

PREVALENCE OF HBV AND HCV IN HIV INFECTED MIGRANT WORKERS IN MUMBAI

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FOR THE DEGREE OF
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IN
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SUBMITTED BY
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UNDER THE GUIDANCE OF
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MUMBAI

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A Thesis Submitted to the

University of Mumbai

For the

**M.Sc. (By Research) Degree,
In Microbiology**

Submitted by

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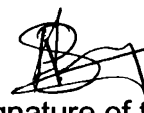
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STATEMENT BY THE CANDIDATE

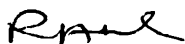
As required by the University Regulation No.2190, I wish to state the work embodied in this thesis titled "**Prevalence of HBV and HCV in HIV Infected Migrant Workers in Mumbai**", forms my own contribution to the research work carried out under the guidance of **Dr. (Mrs.) R. A. Deshmukh** at Department of Virology, Haffkine Institute Parel, Mumbai 400 012. This work has not been submitted for any other degree of this or any other University. Whenever references have been made to previous works of others, it has been clearly indicated as such and included in the Bibliography.



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Dedicated Lovingly to my Family and Friends...

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1. Introduction

Acquired Immune Deficiency Syndrome (AIDS) is the name of a life threatening condition caused by the Human Immunodeficiency Virus (HIV). It is a serious disorder of immune system in which body's normal defense against infection breaks down, leaving it vulnerable to a host of life threatening infections including malignancies.

The HIV/AIDS global epidemic has greatly exceeded earlier predictions and it is now clear that it has the potential to affect all countries and all population groups. The total number of people living with the Human Immunodeficiency Virus (HIV) in 2005 has reached 40.3 million [36.7–45.3 million] ¹. Five million people were newly infected with the virus in 2005.¹

Since the human immunodeficiency virus (HIV) was found in Chennai in 1986, India has had an AIDS epidemic.² In many respects; however, its extent and complexities have only recently begun to be appreciated. India has a population of 1.1 billion people — one sixth of the world's population — and is home to perhaps one of every eight people with HIV infection. In 2006, the estimate of 5.7 million HIV-infected people in India, this as compared with 5.5 million in South Africa has captured wide attention.² It remains uncertain, however, whether India has more infected people than any other country. The epidemiologic data for India (estimates of the number of infected persons range from 3.4 million to 9.4 million) are far less precise than for South Africa (4.9 million to 6.1 million).² The estimate for India is based primarily on anonymous testing data from public clinics for prenatal care and for patients in high-risk

groups or with sexually transmitted infections.³ Although the number of surveillance sites is expanding, the data may still be skewed and inadequate.^{3,4}

Of India's 35 states and territories, 6 had a high HIV prevalence in 2005, as defined by a rate of HIV positivity of more than 1% among women visiting prenatal clinics and a rate of more than 5% among patients visiting clinics for sexually transmitted diseases.²

Within high-prevalence areas, the HIV epidemic reflects diverse social, cultural, religious, and sexual practices.³ Female and male sex workers, men who have sex with men, and injection-drug users have the highest rates of infection — surveys typically find a prevalence of 10 to 20%.³ The epidemic spreads from these groups to others in various ways, including through the clients of sex workers, bisexual men (many of whom are married), and “bridge populations,” the most important of which appear to be long-distance truckers and people who migrate between states for seasonal work in construction and other industries.^{3,5}

In some parts of India, three out of four households include a migrant.⁶ Masses of the poor, landless, illiterate and unskilled agricultural laborers and petty farmers from backward states make quantum jumps towards big metropolises like Calcutta, Mumbai, Delhi, Madras, and so forth, bypassing local small towns and small cities -- which fail to give them even minimum employment.

Migration has historically played a major role in the growth and development of Mumbai, the commercial center of India. According to the

estimates based on the 1999-2000 NSS survey, migrants constitute about 37 per cent of Mumbai population. There has been an increase in female migration in the recent years compared to male migration. About 75 per cent of the migrants originate from the rural areas compared to 23 per cent from the urban areas. The migrants from Maharashtra state in which Mumbai is located constitute about 43 per cent of the cities population according to the survey. Migration from other states, mostly Uttar Pradesh and Bihar, has increased over the last two decades.⁷

Such metropolitan migration is leading to acute urban involution, congestion and decay. Proliferation of filthy urban slums and pavement dwelling, extreme squalor and very poor level of living characterize Mumbai. Overflow of urban poverty, unemployment, extreme housing shortages, and frequent breakdowns of essential urban services (like water, electricity, sewerage, transport) are visible everywhere in Mumbai.

Economic and social impacts on migrants and their families are variable. Migration often involves longer working hours, poor living and working conditions, social isolation and poor access to basic amenities.

Because of their lifestyles, language and culture they are not exposed to the educative media. Hence HIV prevention messages, which impact their interpretation and utilization of risk averse behaviour goes unattended. In fact the culture of migrancy itself affects the extent of risky behaviour practiced, for example the separation from families may drive them to seek commercial sex or casual sex. Thus, migrant workers are more vulnerable to HIV infection.

In several parts of the world, migration has been identified as significant risk factors in the transmission of HIV, and 'migration has become a central theme in the discussion of AIDS.⁸ There are only few studies on Mumbai migrant workers relating to their risk of acquiring HIV. In one such study by Krishna C et al reported a high prevalence of HIV was reported among male migrant returnees to Nepal from Mumbai as compared to non-migrants.⁹

Once the individual is infected, the virus breaks down the immune system and makes the host vulnerable to multiple opportunistic infections (OIs) caused by various pathogens. These opportunistic infections remain principally responsible for the morbidity and mortality associated with HIV disease.

Diseases of the hepatobiliary system are a major problem in patients with HIV infection. An estimated one-third of deaths in HIV patients are directly or indirectly related to liver disease. Liver diseases in HIV infected persons can occur due to Hepatitis B virus (HBV) and Hepatitis C virus (HCV) co-infections, chronic alcoholism, hepatic tuberculosis, or due to the effects of anti-retroviral therapy (ART).^{10,11} Since the principal routes for HIV transmission are similar to that followed by the hepatotropic viruses, as a consequence, infections with HBV and HCV are expected to be high in HIV infected patients.

HIV significantly modifies the natural history of HBV or HCV infection.^{12,13} HIV infection increases the levels of HBV or HCV viremia,^{14,15} especially at the time of HIV seroconversion.¹⁶ HIV co-infection worsens the histological course of HBV and HCV infection by increasing and accelerating the risk of cirrhosis.¹⁷ High levels of viremia lead to an accumulation of viral proteins in the

endoplasmic reticulum and to hepatocyte death.^{18,28} The rate of cirrhosis is increased 2–5-fold among HIV/HBV or HIV/HCV coinfecting patients, and the mean elapsed time between transmission and the onset of cirrhosis is significantly reduced.¹⁹ This increased rate of cirrhosis and shorter disease progression is explained by a significantly increased yearly progression of the fibrosis score in co-infected subjects that seems to depend on the CD4 cell count (>200 cells/mL).²⁰

HCV- related liver disease is now an important cause of morbidity (8.6% of hospitalized patients) and mortality (4.8%) in HIV/HCV coinfecting patients.²¹

In a study it was also observed that liver disease in men who had HIV and chronic hepatitis B had a higher mortality rate attributable to liver disease than in men with either HBV or HIV alone.²²

The prevalence of co-infection of these viruses varies from place to place and is largely dependent on the high-risk practices exhibited by the study population. In Europe and USA, HIV-HBV coinfection has been seen in 6%-14%^{11, 23} of all patients while HIV-HCV co-infection has been variably reported ranging from 25% to almost 50%^{24,25} of these patients. Evidence of exposure to HBV and HCV has been found in 8.7% and 7.8%, respectively, of HIV patients from Thailand²⁶ in Southeast Asia.

Within India also, variable co-infection rates have been reported from region to region. HIV-HBV co-infection is reported in 6% of people from Chennai, South India²⁷ 7.5% in Chandigarh, Northwest India³¹ and 16% in Mumbai, Western India²⁸. Similarly, HIV-HCV co-infection rates also vary from 4.8%-

21.4% in South India ^{27,29} and 30% in Western India ²⁹ to as high as 92% in the North- East.³⁰

HBV vaccination should be provided to all HIV-positive individuals with no serum HBV markers. The response to HBV vaccine is lower in HIV-positive individuals, particularly among those with lower CD4 cell counts. Anti-HBsAg titers should be checked 12 weeks after ending the vaccination cycle and booster doses or extra cycles are advisable when no appropriate humoral responses have been obtained. In patients with CD4 cell counts of less than 500 cells/ml, intensive schedules are initially warranted. ³²

There is a scarcity of published information on the prevalence of HBV and HCV among HIV infected migrant workers, regardless of the elevated risk of HIV/AIDS in migrant workers, and reported increase of infectious hepatitis in HIV infected patients.

