicate that either stimulation or inhibition of IFN-I signalling may yield positive outcomes in chronic ART-suppressed HIV infection [33].

In vivo, the primary targets of HIV-1 infection are CD4⁺ T lymphocytes and cells of the mononuclear phagocytic lineage. The most severe pathogenic consequences of HIV-1 infection arise from dysfunction or premature apoptosis of these hematopoietic cells. Patients with Acquired Immunodeficiency Syndrome (AIDS) often exhibit various hematologic abnormalities, especially during chronic HIV infection. These include anemia, lymphocytopenia, thrombocytopenia, and neutropenia, which are driven by multifactorial mechanisms. Nonetheless, considerable evidence suggests that infection of

bone marrow microenvironment cells by HIV-1 contributes to hematopoietic deficits. Most studies demonstrate that hematopoietic stem cells are not susceptible to HIV-1 infection until partial differentiation occurs, enabling expression of viral entry receptors. However, certain stromal cells within the bone marrow microenvironment—such as marrow microvascular endothelial cells, stromal fibroblasts, and mononuclear phagocytes—support HIV-1 replication [34].

Following a detailed exploration of HIV pathogenesis in various organ systems, it is pertinent to discuss HIV transmission modes. To date, only two human retroviruses have been identified as pathogenic: Human T-cell Lymphotropic Virus type 1 (HTLV-1) and HIV types 1 and 2 (HIV-1, HIV-2). Both primarily target CD4⁺ T lymphocytes. HIV-induced apoptosis of these cells leads to AIDS. In contrast, HTLV-1, after prolonged latency, can transform lymphocytes, resulting in adult T-cell leukemia/lymphoma (ATLL). A subset of HTLV-1-infected individuals may develop a neurological autoimmune disorder known as HTLV-1-associated myelopathy/tropical spastic paraparesis (HAM/TSP). Both viruses are transmitted through sexual contact, breastfeeding, organ/tissue transplantation, and blood transfusion, propagating via cell-free or cell-to-cell mechanisms [35].

A significant proportion of mother-to-child HIV transmission occurs postpartum, with studies indicating reduced transmission rates among infants delivered via caesarean section [36]. Although the relative contributions of different transmission routes remain unclear, vertical transmission can occur before, during, or after delivery. Clinical and laboratory evidence suggests multiple transmission mechanisms, including breastfeeding, fetal exposure to infected maternal fluids during pregnancy and labor, and maternal viral load and disease status. Clarifying the timing of transmission is crucial to inform prevention and treatment strategies. Interventions are more feasible if transmission occurs in late pregnancy or during delivery, while early pregnancy transmission poses greater challenges. Caesarean delivery or reduction of maternal genital viral load may protect against transmission during labor. Neonatal antiretroviral prophylaxis is warranted in cases of suspected intrapartum transmission. Guidelines on infant feeding must consider the risks of postnatal transmission via breastfeeding. Although HIV can infect the placenta throughout gestation, the extent of transplacental transmission remains uncertain. Potential mechanisms include placental breaches, progressive infection of placental layers, and transplacental cellular trafficking. Certain placental cells, including Hofbauer and trophoblast cells, express CD4 and may facilitate infection. Placental vulnerability to

infection may vary with gestational changes and may be exacerbated by factors such as substance abuse, smoking, and infections like chorioamnionitis [37].

In 2002, an estimated 800,000 pediatric HIV cases resulted from vertical transmission, underscoring its ongoing public health significance. Interventions such as antiretroviral therapy (ART), elective caesarean delivery, and breastfeeding avoidance can reduce transmission risk to below 1–2% when implemented appropriately. However, identifying HIV-infected pregnant women remains a challenge, particularly in resource-limited settings where adherence to complex treatment regimens and issues of drug resistance complicate prevention efforts. Encouragingly, clinical trials support the efficacy of shortened antiretroviral regimens for preventing vertical transmission in low-resource environments. Global initiatives, including the Global Fund to Fight AIDS, Tuberculosis, and Malaria, pharmaceutical donation programs, and generic drug production, have further enhanced prevention efforts [38].

Multiple risk factors influence mother-to-child transmission (MTCT). A study from 19 European centers (1984–1991) found a vertical transmission rate of 14.4% among 721 infants. Transmission correlated with maternal p24-antigenemia and CD4+ counts below 700/ μ L. Multivariate analysis identified cesarean delivery (OR 0.56), prematurity (<34 weeks gestation, OR 3.80), and breastfeeding (OR 2.25) as significant factors. Vaginal deliveries involving instrumentation (e.g., forceps, vacuum extraction) correlated with increased transmission only in centers where these practices were routine. Counselling for HIV-positive women regarding these risks, especially in cases of high antigenemia or low CD4+ counts, is essential. Although elective cesarean is not universally recommended, it may confer protective benefits. Premature birth likely increases risk due to peripartum transmission and immature immunity. Breastfeeding remains a significant route of transmission [39].

Individuals living with HIV/AIDS often experience symptoms that impact quality of life. These symptoms may arise from the disease itself, its complications, or adverse effects of Highly Active Antiretroviral Therapy (HAART) and other medications. Symptoms play a vital role in prompting healthcare engagement and guiding clinical assessments of disease progression and well-being [40]. Symptom management is crucial as poorly controlled symptoms compromise adherence, hasten disease progression, and diminish quality of life [41].

HAART has revolutionized HIV management, transforming it into a chronic but manageable condition. However, unlike other chronic diseases, early treatment decisions

in HIV can permanently influence subsequent therapeutic responses [42]. Lifelong management remains challenging due to viral reservoirs, toxicity, resistance, and adherence issues. Nanotechnology offers potential solutions by enhancing drug delivery, targeting latent reservoirs, and improving pharmacological profiles. Nano pharmaceuticals may deliver antiretrovirals to specific sites, carry anti-HIV nucleic acids, and possess inherent antiviral properties. Nonetheless, challenges persist regarding toxicity, biological interactions, and large-scale production [42].

Although combination antiretroviral therapy has extended life expectancy, persistent immune activation persists in many individuals despite undetectable plasma HIV-RNA levels. A deeper understanding of these pathways may inform adjunctive therapies to bolster immune recovery [43].

OBJECTIVE

To assess determinants of early infant HIV diagnosis and treatment success in Punjab, Pakistan.