With this background we set out to determine the prevalence of hepatitis B and C virus infections in HIV-positive migrant workers from Mumbai and sought to identify potential risk factors predisposing to the infections. The results generated would give us the probable disease burden on the study population. This would help us to implement proper interventions to educate the population about the sexually transmitted disease and related risk behaviours, which would help to reduce the number of further infections.

2. Review of Literature

HUMAN IMMUNODEFICIENCY VIRUS

History

The human immunodeficiency virus (HIV) was unknown until the early 1980's but since that time has infected millions of persons in a worldwide pandemic. It is a retrovirus that causes acquired immunodeficiency syndrome (AIDS, a condition in humans in which the immune system begins to fail, leading to life-threatening opportunistic infections). It has caused enormous social economic and behavioral impact on individuals, families, communities and the whole world. AIDS has shattered the global economy with no successful treatment and vaccine in sight.

AIDS was officially recognized for the first time in June 1981 at the Centres for Disease Control, U.S.A. in previously healthy homosexual men dying with pneumocystis carinii pneumonia and Candidiasis. Since then AIDS has been reported from all the continents.³³

In 1983, scientists led by Luc Montagnier at the Pasteur Institute in France first discovered the virus that causes AIDS.³⁴ They called it lymphadenopathy-associated virus (LAV). A year later a team led by Robert Gallo of the United States confirmed the discovery of the virus, but they renamed it human T lymphotropic virus type III (HTLV-III).³⁵ The dual discovery led to considerable scientific disagreement, which was resolved by President Mitterrand of France and President Reagan of the USA. In 1986, both the French and the U.S. names for the virus itself were dropped in favor of the new term, Human Immunodeficiency Virus (HIV).³⁶

Almost 25 years have now elapsed. Twenty-five years, in which HIV infection has changed from a fatal condition to a manageable chronic illness. Twenty-five years, in which the development of antiretroviral therapy (ART) has been one of the dramatic advances in the history of medicine. However, for the vast majority of people living with HIV/AIDS, ART is still light years away—largely inaccessible in resource-poor countries where HIV continues to devastate families, communities and societies, especially the poor and the socially marginalized.

Epidemiology

Global Scenario

HIV continues to grow, as does the number of deaths due to AIDS. A total of 39.5 million [34.1 million–47.1 million] people were living with HIV in 2006—2.6 million more than in 2004. This figure includes the estimated 4.3 million [3.6 million–6.6 million] adults and children who were newly infected with HIV in 2006, which is about 400 000 more than in 2004.³⁷

In many regions of the world, new HIV infections are heavily concentrated among young people (15–24 years of age). Among adults 15 years and older, young people accounted for 40% of new HIV infections in 2006. Sub-Saharan Africa continues to bear the brunt of the global epidemic. Overall sub-Saharan Africa is home to an estimated 24.7 million [21.8 million–27.7 million] adults and children infected with HIV—1.1 million more than in 2004.³⁷

In the past two years, the number of people living with HIV increased in every region in the world. The most striking increases have occurred in East Asia and in Eastern Europe and Central Asia, where the number of people living with HIV in 2006 was over one fifth (21%) higher than in 2004.³⁷

Indian Scenario

The world's second-most populous country, India, is experiencing a highly varied HIV epidemic, which appears to be stable or diminishing in some parts while growing at a modest rate in others. Approximately 5.7 million [3.4 million–9.4 million] people, of whom 5.2 million were adults aged 15–49 years, were living with HIV in 2005.³⁷

The national adult HIV prevalence is 0.8%. However, the aggregate data mask the multiple and diverse HIV sub-epidemics in the country. Of the 28 States of India, 6 States, four in southern India (Andhra Pradesh, Tamil Nadu, Maharashtra, Karnataka) and two in north eastern India (Manipur and Nagaland) have generalized epidemics with HIV prevalence rate of above 1% among pregnant women. These six States account for nearly 80% of all reported AIDS cases in the country.³⁸

In the southern states, heterosexual transmission accounts for majority of the reported cases, whereas in the northeastern states of Manipur and Nagaland, injecting drug use is the predominant mode of transmission.³⁸

In the six States with generalized epidemics, the HIV prevalence has remained constant over the last three years, indicating that the epidemic may have stabilized.³⁸

The average HIV prevalence among women attending antenatal clinics in India is 0.88%. Much higher rates are found among people attending sexually transmitted disease clinics (5.66%), female sex workers (8.44%), injecting drug users (10.16%) and men who have sex with men (8.74%).³⁹

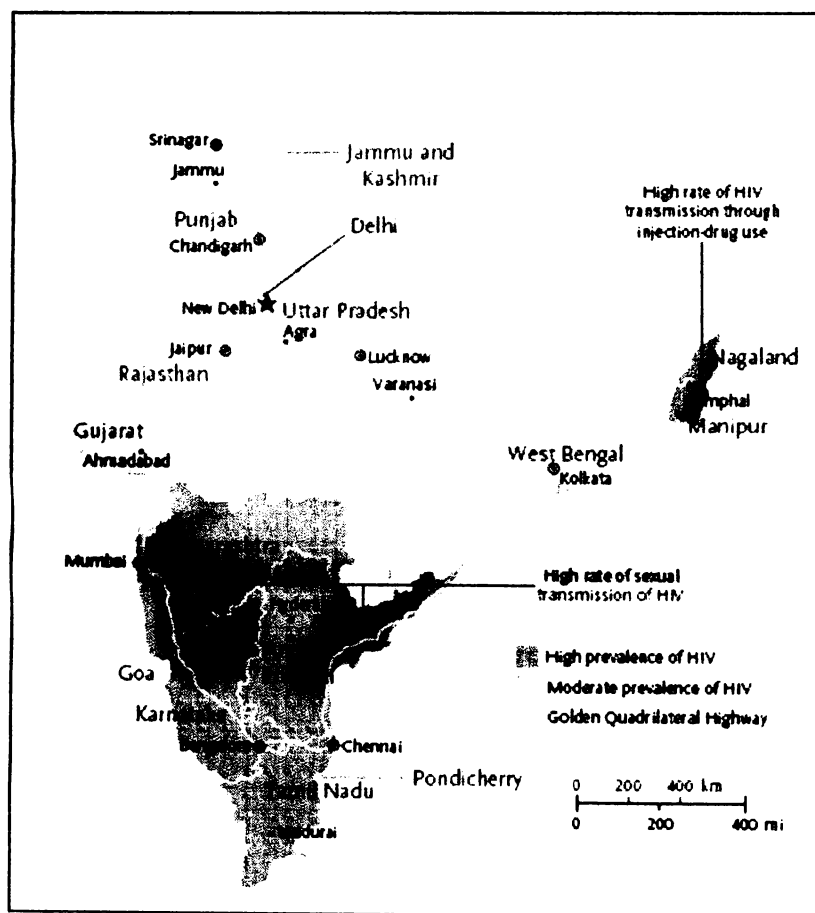


Figure no. 1: HIV Prevalence in India in 2005

Classification

HIV belongs to the class Retroviruses and family Lentivirinae. Two major antigenic types (HIV-1 and HIV-2) have been identified and are readily distinguished by differences in geographical distribution, biological and molecular characterization and extent of transmissibility.

Three classes of HIV-1 have developed across the globe: M (major), O (outlying) and N (new).⁴⁰ Among the M group, which accounts for over 90% of reported HIV/AIDS cases, viral genetic diversity is so great that this group has been sub classified into nine major clades, including A–D, F–H, J and K, as well as several circulating recombinant forms.⁴⁰ Viral diversity is greatest in sub-Saharan Africa, perhaps reflecting its longer residence in the human population.⁴⁰ Viruses within the M group are found worldwide, while the highly divergent O group viruses have been isolated thus far in Africa, Germany, France and other parts of Europe.⁴⁰

The genomic diversity of viral subtypes in different geographical regions is a direct consequence of the very high error rate of the HIV reverse transcriptase (RT) enzyme that is responsible for the copying of viral RNA into DNA, an essential step in the retroviral life cycle. Other factors that contribute to rapid genetic diversification include the high replicative rate of the virus, the number of mutations arising in each replicative cycle, the viral propensity for genomic recombination, and viral fitness. In addition, high rates of genomic evolution may result from host, environment, or therapeutic selection pressures.⁴⁰

The Morphologic Structure of HIV-1

Electron microscopy reveals HIV to be a roughly spherical particle with a diameter of 110nm. The virus contains a dark cone shaped core-measuring 100nm in length with a width of 50nm tapering to 40nm. HIV is covered in a lipid bilayer from which 72 knob like structures are projected. These projections are composed of multimers of two viral glycoproteins called gp120 and gp41. ⁴¹

The viral matrix is composed of the p17 gag protein and is attached to the inner face of the lipid bilayer. The core contains all the genetic and biochemical information required for replicating HIV. This information includes two identical copies of the HIV RNA genome, a cellular transfer RNA (tRNA) captured during the budding process and three virally encoded enzymes called reverse transcriptase (RT), integrase and protease. ⁴¹

The Organization of the Viral Genome

Every HIV particle contains two identical strands of RNA, and each one of these RNA strands contains the entire genetic blueprint coding for the structure and life cycle of HIV. The HIV genome is made up of only 9800 nucleotides and is approximately 100000 times smaller than the human genome. In addition, to the genes encoding the Gag, Pol, and Env polypeptides common to all the retroviruses, the genome also carries open reading frames for several regulatory proteins such as vif, vpr, vpr, tat, rev and nef. ⁴¹

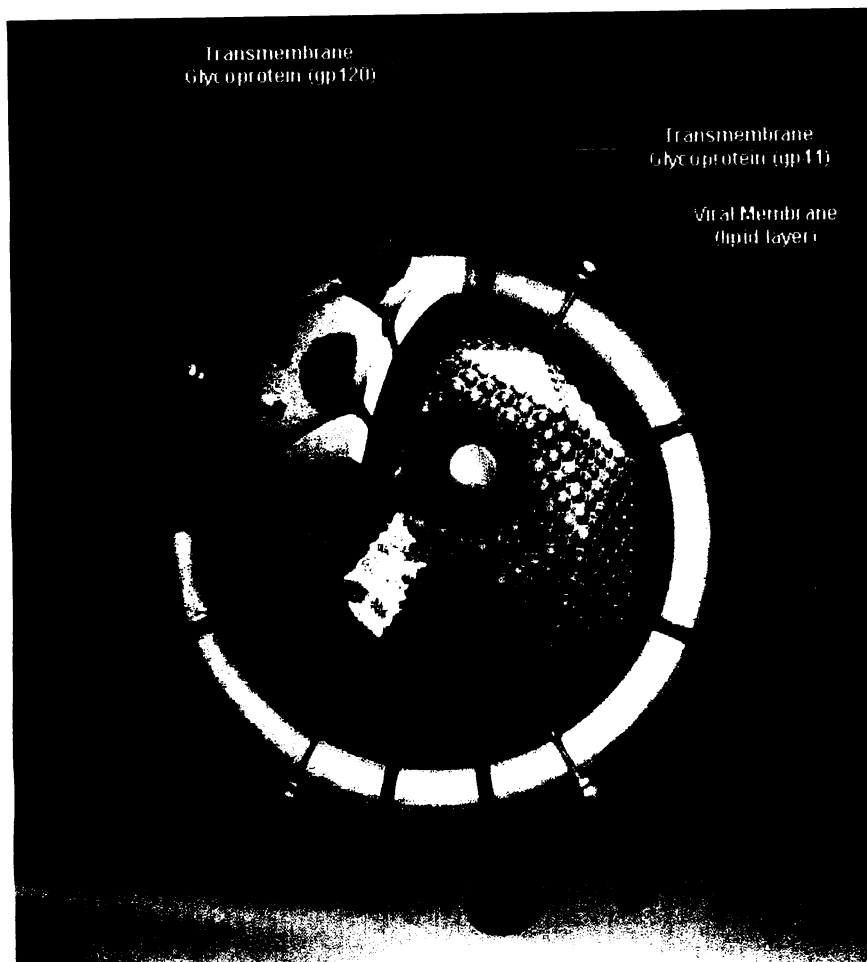


Figure no. 2: Diagrammatic representation of HIV-1

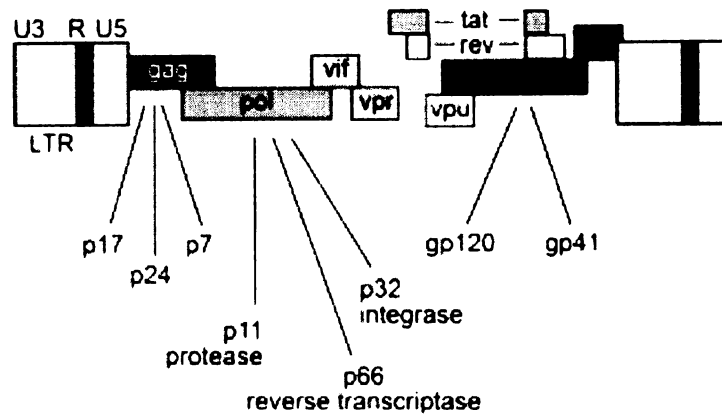


Figure no. 3: Diagrammatic representation of HIV-1 genome

Major Structural and Regulatory Proteins

The major core proteins of HIV are synthesized from *gag* as a large, myristoylated precursor protein (pr55), which is subsequently cleaved to yield the capsid (p24), matrix (p17) and nucleocapsid protein (p7). Several smaller cleavage products, p1, p2 and p6 are also generated.⁴²

The cleavage of *gag-pol* precursor protein (pr170) formed by one nucleotide ribosomal frame shift between the first and second long open reading frames of the HIV-1 genome yields the *gag* protein products and the retroviral enzymes protease (p11), reverse transcriptase/ribonuclease H (p51/p66), and integrase (p32).⁴²

The major protein product of *env* is synthesized in the host cell cytoplasm as an 88-kd polypeptide. This protein undergoes a series of post-translational glycosylation steps in the endoplasmic reticulum and Golgi complex, yielding a

final product of 160 kd, called as gp160. Much of the newly synthesized gp160 is retained in the intracellular compartments and eventually degraded; however, a small fraction is cleaved by the serine protease to generate the transmembrane (gp41) and surface (gp120) subunits. Following proteolytic cleavage, gp120 remains covalently associated with gp41 on the cell surface and is incorporated into the envelope of the budding virions. The envelope glycoproteins are arranged on the surface as tetramers in a typical spike-and –knob configuration with an estimated total of 72 spikes/virion. ⁴²

Nef, Vif, Vpr and Vpu were classified as accessory genes in the past, as they are not absolutely required for replication *in vitro*. However, the regulation and function of these accessory genes and their proteins have been studied and characterized in more detail in the past few years. The accessory genes, *nef*, *tat* and *rev*, are all produced early in the viral replication cycle. ⁴²

Tat and Rev are regulatory proteins that accumulate within the nucleus and bind to defined regions of the viral RNA: TAR (transactivation-response elements), found in the LTR; and RRE (rev response elements), found in the *env* gene, respectively. The *tat* protein is a potent transcriptional activator of the LTR promoter region and is essential for viral replication in almost all *in vitro* culture systems. Cyclin T1 is a necessary cellular cofactor for *tat*. *Tat* and *rev* stimulate the transcription of proviral HIV-1 DNA into RNA, promote RNA elongation, enhance the transportation of HIV RNA from the nucleus to the cytoplasm and are essential for translation. *Rev* is also a nuclear export factor that is important

for switching from the early expression of regulatory proteins to the structural proteins that are synthesized later. ⁴²

Nef has been shown to have a number of functions. Nef induces down regulation of CD4 and HLA class I molecules from the surface of HIV-1-infected cells, which represents an important escape mechanism for the virus to evade an attack mediated by cytotoxic CD8+ T-cells and to avoid recognition by CD4+ T-cells. Nef also interferes with T-cell activation by binding to various proteins that are involved in intracellular signal transduction pathways. ⁴²

Vpr seems to be essential for viral replication in non-dividing cells such as macrophages. Vpr stimulates the HIV-LTR in addition to a variety of cellular and viral promoters. More recently, vpr was shown to be important for the transport of the viral pre-integration complex to the nucleus and arresting cells in the G2 phase of the cell cycle. ⁴²

Vpu is important for the virus 'budding' process, because mutations in vpu are associated with persistence of the viral particles at the host cell surface. Vpu is also involved when CD4-gp160 complexes are degraded within the endoplasmic reticulum and therefore allows recycling of gp160 for the formation of new virions. ⁴²

Some recent publications have highlighted a new and important role for vif in supporting viral replication. Vif-deficient HIV-1 isolates do not replicate in CD4+ T-cells, some T cell lines ('non-permissive cells') or in macrophages. Vif-deficient isolates are able to enter a target cell and initiate reverse transcription, but synthesis of proviral DNA remains incomplete. In vitro fusion of 'permissive' and

'non-permissive' cells leads to a 'non-permissive' phenotype, suggesting that the replication of HIV depends on the presence or absence of the cellular inhibitor.⁴²