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Chapter 2

LITERATURE REVIEW

HIV continues to present severe health challenges globally. In 2010, HIV was recognized as the fifth leading health condition contributing to disabilities, particularly affecting individuals aged 30 to 44 years. Scientific investigations have established that HIV belongs to the *Retroviridae* family, under the *Lentivirus* genus, as confirmed through analyses of its biological structure and genetic composition. HIV primarily targets immune cells, particularly T-helper cells and those involved in immune activation and communication. HIV exists in two forms: HIV-1, which is more virulent and widespread, and HIV-2, which is less transmissible and primarily confined to West Africa. Advances in diagnostic technologies have facilitated more effective treatments and contributed to reductions in HIV incidence globally [44].

Data from various nations indicate that approximately 40% to 51% of people living with HIV (PLHIV) initiated treatment too late, often facing delays in care linkage. However, once enrolled in care, PLHIV demonstrated high retention rates and adherence to prescribed treatments. For instance, after thirty-six months, HIV care retention rates dropped to 78% in Nepal but exceeded 90% in Laos. ART adherence rates reached 91% in Bangladesh and surpassed 95% in the Philippines and Vietnam, whereas Laos reported 70% and the Philippines 89%. Patients with tuberculosis (TB), those who maintained effective communication with healthcare providers, and those with sound knowledge of HIV care, demonstrated better ART initiation and adherence. Several socio-demographic and structural factors contributed to treatment interruptions, including younger age, engagement in sex work, incarceration, transgender identity, illiteracy, rural residency, substance abuse, and concerns related to health status, health insurance, confidentiality, and stigma associated with self-initiated HIV testing.

HIV program planners must ensure universal access to testing and expedite linkage to care for PLHIV. The UNAIDS 90-90-90 targets require comprehensive strategies that address both health system inefficiencies and socio-economic barriers throughout the HIV care continuum in the Asia-Pacific region [45].

Since its emergence as a global health threat in December 1981, AIDS has been closely linked to transmission among people who inject drugs (PWID). PWID populations exist in 148 countries worldwide, with HIV transmission among this group confirmed in 61 nations. Recurrent HIV outbreaks in PWID communities often arise under circumstances

that promote rapid viral transmission. In response, harm reduction programs targeting PWID have been established, incorporating services such as needle and syringe exchanges, medication-assisted therapies, and antiretroviral treatment (ART). Combined prevention strategies have successfully reduced new HIV infections among PWID populations in regions including New York City, Vancouver, and France, where such approaches have effectively curbed local epidemics. Recent outbreaks have been reported in Athens (Greece), Dublin (Ireland), Tel Aviv (Israel), and Scott County (Indiana, USA). These cases underscore the persistent challenges in protecting PWID from HIV infection and the importance of integrated, evidence-based interventions.

Between 2014 and 2015, a significant HIV-1 outbreak occurred in the Roka commune of Cambodia, involving 242 identified cases among individuals aged 2 to 89 years. Clinical investigations determined that unsafe injection practices were the primary driver of this outbreak. Researchers conducted comprehensive case identification and epidemiological investigations to trace the transmission pathways. Among 209 HIV-positive individuals (86.4%), additional testing identified high rates of co-infection with hepatitis C virus (HCV) and hepatitis B virus (HBV), detected in 78.5% and 12.9% of cases, respectively. Recent HIV infections accounted for 37.8% of cases. Phylogenetic analyses of 202 HIV *env* C2V3 sequences revealed that most CRF01_AE strains originated from a single evolutionary lineage traced back to September 2013. The highest transmission peak occurred in September 2014, averaging 15 new infections per day. Areas with higher HIV case concentrations corresponded with greater utilization of services provided by unlicensed practitioners ($p = 0.04$). Additionally, 38.6% of 153 patients had recently acquired HCV. Analysis of 90 NS5B HCV sequences identified three major clusters, with one linked to viral variants from 2012 and another from 2002–2003 [47].

In April 2019, an HIV outbreak primarily affecting children was reported in Larkana District, Sindh, Pakistan. By December 2019, 881 children, comprising 4% of 21,962 tested, had been diagnosed with HIV. This study aimed to identify the factors contributing to HIV transmission within this pediatric population. Researchers recruited HIV-positive children under 16 years of age who were receiving care at Shaikh Zayed Children's Hospital, Larkana. Each case was matched with an HIV-negative control from the same neighborhood, matched by age and sex. Face-to-face interviews collected data on potential risk factors, and all participants were screened for HBV and HCV. Mothers of participants were also tested for HIV. Conditional logistic regression was applied to analyze factors associated with HIV transmission.

Data from 401 matched pairs revealed HBV surface antigen positivity in 18.2% of cases compared to 5.2% of controls. Similarly, HCV antibodies were detected in 6.5% of cases versus 1.0% of controls. HIV positivity among mothers occurred in 7% of cases but not in any control group mothers. In the six months preceding recruitment, 56% of case patients had received over ten injections, compared to only 8% of controls. Furthermore, 73% of case patients received intravenous infusions, markedly higher than the 19% reported among controls. Notably, 14% of HIV-positive children had undergone blood transfusions within the past two years, compared to only 1% in controls [48].

These findings highlight the importance of reducing risky injection practices, which contribute significantly to HIV transmission. Harm reduction strategies, including syringe services programs (SSPs), have been instrumental in lowering infection risks among PWID. Such interventions underscore the necessity of providing sterile injection equipment and education on safe injection practices, particularly in the context of HIV outbreaks.

A major barrier to initiating ART in infants is the attrition of HIV-positive mothers between pregnancy diagnosis and childbirth. A study conducted in Zambézia Province, Mozambique, sought to understand why HIV-exposed infants often fail to receive necessary healthcare in rural settings. The study followed 443 HIV-positive mothers and their infants to assess follow-up success and timing of early infant diagnosis (EID) services. Multivariate models were employed to identify factors influencing infant testing uptake.

While nearly half of the mothers engaged with adult HIV services, only a quarter brought their infants for early HIV testing. Positive follow-up outcomes correlated with factors such as household support, maternal income, longer travel distances, and maternal ART adherence. Testing of 105 infants occurred between two and seven months of age, with a median of five months. Despite 16% HIV positivity in tested infants, 75% of HIV-positive mothers failed to present their infants for timely diagnosis. Maternal ART use during pregnancy was associated with improved maternal health and increased infant testing uptake. Collaborative efforts between healthcare providers and local communities aim to enhance early HIV diagnosis and treatment for infants.

Globally, fewer than half of infants born to HIV-positive mothers undergo HIV testing between 4 to 6 weeks of age, and testing rates decline further as infants age. Among those tested, only half receive necessary ART. This analysis highlights the need for strategies to improve early diagnosis, reduce result turnaround times, and ensure timely linkage to

care to enhance treatment outcomes for HIV-infected children.