Replication

The pathogenesis of HIV infection is a function of the virus life cycle, host cellular environment, and quantity of viruses in the infected individual. HIV primarily infects cells that have CD4 cell-surface receptor molecules, using it to penetrate the cell. Cells with CD4 receptors susceptible to HIV infection include cells of the mononuclear phagocyte system, principally blood monocytes and tissue macrophages, T lymphocytes, natural killer (NK) cells, dendritic cells (Langerhans cells of epithelia and follicular dendritic cells in lymph nodes), hematopoietic stromal cells, and microglial cells in brain. The galactosylceramide receptors on cells within the brain and bowel may play a role as well. In addition to the CD4 receptor, a co-receptor known as a chemokine co-receptor (CCR5 and CXCR4) is required for HIV to infect the cells.⁴³

HIV entry into a host cell through binding of gp120 to CD4 receptor induces a conformational change in gp120, exposing the co-receptor binding sites. The V3 loop region of gp120 determines whether the host cell CCR5 or CXCR4 chemokine co-receptor will be engaged. After the chemokine co-receptor is engaged, the gp41 on the HIV surface undergoes a conformational change. The gp41 transmembrane co-receptor consists of HR1 and HR2 helical regions along with a fusion peptide. Conformational change in gp41 through HR1 and HR2 interaction leads to formation of a stable structure that allows fusion of

HIV and host cell membranes, with a fusion pore through which the viral core enters the host cell. The core can utilize host cell microtubules to move toward the cell nucleus.⁴³

The isolates of HIV-1 that selectively interact with the CXCR4 chemokine co-receptor to infect lymphocytes are known as T-tropic strains and the HIV-1 isolates that selectively interact with the CCR5 chemokine co-receptor, and also CCR2 and CCR3, to infect macrophages and dendritic cells are called M- tropic strains. T-cell-tropic HIV-1 isolates have classically been identified as being those that are easily propagated in T-cell lines, and grow poorly in macrophages, but are able to easily infect primary T-cells from peripheral blood samples. Conversely, M-tropic HIV-1 isolates are classically those viruses that, are unable to infect Tcell lines (i.e., immortalized T-cells), are most easily propagated in macrophage cultures, but are also able to easily infect primary T-cells from peripheral blood samples. Thus, it should be noted that both T-tropic and M-tropic HIV-1 variants can easily infect primary human non-immortalized T-cells *in vitro*.⁴² CCR8 co-receptor has been identified as a cofactor to permit infection by either T-cell tropic or by M-tropic strains of HIV. Dual tropic HIV strains have been identified that can use more than one chemokine co-receptor.⁴³

After binding and penetration, the viral ribonucleoprotein complex is rapidly uncoated in the cell cytoplasm. This complex contains two full-length HIV transcripts. Conversion of RNA to DNA is carried by coordinated polymerase and ribonuclease H activities of the viral enzyme. Reverse transcriptase (RNA-dependent DNA polymerase) first synthesizes a complementary strand of DNA

from the viral genome RNA template, while ribonuclease H then selectively degrades original RNA template. The enzyme then synthesizes a second strand of DNA, yielding a double copy of the original RNA genome.⁴²

Reverse transcriptase is initiated in the cytoplasm and proceeds concurrently with the nuclear import of the viral genetic material. Translocation of the viral genome to the nucleus occurs in association with a large “pre-integration” complex. This nucleoprotein complex consists of HIV RNA and DNA in addition to the viral matrix protein, Vpr, integrase, reverse transcriptase and other cellular proteins. After translocation to the nucleus, the double stranded, linear DNA is integrated in the host cell chromosome by virally encoded integrase enzyme.⁴²

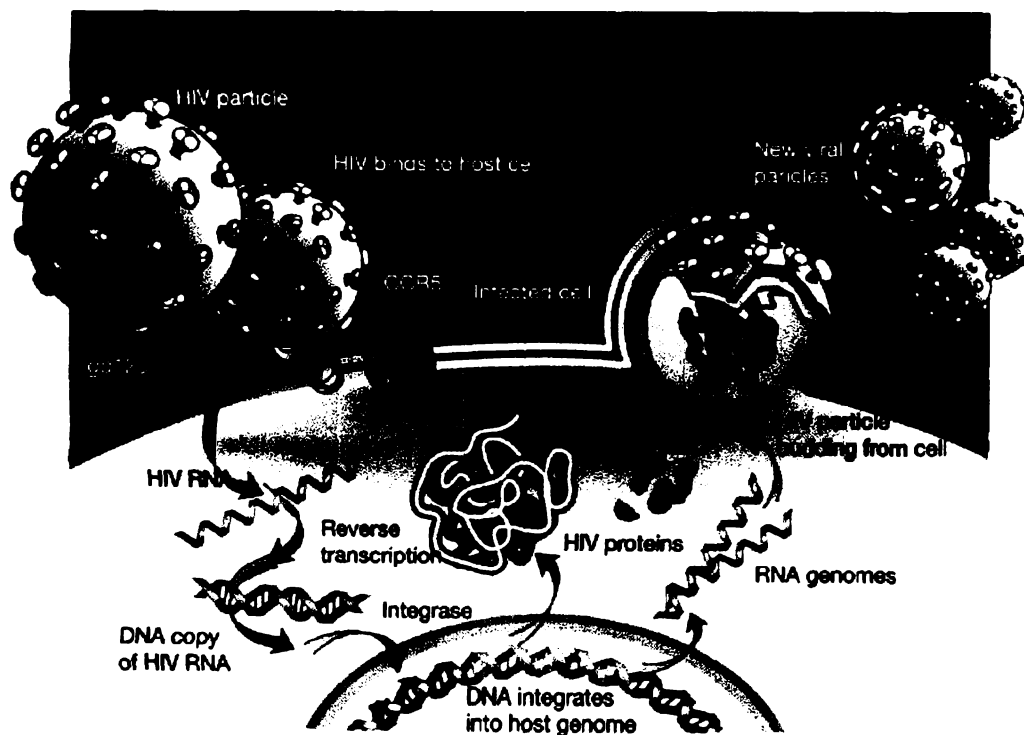


Figure no. 4: Replication of HIV

Once integrated activation of HIV transcription is dependent on the coordinated activity of both cellular and viral factors. Low levels of transcription are initiated by the interaction of several cellular transcription factors with specific recognition sites within the HIV LTR. These early transcripts are transported to the cytoplasm as multiply spliced 2.0kb messages that are differentially translated into the regulatory proteins Tat, Rev and Nef. Tat functions to increase the LTR driven transcription significantly through the specific interaction with RNA stem-loop structure known as transcription response (TAR) element, located immediately downstream of the transcription initiation site on the HIV LTR.⁷⁵ Interaction of Tat with TAR element leads to increase in transcription and provides a powerful feedback mechanism for increasing viral gene expression.⁴²

The Rev is produced from multiply spliced early transcripts. Rev is a 10kd protein, which mediates the critical transition from early expression of the regulatory proteins to the late expression of viral structural and enzymatic proteins. This is brought about by interaction of Rev with another RNA stem-loop structure, the Rev response element, located within the viral *env* gene. In the absence of Rev, singly spliced and unspliced transcripts encoding structural and enzymatic proteins, as well as the viral genome RNA fail to be transported from the nucleus to the cytoplasm. Rev functions to alter the cytoplasmic transport of these transcripts either by activating the nuclear export of incompletely spliced mRNA or by protecting the unspliced messages from the splicing complex allowing the nuclear export to occur. Once transported to the cytoplasm these messages are translated into the viral structural and enzymatic proteins.⁴²

Before the virion can be released from the cell, the structural proteins must co-ordinately assemble. HIV has been observed to assemble both at the plasma membrane and within the intra-cytoplasmic vacuoles. Unspliced viral transcripts are used to synthesize gag and gag-pol proteins in the cytosolic compartment. These polyproteins are subsequently transported to the cytoplasmic side of the plasma membrane where they mediate budding, and two copies of viral genome are incorporated into the particle. Spliced RNA transcripts are used to make ENV protein on the membrane of the rough endoplasmic reticulum. These proteins are transported to the external surface via the secretory pathway and then move to the sites of the budding for packaging immediately after budding, the gag and the gag-pro-pol proteins within the immature virions are cleaved by viral protease to produce a mature infectious virion. The gag and the gag-pol proteins begin to encapsidate the HIV genomic RNA and the plasma membrane forms a lipid bilayer over the viral core. Precursor gp160 ENV undergoes glycosylation and oligomerisation in the endoplasmic reticulum. It is then transferred to the golgi complex where cellular enzymes process the glycoprotein to the surface gp120 and the transmembrane gp41. They then associate non-covalently and are transported to the cell surface. Finally, the inner core shell condenses and is and is linked with the glycoprotein incorporated lipid membrane.⁴⁴

Transmission

HIV is transmitted by certain specific behaviors involving the exchange of bodily fluids between people. Unlike many other diseases, HIV is not transmitted through routine casual contact, through the air, or through insect bites. The efficiency of HIV transmission is determined by the amount of virus in the body fluid and the extent of contact.

Since the beginning of the pandemic, three main transmission routes for HIV have been identified:

1. Sexual Route

One of the most common ways HIV is transmitted is through sexual contact, primarily through unprotected vaginal or anal intercourse. In every act of sexual penetration, there is an inserter and a receiver. The receiver is generally at greater risk than the inserter, although if the penis of the inserter has open cuts, sores, or ulcers, then the inserter's risk will be increased.

Unprotected anal sex between two men or between a man and a woman is particularly risky because the chance of damage (small tears and lesions) to the thin lining of the rectum is high. This facilitates HIV transmission by enabling the virus in semen to quickly enter the bloodstream.

With penile-vaginal sex, the female partner is generally at greater risk because of the greater exposed surface area in the female genital tract than in the male genital tract, the higher concentrations of HIV in seminal fluids than in vaginal fluids, and the larger amount of semen than vaginal fluid is exchanged during intercourse.

Although HIV transmission through unprotected oral sex—cunnilingus (oral-vulva contact) or fellatio (oral-penile contact)—can occur, the risk is much lower than for unprotected vaginal or anal sex. But this behavior is not free of risk.

HIV transmission has also been reported through infected semen used for artificial insemination. Reputable sperm banks now test all samples before using them.⁴⁵

2. Blood or blood product route

2a. Sharing injection drug works

The sharing of HIV-contaminated needles, syringes, drugs, and other drug paraphernalia can lead to the transmission of HIV. Even if syringes and needles are sterile, drugs that are mixed in containers (including spoons or bottle caps) and are shared, or drugs shared from a common container, make for very risky injections.

While intravenous injections hold the greatest risk for infection, it is possible to be infected from subcutaneous and intramuscularly injections as well.⁴⁵

2b. Transfusions and organ transplants

Transfusions or treatments with infected blood or blood products can lead to HIV transmission. Many parts of the world now routinely test donated blood for HIV before approving its use, but many countries lack the resources to do so.

Organs or tissues taken from individuals with HIV can also transmit the virus to the people receiving them.⁴⁵

2c. Sharing skin-cutting or -piercing tools

HIV can be transmitted by skin piercing, cutting, and tattooing instruments (needles, razor blades, circumcision instruments) that have been in contact with infected blood or body fluids and have not been properly processed before reuse.⁴⁵

2d. Transmission in health care settings

Health care workers, including cleaners and lab technicians, will be at risk for becoming infected with HIV if they are exposed to blood and other body fluids of infected individuals during their work.

One form of exposure among workers in health care settings is needlestick injuries with HIV-contaminated needles. The risk for HIV transmission to clients during clinical or surgical procedures exists when clients are exposed to blood or body fluids containing HIV from other clients.⁴⁵

3. Mother-to-Child Transmission (MTCT)

A woman infected with HIV can pass the virus to her baby during pregnancy, labor and delivery, or breastfeeding. Treatment options are now available in some settings that can greatly reduce the rate of HIV transmission from mother to child.

The risk for HIV transmission from breastfeeding, which has been estimated to be between 10 and 20%, increases with a longer duration of

breastfeeding. The risk for HIV infection through breastfeeding appears to be greatest in the first few months of life and is lower among infants who are fed breast milk exclusively than among those who are breastfed and also receive supplemental foods or liquids. In a recent study in South Africa, babies who were exclusively breastfed were significantly less likely to become infected in the first three months than were those who had mixed feeding.

The risk for HIV transmission from an individual woman to her child is affected by a variety of factors, including:

- **Stage of infection:** If a woman having a higher viral load, she will be more likely to transmit the virus to the child. Also, if she becomes infected during or just before pregnancy, the initial spike in viral load at the time of infection may increase the risk of MTCT.
- **Breastfeeding pattern:** Exclusive breastfeeding has been found to present a decreased risk over mixed feeding, which is the norm in many countries.
- **Duration of breastfeeding:** Risk increases with the duration of breastfeeding.
- **Oral or breast lesions:** Oral lesions in the baby or lesions on the mother's breasts increase the risk for transmission due to the increased portals of entry for the virus and exposure to blood.
- **Gastrointestinal illness:** A weakened gut may increase portals of entry for the virus found in breast milk in a baby who is breastfeeding.

- **Antiretroviral therapy:** This can significantly reduce the risk for MTCT during pregnancy and labor and delivery.
- **Cesarean section:** This can significantly reduce the risk for MTCT, but it is technically and financially intensive, and not necessarily realistic in resource-poor settings.
- **Invasive procedures:** Avoidance of invasive procedures during delivery can significantly reduce risk for MTCT.

It is important to note that all children born to HIV-positive women will test positive for HIV antibodies at birth, whether or not the children are actually infected. This is due to the presence of the mothers' antibodies in the children's blood. Antibody testing can accurately determine infection after the age of 18 months.⁴⁵

Diagnosis

The diagnosis of an HIV infection is normally made indirectly, i.e. through the demonstration of virus-specific antibodies by Enzyme Linked Immunosorbent Assay (ELISA). The antibodies are found in virtually 100% of those infected with HIV and constitute a marker of a humoral immune response against the agent. Cases in which individuals persistently fail to have detectable antibodies against HIV despite the presence of HIV infection are exceedingly rare and so far play little or no role in clinical practice.⁴⁶

Besides indirect diagnosis based on detection of antibodies, a direct diagnosis of HIV infection is also possible: either through the demonstration of

infectious virus (using cell culture this is only possible in laboratories of at least biological safety level 3), of viral antigens (p24 antigen ELISA) or of viral nucleic acid (i.e. viral genome); the latter is also termed nucleic acid testing (NAT). Viral genome detection is nowadays most often used, as it does not require a high security laboratory, is more sensitive than antigen detection and allows quantification.⁴⁶

To determine the infection status of a patient, direct virus detection by qualitative tests is only useful under certain circumstances, such as a suspected primary infection or in the case of babies born to HIV infected mothers. However, quantitative viral genome assays have gained great importance: The determination of the so- called "viral load", i.e. the concentration of viral RNA in plasma, has become an indispensable tool for guiding antiretroviral therapy. The term "HIV test" (still occasionally but inaccurately referred to as the "AIDS test"), however, almost always refers to testing for HIV-specific antibodies as a marker of infection.⁴⁶

Pathogenesis

In the vast majority of individuals, infection with HIV-1 results in a gradual yet sustained loss of CD4 + T cells and the consequent development of immune dysfunction and opportunistic infections that are the hallmark of AIDS. In most cases, progression to AIDS occurs after a prolonged period of clinical stability that lasts on average from 8 to 10 years. However, in a small number of people, disease progression occurs rapidly within 1 to 3 years after infection. Variation in the natural history of disease in humans reflects the complex roles of viral and host cell factors in HIV infection. These differences may emerge as a result of variation in the host immune response and differences in the cytopathicity or tropism of the virus.

Despite variation in the rate of clinical progression, infection with HIV commonly proceeds in three stages: (1) primary infection, (2) clinically asymptomatic infection, and (3) symptomatic disease progression. Each stage is associated with specific biologic and molecular changes in the virus.

Primary Infection

Primary infection with HIV-1 is marked by a period of explosive virus replication. Quantitative estimates of the viral burden during this time indicate that the levels of circulating HIV-1 may reach 10^7 particles/ml plasma, comparable to/or higher than that found in individuals with advanced AIDS. Despite profound viremia, some infected individuals remain clinically

asymptomatic, while others exhibit flu-like symptoms, including fever, chills, myalgia, rash, and gastrointestinal problems. Resolution of acute illness occurs within a few weeks after exposure to the virus and is associated with the development of HIV -1-specific antibodies and seroconversion. The levels of HIV-1 in the circulation also decrease dramatically during the period, suggesting that host immune mechanisms effectively control virus replication and diminish viral burden.⁴⁷

Controlling HIV -1 viremia during primary infection involves both the humoral and cellular arms of the immune system. Antibodies to HIV-1 may contribute to reducing the levels of circulating virus either directly by virus neutralization, or indirectly by the formation of immune complexes that are subsequently cleared by the reticuloendothelial system. HIV-1 specific cytotoxic T lymphocytes (CTLs) may act in concert to eliminate HIV -infected cells and suppress further replication by secretion of antiviral cytokines.⁴⁷

In most individuals, the viral load in plasma stabilizes following acute infection. This plateau varies considerably from person to person but generally ranges from 10^3 to 10^5 viral RNA copies/ml plasma. The steady-state level of HIV-1 in the plasma after primary infection is predictive of the rate of disease progression. Plasma HIV RNA levels exceeding 10^5 copies/ml after seroconversion is a strong predictor of rapid progression to AIDS, while levels of HIV-1 RNA below 10^3 copies/ml is associated with a more stable clinical course.⁴⁷