Different countries have adopted varied strategies for determining the optimal timing of infant HIV testing. Although EID programs require further refinement, successful interventions include SMS-based result systems, maternal education, and community-driven approaches. Experts recommend dual testing approaches—at birth and at treatment facilities—to strengthen EID programs [50].

By the end of 2009, an estimated 2.5 million children globally were living with HIV-1, with vertical transmission accounting for most cases, and over 90% residing in sub-Saharan Africa. In 2008, the World Health Organization (WHO) recommended the initiation of ART for all HIV-infected infants diagnosed within their first year of life, expanding in 2010 to include those under two years of age, regardless of CD4 count or clinical staging. A study evaluated EID program implementation across three Tanzanian regions with differing HIV prevalence and logistical capacities for HIV DNA testing.

Data from Prevention of Mother-to-Child Transmission (PMTCT) registers (2009–2011) and Care and Treatment Clinics (CTCs) (2005–2011) across Kilimanjaro, Mbeya, and Tanga regions were analyzed using Poisson regression and rank-sum tests. Among 4,860 infants born to HIV-infected mothers (2009–2011), 4,292 (88.3%) underwent HIV testing. Testing coverage improved from 77.2% in 2009 to 97.8% in 2011. Initial test results identified HIV in 10.5% of infants. HIV prevalence was higher in Mbeya compared to Kilimanjaro (RR 1.872, 95% CI 1.408–2.543, $p < 0.001$). Sample turnaround times were significantly shorter in Mbeya (2.7 weeks) and Tanga (5.0 weeks) than in Kilimanjaro (7.0 weeks) ($p < 0.001$). Despite improvements in testing coverage, high loss-to-follow-up (LTFU) persisted, with 61% of treated infants LTFU during the study period. Over the three-year period, the study demonstrated progress in EID service delivery, albeit with geographic disparities in HIV prevalence and ongoing challenges related to sample turnaround times and retention in care [51].

The global population of children living with HIV continues to rise. Evidence shows that early diagnosis and appropriate management significantly enhance survival and reduce mortality. Polymerase chain reaction (PCR) technology has facilitated reliable early diagnosis worldwide. However, most HIV programs in low-income countries remain donor-dependent, with limited progress towards financial sustainability. Strengthening healthcare systems in these regions is essential to address operational challenges in identifying HIV-exposed infants and ensuring timely enrollment in care. This study highlights persistent barriers to early infant HIV diagnosis in Nigeria [52].

The management of Human Immunodeficiency Virus (HIV) has undergone significant transformation through the introduction of Highly Active Antiretroviral Therapy (HAART), which has redefined HIV as a chronic, manageable condition. Early decisions regarding HIV treatment substantially influence patient outcomes, following patterns like other chronic diseases. Despite the availability of numerous approved antiretroviral medications, treatment options remain limited due to issues of cross-resistance across the three primary drug classes.

Effective HIV treatment requires healthcare providers with specialized expertise in antiretroviral therapy. Initiation of treatment is recommended only after comprehensive patient education on the importance of adherence and after the patient demonstrates readiness to comply fully with the prescribed regimen. Selecting an appropriate antiretroviral therapy (ART) regimen requires careful consideration of the patient's potential for adherence and the risk of developing drug resistance. In cases of treatment failure, the timing of subsequent therapy changes significantly influence long-term outcomes. Resistance testing plays a critical role in guiding the selection of future treatment options. Achieving durable viral suppression is particularly challenging in patients with extensive prior antiretroviral exposure. Although well-tolerated regimens offer benefits, they may not completely prevent the development of resistance to future therapies.

Both healthcare providers and patients contribute to the development of drug resistance and treatment failure. Therefore, prevention of resistance remains a cornerstone strategy in managing treatment-experienced patients [53].

Research advancements in HIV/AIDS have successfully redefined the disease as a controlled medical condition. However, new challenges have emerged due to the accelerated aging of people living with HIV (PLHIV), cumulative drug toxicity, and the increased prevalence of comorbid conditions. HIV/AIDS continues to negatively impact patients' quality of life (QoL). Given these developments, a re-evaluation of traditional definitions of treatment success is warranted. Historically, treatment success has been measured by CD4 cell count recovery, suppression of plasma HIV RNA, and prevention of drug resistance. However, contemporary definitions should also incorporate factors beyond viral suppression, such as QoL and treatment tolerability.

In response to these evolving needs, a consortium of experts developed the RET Project, which identified five essential pillars for redefining HIV treatment success: rapid initiation, treatment effectiveness with ease of administration, safety, and quality of life.

Extensive literature reviews supported these pillars, which were subsequently validated by 32 HIV/AIDS specialists, including 4 coordinators and 28 field experts. The resulting guidelines provide updated recommendations, address contentious issues, and reflect consensus on key aspects of HIV care [54].

Our study assessed the prevalence of hepatitis C virus (HCV), HIV, and other sexually transmitted infections (STIs) among injection drug users (IDUs) in Pakistan, focusing on identifying transmission risks in target populations. Two separate testing sessions were conducted to recruit IDUs from their communities for screening of HCV, HIV, and other STIs. Behavioral data was collected through structured questionnaires. Comparative analysis of background characteristics and risk behaviors was conducted between IDUs in Rawalpindi and Abbottabad. Both univariate and multivariate analyses were performed to identify factors associated with heightened transmission risks [55].

In Rawalpindi, among 302 participants, the HIV prevalence was 2.6% (95% CI: 0.83–4.5%), whereas no HIV cases were identified among 102 participants in Abbottabad. HCV prevalence was higher in Rawalpindi (17.3%) than in Abbottabad (8%). Prevalence rates of gonorrhea and chlamydia were below 2% in both cities, while active syphilis was detected in less than 3% of participants. Risky injection behaviors were more prevalent in Rawalpindi. Use of informal syringe sources and prior drug treatment were associated with increased HCV risk (OR 2.8 and 3.7, respectively). Interestingly, in Rawalpindi, reporting STI symptoms was associated with a lower risk of HIV infection (OR 0.02, 95% CI: 0.03–0.9). These findings highlight ongoing transmission of HIV and HCV among IDUs, underscoring the need for enhanced harm reduction services, including safe needle and syringe distribution. Inadequate infection control practices in medical settings also contribute significantly to HIV epidemics across Africa. Both the U.S. and WHO have introduced infection safety initiatives, emphasizing the elimination of syringe reuse, a practice now regulated in several African countries [56].

In sub-Saharan Africa, the high prevalence of IDU, particularly involving heroin and stimulants, contributes substantially to HIV transmission. Adolescent drug users are especially vulnerable due to their engagement in high-risk sexual behaviors, exacerbating the epidemic. Despite evidence supporting harm reduction, many African nations maintain punitive approaches, hindering effective intervention programs. Public misunderstanding and stigma further impede progress. Awareness regarding sexual transmission of HIV is comparatively higher than understanding of risks associated with needle sharing. There is an urgent need for African nations to adopt proven harm reduction

strategies utilized in Europe, Asia, and North America to address the growing risks over the next decade [57].

A study conducted in Xinjiang, China, examined HIV incidence, retention in care, and predictors of these outcomes among high-risk IDUs. A cohort of 508 HIV-negative individuals was enrolled and tested at baseline, 6 months, and 12 months, accompanied by counseling and collection of behavioral and demographic data. At 12 months, HIV incidence was 8.8 per 100 person-years (95% CI: 6.3–12.0) with a retention rate of 93%. Marital status at baseline was the sole predictor of HIV incidence. Despite the high incidence, few baseline factors predicted retention or infection risk. High retention suggests the feasibility of future HIV prevention research in this population [58, 59].

Surveillance data from Pakistan confirms an emerging HIV epidemic among IDUs in urban centers. Linkages between IDU networks and high-risk sexual behaviors create pathways for HIV transmission to broader populations. Second-generation surveillance, incorporating geographic, biological, and behavioral data across eight major Pakistani cities, involved mapping IDU populations, behavioral surveys of 2,432 IDUs, and HIV testing of blood samples [60].

In South Africa, the narrative that the HIV epidemic is driven solely by sexual transmission has minimized scrutiny of unsafe medical practices. However, evidence points to significant infection control lapses, especially in maternity, pediatric, and dental settings. Approximately 20% of medical injections in some provinces involve reused equipment, and 25% of new adult HIV infections were reported among individuals denying sexual activity within the previous year. HIV transmission through immunization practices has also been documented in children, who subsequently infected mothers during breastfeeding. South Africa remains among the few sub-Saharan nations not mandating auto-disable syringes for all immunizations. Reuse of needles due to resource constraints is ethically indefensible given the availability of safer alternatives.

A global burden of disease (GBD) analysis quantified the impact of HBV, HCV, and HIV transmission through unsafe injections in 2000. Excluding four regions with negligible unsafe practices, the remaining ten regions averaged 3.4 injections per person annually, with 39.3% involving reused equipment. Contaminated injections led to 21 million HBV, 2 million HCV, and 260,000 HIV infections in 2000, accounting for significant proportions of global cases and contributing to 9.2 million disability-adjusted life years (DALYs) between 2000 and 2030. These findings highlight the critical need for policies

promoting injection safety, particularly in regions with substandard healthcare practices [61].

In Vietnam, IDUs constitute the largest group affected by HIV. This study explored the perceptions and behaviors of HIV-positive IDUs regarding safe injection and sexual practices through qualitative methods, including in-depth interviews, focus group discussions, and participant observation. Findings indicated a shift toward reduced-risk behaviors, such as seroconcordant needle sharing and lending rather than borrowing needles. Nonetheless, high-risk behaviors persisted, including equipment reuse and "syringe pulling" to extract residual drugs. Despite the existence of harm reduction programs, many IDUs avoided accessing services due to fears of arrest for possession of syringes or drugs. Disclosure of HIV status remained challenging, contributing to inconsistent condom use, especially among recently diagnosed individuals. Law enforcement practices and anti-drug policies further hinder harm reduction efforts. These findings underscore the need for policy reforms to address the high HIV prevalence among IDUs in Vietnam [62].

Chapter 3

METHODOLOGY

3.1. Research Design

This study employed a retrospective cohort study conducted to assess early infant HIV diagnosis and treatment success in HIV-exposed infants in Punjab, Pakistan.

3.2. Clinical Settings

This study was conducted at ADVANCED DIAGNOSTIC LAB (BSL-3) of Punjab Aid Control Program Lahore.

3.3. Sample Size

According to Slovin's formula N is number of populations is taken approximately 500 while e is margin of error is 0.05.

$$n = N / [1 + Ne^2]$$

$$N=500, e = 0.05$$

$$n=500/1+500[0.05]^2$$

$$n=214$$

3.4. Sampling Technique

A convenient sampling technique was utilized for this study.

3.5. Duration of Study

The duration of study was 04 months.

3.6. Selection Criteria

3.6.1. Inclusion Criteria

All Infants aged 0-18 months, born to HIV-positive mothers who have been on antiretroviral therapy (ART) for at least six months, and who underwent GeneXpert or PCR testing for HIV as part of routine medical care, are included in the study. Only those infants with complete clinical data and HIV test results were included in study.

3.6.2. Exclusion Criteria

Infants who were not tested for HIV during the study period, those with missing or incomplete medical records (including HIV test results), and infants diagnosed with HIV using methods other than GeneXpert or PCR were excluded from the study.

3.7. Ethical Consideration

a) Confidentiality and Privacy

We maintained confidentiality by ensuring all data is anonymized and securely stored.

b) Ethical Approval

Ethical approval was obtained from the Institutional Review Board [IRB] of Superior University and Ethical review committee of PACP before commencing the study. The IRB have reviewed the research proposal and ensured it adheres to ethical guidelines and protects participant rights.

3.8. Data Collection Procedure

Data was collected retrospectively from the Punjab AIDS Control Program (PACP) EID project database. Relevant variables on infant HIV diagnosis, maternal status, and treatment outcomes were extracted, cleaned, and analyzed using statistical software.

3.9. Data Analysis

Data was analyzed using the Statistical Package for the Social Sciences [IBM® SPSS® Statistics, Version 29].

Chapter 4

RESULTS

Among 214 infants, 179 infants were HIV negative and 35 were positive. Positivity ratio was 16%.

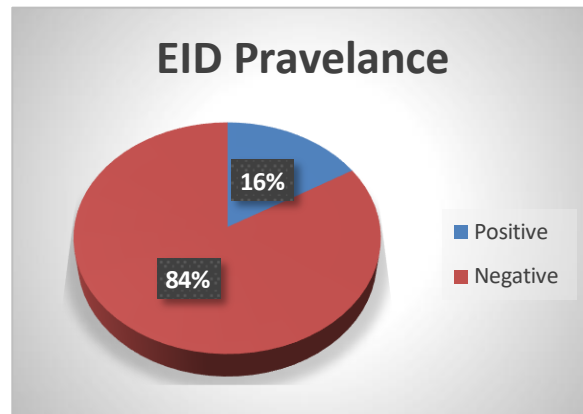


Figure 4.1 Early infant Diagnosis Prevalence

Among the 214 infants screened, the majority (88) were between 0-5 months old, of whom 14 tested positive and 74 were negative. In the 6-10 months age group, 7 out of 48 infants were HIV-positive, whereas 41 tested negatives. Among infants aged 11-15 months, 12 out of 75 were diagnosed as positive, and 63 were negative. In the smallest age group of 16-18 months, 3 children were tested, with 2 found positive and only 1 negative.