Asymptomatic Infection

With the resolution of acute illness, many individuals enter an asymptomatic period of variable duration characterized by low levels of HIV-1 antigens in the peripheral blood and relatively few clinical manifestations; however, it is now evident that clinical latency does not equate with viral latency. Although this phase may last for many years, immune function continues to deteriorate progressively. This apparent paradox has raised questions concerning the relationship between viral load and immune status during this stage of infection. The viral burden in the peripheral blood and lymph nodes during asymptomatic infection is now recognized to be several orders of magnitude higher than was previously believed. The frequency of circulating CD4+T cells containing HIV -1 DNA is estimated to range from 1 in 1,000 to 1 in 100,000 cells in asymptomatic individuals, while in AIDS patients the frequency can easily rise to 1 in 100 cells. HIV-1 RNA is found in the plasma at all stages of infection, providing clear evidence that virus replication is not truly latent within the host.⁴⁷

Treatment of infected individuals with anti-HIV drugs during this stage results in a dramatic reduction in the amount of free virus in the circulation and a rise in the number of CD4+T cells. Clearance of virus reflects the rapid removal of circulating virions, coupled with the destruction of infected CD4+T cells.⁴⁷

The number of circulating CD4+T cells is maintained, by the immune system by replenishing the CD4 + cell population, leading to a constant rapid turnover of cells and virus. The fact that most asymptomatic patients maintain

fairly stable CD4+T-cells counts for prolonged periods in the face of such extensive virus replication reflects the resiliency of the immune system in supplying new CD4 + cells to the existing pool. Although this balance may be maintained for many years, there is ultimately a progressive loss of CD4 + T cells and subsequent deterioration of immune function.⁴⁷

Symptomatic Disease Progression

Declining CD4+T-cell counts accompanied by frequent opportunistic infections generally signal the onset of AIDS. In some patients, CD4+T-cell counts decline gradually throughout the course of infection, while others experience a sharp drop after a period of relative stability. Longitudinal analyses demonstrate that the levels of virus present in the peripheral blood increase prior to the onset of AIDS, indicating that viral load in vivo is directly related to the immunologic and clinical deterioration of the patients. Viral isolates obtained during this period replicate rapidly in culture, and in about 50% of patients this is accompanied by a switch to a more cytopathic, syncytium-inducing viral phenotype.⁴⁷

Diversity within the viral genome increases throughout infection, reach infection reaching a plateau at the time of significant CD4 + T-cell depletion. Even during clinically asymptomatic infections patients may harbour up to 1 million genetically distinct variants. Phylogenetic studies show that throughout the course of infection waves of new viral quasispecies appear, only to be eliminated and replaced by new populations. It is likely that disease

progression is linked to the emergence of variants with increased virulence or, alternatively, variants that can escape detection by the immune system.⁴⁷

In addition to depletion of CD4+T cells, HIV-1infected individuals exhibit defects in other arms of the immune system. B cells isolated from AIDS patients are often in a state of chronic activation leading to hypergammaglobulinemia and elevations in serum, β_2 -microglobulin and neopterin levels. Cytokine profiles are also altered, leading to increased levels of interleukin-6 (IL-6) and tumor necrosis factor- α (TNF- α) in the plasma of AIDS patients. These cytokines have been shown to up-regulate HIV-1 expression *invitro* and may act via autocrine/paracrine pathways to increase expression of the virus in infected cells and to modulate activation of other immune cells. Despite declining numbers of CD4 + T cells, CD8 + T-cell counts may rise as disease progresses, leading to an inversion of the normal CD4/CD8 cell ratio. However, in the absence of CD4+T-cell help, HIV-1-specific cytotoxic activity mediated by CD8+ T cells is often significantly reduced or absent in AIDS patients.⁴⁷

Treatment

There is currently no vaccine or cure for HIV or AIDS. Current treatment for HIV infection consists of highly active antiretroviral therapy, or HAART. This has been highly beneficial to many HIV-infected individuals since its introduction in 1996, when the protease inhibitor-based HAART initially became available.⁴⁸

Current HAART options are combinations (or "cocktails") consisting of at least three drugs belonging to at least two types, or "classes," of anti-retroviral agents. Typically, these classes are two nucleoside analogue reverse transcriptase inhibitors (NARTIs or NRTIs) plus either a protease inhibitor or a non-nucleoside reverse transcriptase inhibitor (NNRTI). Because AIDS progression in children is more rapid and less predictable than in adults, particularly in young infants, more aggressive treatment is recommended for children than adults. Doctors assess their patients thoroughly: measuring the viral load, how fast CD4 declines, and patient readiness. They then decide when to recommend starting treatment. ⁴⁸

HAART allows the stabilization of the patient's symptoms and viremia, but it neither cures the patient, nor alleviates the symptoms. Moreover, it would take more than a lifetime for HIV infection to be cleared using HAART. Despite this, many HIV-infected individuals have experienced remarkable improvements in their general health and quality of life, which has led to a large reduction in HIV-associated morbidity and mortality in the developed world. ⁴⁸

HEPATITIS B VIRUS

History

Clinical and epidemiological studies to differentiate among various types of acute hepatitis began after World War II. The groundbreaking studies of Krugman and colleagues in 1967 firmly established the existence of at least two types of hepatitis, one of which (then called serum hepatitis, and now called hepatitis B) was parenterally transmitted. Links to the virus responsible for this form of hepatitis were derived by serologic studies conducted independently by Prince and colleagues ⁴⁹ and by Blumberg and colleagues. ⁵⁰ Blumberg and colleagues, searching for serum protein polymorphisms linked the diseases, to an antigen (termed Au) identified in serum from patients with leukemia, leprosy, and hepatitis, though the relationship of this antigen to hepatitis was initially unclear. By systematically studying patients with transfusion associated hepatitis, Prince and coworkers independently identified an antigen, termed SH, that appeared in the blood of these patients during the incubation period of the disease, and further work established that Au and SH were identical. ⁵¹ The antigen represented the hepatitis B surface antigen (HBsAg). ⁵² These seminal studies made possible the serologic diagnosis of hepatitis B and opened up the field to rigorous epidemiologic and virologic investigation.

Epidemiology

Global Scenario

Hepatitis B virus (HBV) infection is a major global public health problem. Of the approximately 2 billion people who have been infected worldwide, more than 350 million are chronic carriers of HBV.⁵³ HBV infection accounts for 500 000 to 1.2 million deaths each year and is the 10th leading cause of death worldwide.

The prevalence of HBV and its patterns of transmission vary throughout regions of the world, with about 45% of the world's population living in areas of high endemicity, defined as areas where 8% of the population has a positive test for the HBsAg (the main surface antigen of the HBV viral envelope) the areas include sub-Saharan Africa, the Pacific, and particularly Asia. Additionally, another 43% of the world's population lives in areas of moderate endemicity (i.e. 2%-7% of the population is HbsAg positive) the areas include southern parts of Eastern and Central Europe, the Amazon basin, the Middle East, and the Indian subcontinent. Only 12% of the world's population lives in areas of low endemicity (i.e., <2% of the population is HbsAg positive), the areas include western and northern European countries and North America. The map below outlines these areas.⁵³

Indian Scenario

Prevalence of hepatitis B surface antigen (HBsAg) in India varies from 1 to 13 per cent, with an average of 4.7 per cent.⁵⁴ High prevalence rates of HBsAg have been noted among the Indian tribal population. In similar study, Joshi SH et al found 2.99 to 21.54 per cent HBsAg carrier rate among the various tribes in Madhya Pradesh.⁵⁵ Further in a study conducted by Qamer S et al in children, overall 4.35% of the 460 children were tested HBsAg positive.⁵⁶ There are only a few community-based studies that have looked into the prevalence of hepatitis B infection. In one such study by Chowdhury A, et al a high HBsAg prevalence of 5.3% was observed in rural population.⁵⁷

Classification

Hepatitis B virus (HBV) is the prototype member of the Hepadnaviridae (hepatotropic DNA virus) family. Hepadnaviruses have a strong preference for infecting liver cells, but small amounts of hepadnaviral DNA can be found in kidney, pancreas, and mononuclear cells. However, infection at these sites is not linked to extra hepatic disease.⁵⁸

Viral Structure, Antigen and Genomic Organization

HBV virions are double-shelled particles, 40 to 42 nm in diameter, with an outer lipoprotein envelope that contains three related envelope glycoproteins (or surface antigens). 15 Within the envelope is the viral nucleocapsid, or core. The core contains the viral genome, a relaxed-circular, partially duplex DNA of 3.2 kb, and a polymerase that is responsible for the synthesis of viral DNA in infected cells. In addition to virions, HBV-infected cells produce two distinct sub viral lipoprotein particles: 20-nm spheres and filamentous forms of similar diameter. ⁵⁹