Out of the total infants tested, 96 males were negative, and 16 were positive, while 83 females were negative, and 19 were positive.

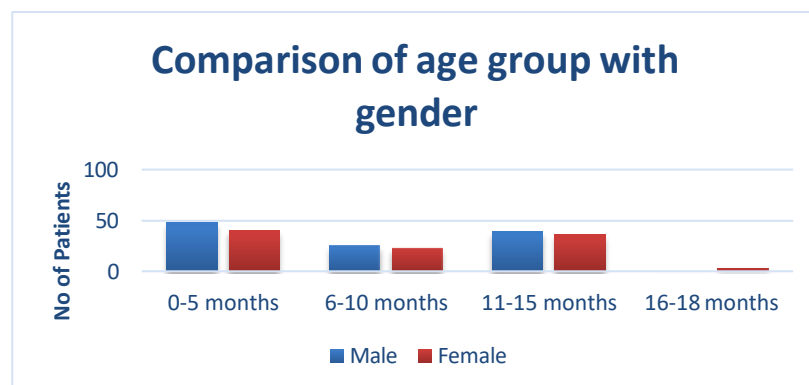


Figure 4. 2 Age and gender wise distribution of HIV in infants

Parental Age and HIV Transmission

The parental age group of 20-24 years had 50 infants, with 4 HIV-positive cases and 46 negatives. In the 25-29 years age group, 14 infants out of 64 tested positive, while 50 were negative. Among 89 parents aged 30-34 years, 16 infants were diagnosed as positive, whereas 73 were negative. In parents aged 35-39 years, only 1 out of 11 infants was HIV-positive.

Table 4. 1 Distribution of Case Prevalence by Parental Age

Age of Parents	No. of Patients	Positive	Negative
20-24 years	50	4	46
25-29 years	64	14	50
30-34 years	89	16	73
35-39 years	11	1	10

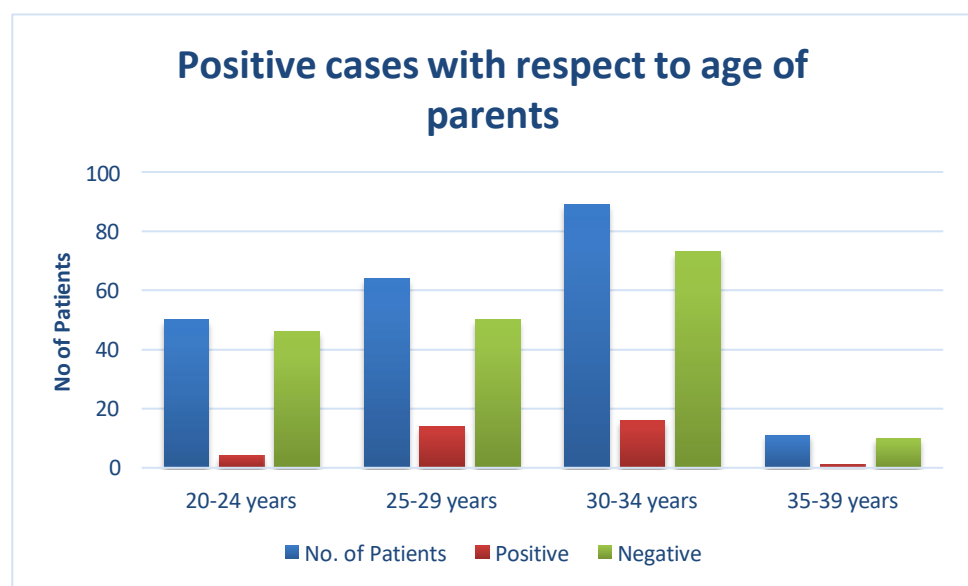


Figure 4.3 Distribution of Case Prevalence by Parental Age

Infant Feeding Practices

Breastfeeding was associated with 26 positive cases, while 178 infants tested negative. In contrast, among infants fed formula milk, 9 were positive, and one was negative, indicating a potential risk factor linked to infant feeding choices.

Table 4.2 Infant Feeding Types and Case Outcomes

Feeding Status		
Feeding Status	Positive	Negative
Breast Feed	26	178
Formula Milk	9	1

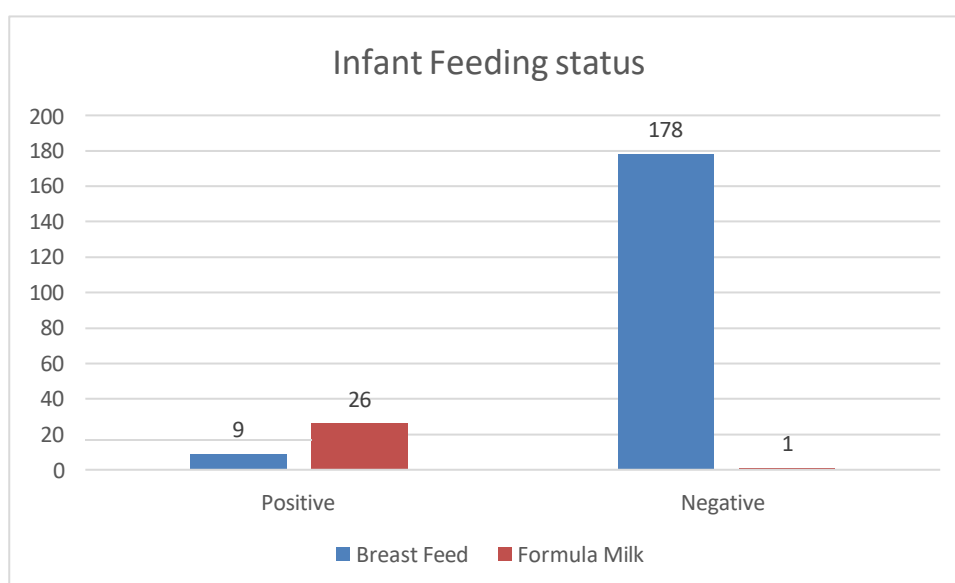


Figure 4.4 Infant Feeding Types and Case Outcomes

HIV Viral Load in Parents

Among parents of HIV-negative infants, 17 had a viral load of 0-50 CP/ML, 145 had a range of 51-100 CP/ML, 7 had a viral load between 101-150 CP/ML, and 10 had a range of 151-200 CP/ML. In contrast, parents of HIV-positive infants had significantly higher viral loads, with 7 cases in the 500-1000 CP/ML range, 2 cases in the 1001-1500 CP/ML range, and 27 cases exceeding 2000 CP/ML.

Table 4. 3 HIV Viral Load Status of Mothers With EID-Negative Infants

HIV viral load of Mothers (Negative Infants)	
Range in (CP/ML)	No. of Patients
0-50	17
51-100	145
101-150	7
151-200	10

Table 4. 4 HIV Viral Load Status of Mothers With EID-Positive Infants

HIV viral load of Mothers (Positive Infants)	
Range in (CP/ML)	No. of Patients
500-1000	7
1001-1500	2
1501-2000	0
>2000	27

CD4 Count of Mothers

The immune status of mothers was assessed through CD4 marker levels. Among mothers of HIV-positive infants, 13 had a CD4 count of 0-200, 14 had a count of 201-400, 8 had a range of 401-600, and only 1 had a CD4 count between 601-800. Conversely, among mothers of HIV-negative infants, 15 had CD4 counts in the 0-1000 range, 162 had values between 1001-2000, and only 1 mother each had CD4 levels in the 2001-3000 and 3001-4000 ranges.

Table 4. 5 CD4 count Status of Mothers With EID-Positive Infants

CD4 Marker of Mothers (Positive)	
Range	No. of Patients
0-200	13
201-400	14
401-600	8
601-800	1

Table 4. 6 CD4 count Status of Mothers With EID-Negative Infants

CD4 Marker of Mothers (Negative)	
Range	No. of Patients
0-1000	15
1001-2000	162
2001-3000	1
3001-4000	1

Parental Risk Factors

The risk group categorization of parents showed that 58 belonged to the general population, 43 were classified as highly suspected cases, 46 were injecting drug users (IDU), 28 were men who have sex with men (MSM), 7 were tuberculosis (TB) patients, and 6 were female sex workers (FSW).

Table 4. 7 Parental risk group distribution of EID Infants

RISK GROUP OF PARENTS	
Name of risk group	No. of patient
General population	58
Highly suspected	43
IDU	46
MSM	28
Tb patient	7
FSW	6

Parental HIV Transmission Factors

Among HIV-positive parents, blood transfusion was a transmission factor in 30 cases, while 20 cases were linked to FSW contact, 12 to MSM relations, and 28 to spousal transmission. Surgical procedures contributed to 12 cases, 55 cases had unknown transmission origins, and 23 cases were associated with IDU.

Table 4. 8 Determinants of HIV Transmission in Mothers of EID Infants

Reason of transmission of Parents	
Reasons	No. of patients
Blood transfusion	30
FSW	20
MSM	12
Spouse	28

Surgical	12
Unknown	55
IDU	23

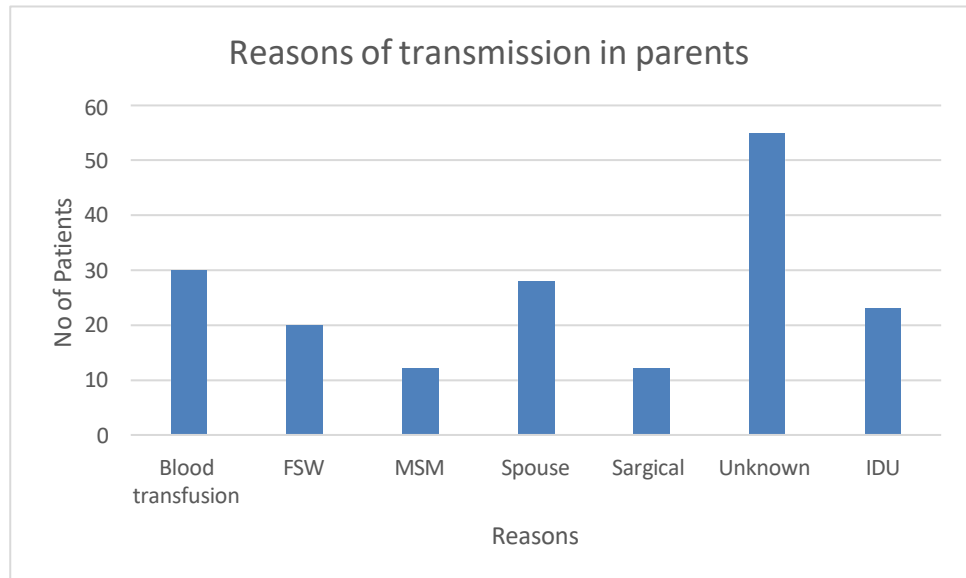


Figure 4. 5 Determinants of HIV Transmission in Mothers of EID Infants

Educational Background of Parents

Among the parents of HIV-positive children, 3 were uneducated, 3 had primary education, 1 had secondary education, and 2 had a bachelor's degree. Among parents of HIV-negative children, 14 were uneducated, 84 had primary education, 76 had secondary education, 4 had bachelor's degrees, and only 1 had a master's degree.

Table 4. 9 Parental Education Status in Relation to Infant HIV Outcomes

Education of Parents		
Education	Positive children	Negative children
Uneducated	3	14
Primary	3	84
Secondary	1	76
Bachelor	2	4
Master	0	1