The HBV genome is relaxed circular species, with circulatory being maintained by hydrogen bonding of complementary 5' cohesive termini of approximately 250 base pairs. 56-58 One strand of the DNA (minus strand) is unit length (3.3kb). Its complement (the plus strand) is less than the unit length; the 5' end of the plus strand is fixed but its 3'end can be anywhere along the genome, even in the virions derived from infection by single cloned genome. ⁵⁹

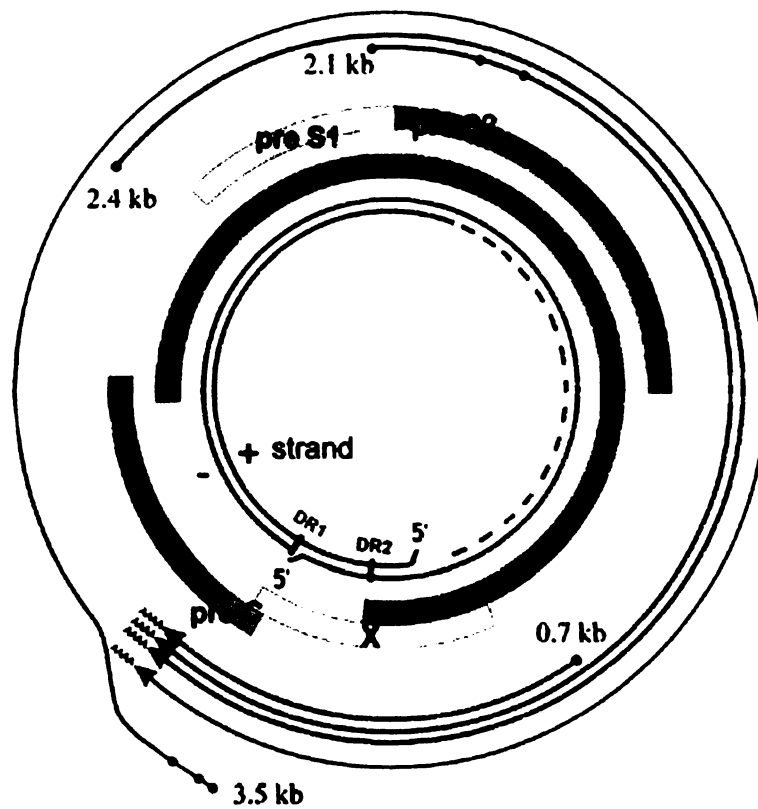
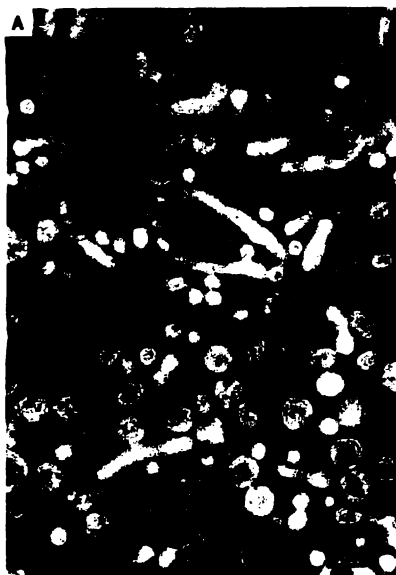
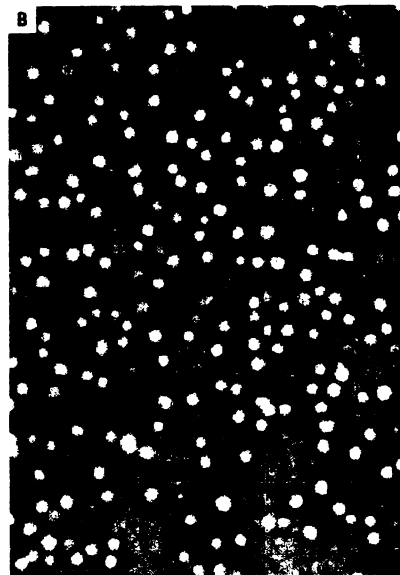


Figure no.5: Diagrammatic representation of Hepatitis B Virus genome



Picture A



Picture B

Figure no. 6: Structure of HBsAg-Associated Particles (Phosphotungstic Acid–Negative Stain). Picture A shows HBV virions (Dane particles) and filaments.

Picture B shows 20-nm HBsAg particles.

The genome has four open reading frames (ORF); they encode the nucleocapsid the envelope and the X and polymerase proteins. The nucleocapsid ORF has two in phase-phase start codons that allow the translation of two closely related polypeptides. The first start codon defines the precore polypeptide and is secreted as hepatitis B e Ag (HBeAg). The HBeAg itself is not required for viral replication in fact, naturally occurring mutant of hepatitis B viruses have been described that do not synthesize HBeAg due to precore stop codon. The second start codon in the nucleocapsid ORF defines 183 amino acid of the hepatitis B virus antigen (HBcAg). Nucleocapsid particles traverse two different pathways inside a cell, one of which transports the viral genome to the

cell nucleus while the other transports it to the endoplasmic reticulum (ER), where the nucleocapsid particle binds to the viral envelope proteins and mature into complete virion to be secreted from the cell. Apart from this important role in the viral life cycle, HBcAg may exert an indirect proviral function by inhibiting the transcription of the antiviral cytokine beta interferon in the infected cell.⁵⁶

The envelope ORF contains three in phase start codons that separate the preS1, PreS2 and S sequences. The product of the S gene is small envelope protein which is also designated the major envelope protein due to its abundance in the serum. The product of the combined preS2 and S gene is the middle envelope protein and the sequence of the pre-S1 and ,pre-S2 and S-gene code for the large envelope protein. While the small and the middle-envelope proteins are present in all viral and sub viral envelope particles, the large-envelope protein is preferentially present in the sub viral filaments and virions.⁵⁸

The polymerase ORF encodes the longest HBV polypeptide. The 832 - amino acid HBV polymerase protein, which is translated by internal ribosomal initiation of the 3.5-kb core mRNA contains reverse transcriptase, DNA-dependent DNA polymerase, and RNase H activity. Polymerase not only plays a role in viral encapsidation but also contains both the primer and the enzyme for reverse transcription.⁵⁸

The X ORF encodes a 154-amino acid protein. The X protein is found in the cytoplasm of infected cells and functions as a transcriptional transactivator of other viral and cellular promoters.⁵⁸ It exerts this function in an indirect way by

binding and activating cellular transcription factors, which modifies their ability to bind to regulatory elements.