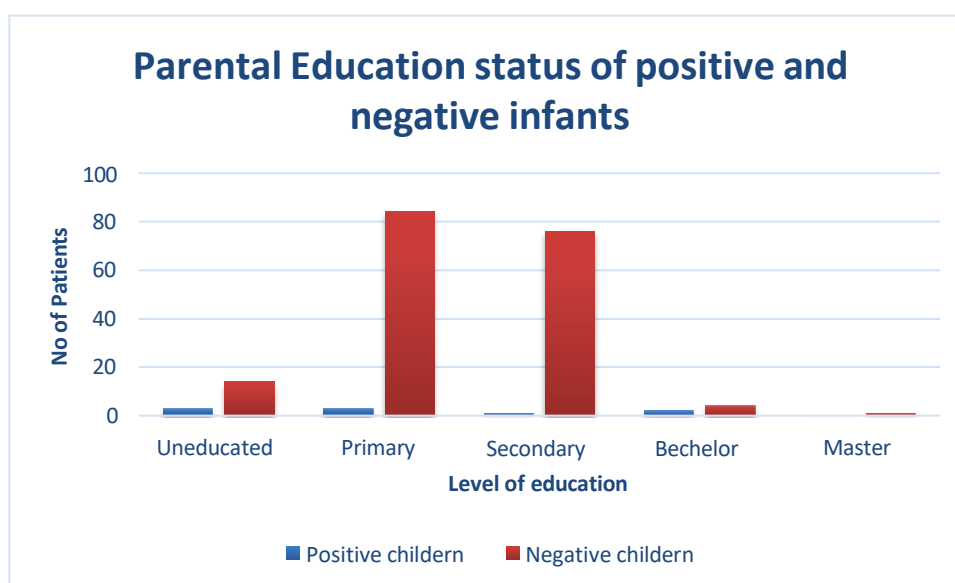


Figure 4. 6 Parental Education Status in Relation to Infant HIV Outcomes

Geographical Prevalence

The highest number of cases was recorded in Lahore, with 101 infants, followed by DG Khan with 16 cases and Faisalabad with 13 cases. Other regions, including Sheikhpura (12 cases), Vehari (6 cases), and Okara (8 cases), reported lower numbers. Cities such as Bahawalnagar, Bahawalpur, Gujranwala, Hafizabad, and Khushab had only 1 to 3 reported cases.

These findings provide crucial insights into the demographic and clinical aspects influencing EID outcomes in Punjab, Pakistan, emphasizing the role of early screening, parental health status, and feeding practices in determining infant HIV status.

Table 4. 10 Regional Prevalence of Early Infant Diagnosis (EID) Cases in Punjab

Area Vise prevalence	
City name	No. of patients
Bahawalnagar	3
Bahawalpur	3
DG Khan	16
Faisalabad	13
Gujranwala	2
Hafizabad	2
Jhang	1
Khushab	1
Coat Moman	2

Lahore	101
Mandi Bhauddin	1
Okara	8
Rajan pur	2
Sahiwal	1
Sargodha	2
Shah Kot	1
Sheikhupura	12
Sialkot	2
Vehari	6

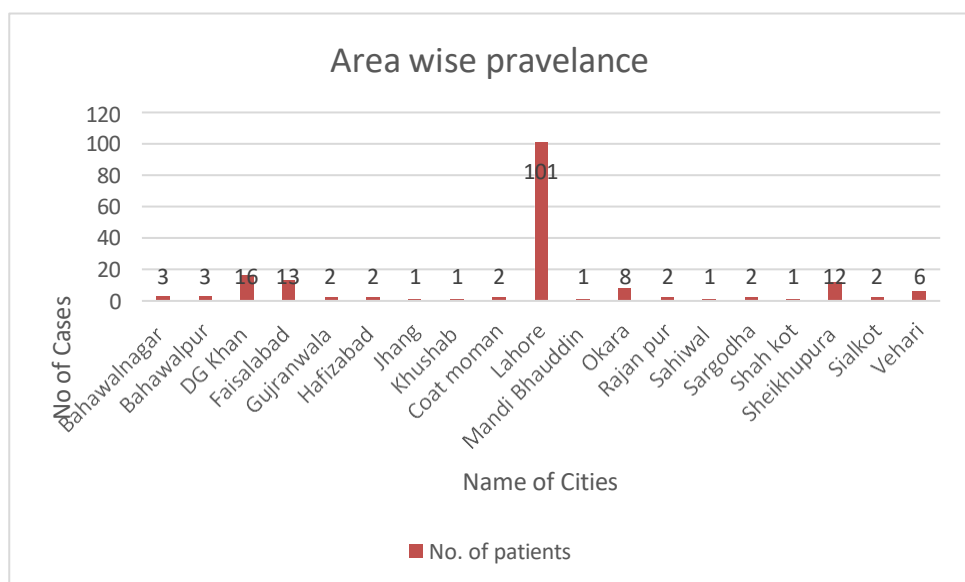


Figure 4. 7 Regional Prevalence of Early Infant Diagnosis (EID) Cases in Punjab

Chapter 5

DISCUSSION

The findings of this study provide crucial insights into the early infant diagnosis (EID) and treatment outcomes of HIV-exposed infants in Punjab, Pakistan. The results are consistent with global and regional studies that emphasize the importance of early testing, maternal health status, and feeding practices in reducing HIV transmission and improving infant survival rates. The discussion integrates the present study's results with recent literature to provide a comprehensive understanding of the factors influencing EID outcomes.

Our study found that the highest number of HIV-positive infants were in the 0-5 months (14 out of 88) and 11-15 months (12 out of 75) age groups. These findings align with research conducted in India by Kamble et al. (2023), which highlighted those delays in early HIV testing led to late ART initiation, resulting in worse clinical outcomes [63]. These findings reinforce the importance of strengthening EID programs in Punjab, particularly ensuring that infants receive their first virological test within six weeks of birth as recommended by the WHO.

The present study observed that female infants had a slightly higher HIV positivity rate (19 cases) than males (16 cases), although the difference was not statistically significant. This trend is consistent with findings in sub-Saharan Africa, where gender disparities in healthcare access, cultural norms, and care-seeking behavior influence EID outcomes. However, biological factors are not known to play a significant role in gender-based HIV susceptibility among infants. The slight difference in our study could be attributed to variations in healthcare-seeking behavior among parents, as some studies suggest that male infants are often prioritized for medical care in certain communities [64].

The results indicated that infants born to parents aged 25-34 years had the highest number of HIV-positive cases. These findings agree with studies from Zimbabwe, where Mafaune et al. (2017) reported that younger parents often have lower adherence to prevention of mother-to-child transmission (PMTCT) programs due to socioeconomic challenges [65]. Older parents (35-39 years) in our study had a lower transmission rate, which may be attributed to better healthcare awareness and adherence to ART. Strengthening targeted counseling and support programs for younger parents in Punjab could help reduce transmission rates further.

A striking finding of our study was the significant difference in HIV prevalence between

breastfed and formula-fed infants. While 26 breastfed infants tested positive, and 9 formula-fed infants were HIV-positive. This finding supports WHO recommendations that exclusive breastfeeding with maternal ART adherence is not safer than mixed or formula feeding in resource-limited settings. Nkenfou et al. (2024) in Cameroon reported similar findings, where mixed feeding increased the risk of HIV transmission compared to exclusive breastfeeding [66]. In contrast, Kamble et al. (2025) found that formula-fed infants in India had higher mortality rates due to malnutrition, further emphasizing the need for proper maternal care and nutritional support for HIV-exposed infants [67]. These findings highlight the need to strengthen infant feeding counseling programs in Punjab to ensure that HIV-positive mothers adhere to appropriate feeding practices based on their ART status.

Our study found that parental viral load had a direct impact on infant HIV outcomes. Among parents of HIV-positive infants, 27 had a viral load above 2000 CP/ML, whereas most parents of HIV-negative infants had viral loads below 100 CP/ML. This finding is consistent with Chapuma et al. (2024), who demonstrated that infants born to mothers with a viral load above 1000 CP/ML were five times more likely to be diagnosed as HIV-positive [68]. Furthermore, our study observed that mothers of HIV-positive infants had lower CD4 counts, with 13 cases falling below 200. These results align with Wahl et al. (2020), who found that advanced maternal immunosuppression increases vertical transmission risks [69]. These findings underscore the importance of maintaining low maternal viral loads through strict ART adherence during pregnancy and breastfeeding to minimize the risk of transmission.

Retention in HIV care remains a significant challenge, as observed in our study, where infants lost to follow-up after the initial test accounted for a considerable proportion of cases. A study in South Africa by du Plessis et al. (2024) found that only 65% of HIV-exposed infants remained in care for a full year after initial EID testing, with social stigma and transportation costs being major barriers [70]. Similarly, Nikhare et al. (2024) in India found that caregivers faced difficulties accessing EID services due to economic

constraints [71]. Addressing these issues in Punjab requires enhanced follow-up mechanisms, community outreach programs, and financial support for low-income families to improve retention rates.

Financial constraints remain a significant barrier to the widespread implementation of PoC EID testing. Our study highlights the need for cost-effective strategies to expand testing in resource-limited settings. Emmert-Fees et al. (2022) conducted a global review and found that while PoC testing is more expensive than centralized laboratory testing, it provides long-term cost savings by reducing infant HIV complications [72]. Adopting similar approaches in Pakistan could help balance cost-effectiveness with improved health outcomes.

The highest number of cases in our study were reported in Lahore (101), followed by DG Khan (16) and Faisalabad (13). These findings align with global trends, where urban centers report higher HIV prevalence due to increased mobility, healthcare access disparities, and socio-economic factors (Mugisa et al., 2022) [73]. These geographical variations suggest the need for region-specific interventions in Punjab, such as targeted awareness campaigns and improved healthcare services in high-prevalence areas.

CONCLUSIONS

This study on Early Infant Diagnosis (EID) among HIV-exposed infants in Punjab, Pakistan, reveals a 16% positivity rate, emphasizing the ongoing risk of vertical transmission despite preventive efforts. Most positive cases were concentrated in younger age groups, with higher prevalence noted among breast-fed infants, highlighting feeding practices as a potential contributor to transmission. A gender-wise analysis showed slightly more female infants testing positive than males, while geographic data identified Lahore as the region with the highest case burden.

Parental factors were strongly linked to infant HIV outcomes. High maternal viral loads and low CD4 counts were common among mothers of HIV-positive infants, demonstrating the importance of effective viral suppression and immune health in preventing transmission. Additionally, risk behaviours such as injection drug use (IDU), MSM contact, and spousal transmission emerged as significant factors. Education levels and socio-economic status also appeared to influence outcomes, indicating a need for targeted awareness and support programs. The findings underscore the importance of strengthening maternal care, ensuring ART adherence, promoting breastfeeding safety, and scaling up EID services to reduce HIV transmission in infants.

Limitation

The study did not include data on how consistently mothers adhered to antiretroviral therapy or the duration of their treatment, both of which are important factors in determining the risk of HIV transmission to infants.

Recommendations

To reduce HIV transmission among infants, early diagnosis services must be strengthened and made widely accessible. Enhancing maternal health monitoring, promoting safe feeding practices, and focusing on high-risk parental groups are essential. Future research should adopt prospective approaches to improve evidence-based strategies.

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APPENDICES

ENGLISH CONSENT FORM

Description of Research and Your Participation

You are invited to participate in a research study conducted by Azka Mubeen

The purpose of this research is to evaluate the “Early infant diagnosis and outcomes in HIV-exposed infants in Punjab, Pakistan”

Risks and Discomforts

There are no risks and discomfort associated with this research.

Potential Benefits

The participants will get indirect benefits as the findings of the study will help to overcome the factors associated with non-adherence to anti-tuberculosis treatment.

Protection of Confidentiality

We will do everything we can to protect your privacy. Your identity will not be revealed in any publication resulting from this study.

Voluntary Participation

Your participation in this research study is voluntary. You may choose not to participate and you may withdraw your consent to participate any time. You will not be penalized in any way should you decide not to participate or to withdraw from this study.

CONSENT

I have read this consent form and have been given the opportunity to ask questions.

I give my consent to participate in this study.

Participant's Signature _____

Date: _____

A copy of this consent form should be given to the participant.

URDU CONSENT FORM

میں _____ تصدیق کرتا / کرتی ہوں کہ **Azka Mubeen** نے اپنی اس تحقیق

“EARLY INFANT DIAGNOSIS AND OUTCOMES IN HIV-EXPOSED INFANTS IN PUNJAB, PAKIST”

زیرنگرانی **Hasnain JavedDR.** کے متعلق بتا دیا ہے۔ مجھے اس تحقیق کی نوعیت، مقاصد، اہداف، توقعات، فوائد اور خطرات کے متعلق، ساری معلومات فراہم کر دی گئی ہیں اس تحقیق کے دوران ساری معلومات صیغہ راز میں رہیں گی اور مریض کا نام اور دیگر معلومات صرف تحقیق کے لیے استعمال ہوں گی۔ مجھے یہ بھی بتا دیا گیا ہے کہ میں اس تحقیق سے متعلق ہر قسم کے سوال پوچھنے کا مجاز ہوں اور یہ تحقیق صرف ایک شخص کے مفاد میں نہیں ہے بلکہ بحیثیت مجموعی انسانیت کا مفاد اس سے وابستہ ہے۔ تمام تفصیلات جاننے کے بعد یس تحقیق میں شامل ہونے یا نہ ہونے پر کسی کا قائل نہیں ہوں۔ اس تحقیق سے کسی بھی وقت علیحدہ ہونے پر مجھ پر کوئی پابندی نہیں ہو گی۔ میں بذاتِ خود بقائمى حوش و حواس اور رضا مندی سے اس تحقیقاتی عمل میں شامل ہوتی / ہوتا ہوں

_____ دستخط محقق

_____ دستخط شرکت کار

_____ تاریخ

PERMISSION LETTER

ETHICS COMMITTEE LETTER

PLAGIARISM REPORT