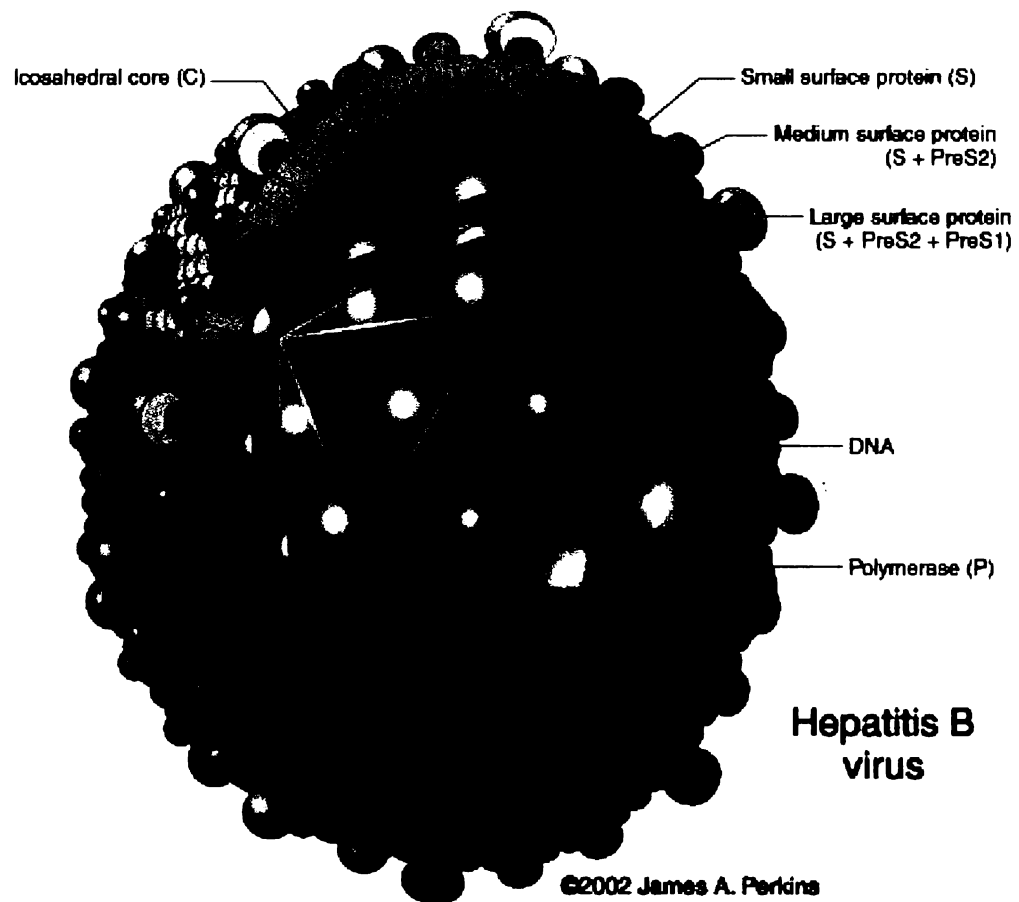


Figure no. 7: Diagrammatic representation of Hepatitis B Virus

Genotypes

DNA sequence of HBV isolates has shown the existence of 8 viral genotypes A-H and these vary in geographic distribution. Genotype A is found primarily in North America, Northern Europe, India, and Africa. Genotype B and C are common in Asia; genotype D, in southern Europe, the Middle East, and India; genotype E, in West Africa and South Africa; genotype F, in South and Central America; genotype G, in the United States and Europe. Genotype H was recently identified in individuals from Central America and California. Several genotypes may be associated with the severity of the disease but the relationship between the genotype and the developing hepatocellular carcinoma has not been established. In China and Japan, some studies have found more severe liver disease to be associated with genotype C than compared with genotype B; other studies have found no such association. There is some evidence that shows HBeAg seroconversion occurs at a younger age among individuals infected with genotype B. Genotype D has been associated with anti-HBe-positive chronic hepatitis B infection in the Mediterranean region.⁶⁰

Replication

The cardinal feature HBV replication is the replication of the DNA genome by reverse transcription of RNA intermediate. Incoming HBV virions are bound by cell-surface receptors, the identity of which remains unknown.⁵⁹

Inside the cell, HBV plus-strand DNA synthesis is completed within the nucleocapsid particle, which transports the viral genome towards the cell

nucleus. Since nucleocapsid particles cannot enter through the small nuclear pores, it has recently been suggested that the open circular viral genome is released by nucleocapsid disassembly on the cytoplasmic face of the nuclear membrane, whereupon it enters the nucleus alone. There, the DNA genome is converted to a covalently closed circular (ccc) form, which it serve as the transcriptional template for host RNA polymerase II. This enzyme generates a series of genomic and sub genomic transcripts. All viral RNA is transported to the cytoplasm, where its translation yields the viral envelope, core, and polymerase proteins, as well as the X and preC polypeptides. Next, nucleocapsid proteins are assembled in the cytosol, and during this process a single molecule of genomic RNA along with viral polymerase is incorporated into the assembling viral core.⁵⁹

Once the viral RNA is encapsidated, reverse transcription begins. The synthesis of the two viral DNA strands is sequential. The first DNA strand is made from the encapsidated RNA template; during or after the synthesis of this strand, the RNA template is degraded and the synthesis of the second DNA strand proceeds, with the use of the newly made first DNA strand as a template. Some cores bearing the mature genome are transported back to the nucleus, where their newly minted DNA genomes can be converted to covalently closed circular DNA (cccDNA) to maintain a stable intranuclear pool of transcriptional templates. Most cores, however, bud into regions of intracellular membranes bearing the viral envelope proteins. In so doing, they acquire lipoprotein envelopes containing the viral L, M, and S surface antigens and are then exported from the cell.⁵⁹

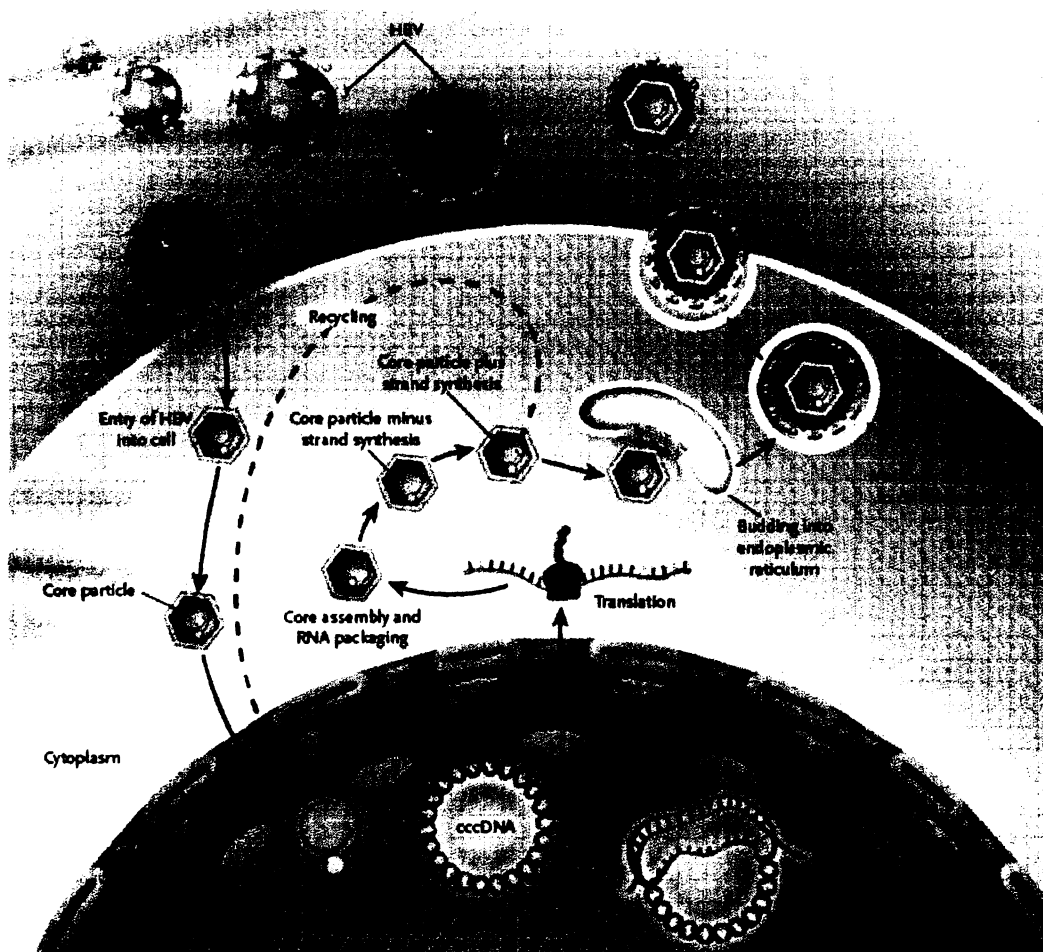


Figure No. 8: The Replication Cycle of Hepatitis B virus

Transmission

HBV is parentally transmitted virus that is acquired from blood or blood products, sexual contact or perinatal exposure.⁵⁹

Worldwide, HBV is most prevalent in people born in regions of high endemicity, and their descendants. Infants born to HBeAg positive mothers who have high levels of viral replication have a 70% to 90% risk of perinatal acquisition. In addition children of HBsAg –positive mothers who do not get infected at birth remain at high risk of early childhood infection.⁵⁹

Sexual transmission is the single most important mode of HBV transmission in areas of the world, where the prevalence is low. In sexual transmission, factors associated with increased risk of HBV infection include multiple sexual partners, anal receptive intercourse, and duration of sexual activity. Sexual partners of injectible drug users, prostitutes and clients of prostitutes are at particularly high risk for infection.⁵⁹

In Western Europe and US injectible drug use remains a very important mode of HBV transmission. Risk of infection increases with duration of use.⁵⁹

Diagnosis

Serological markers of HBV infection remain a corner stone in diagnosis as well as important gauge of viral activity and disease progression .The serological elements include three viral antigens and their corresponding

antibodies, generated by the host. The appearance and disappearance of these markers often herald important alterations in the clinical stage of disease.⁵⁸

During the acute hepatitis B, HBsAg is detectable in the serum and usually persist through early convalescence. As acute hepatitis resolves typically, HBsAg becomes undetectable and anti-HBs appear in the serum. Anti-HBs is a neutralizing antibody, the presence of which in high titre is associated with resolution of infection and correlates with immunity; it is the sole antibody produced in response to hepatitis B vaccination. Persistence of serum HBsAg is indicative of progression to chronic Hepatitis B.⁵⁸

HBcAg is a nonsecreted protein expressed on the linear nucleocapsid core of the virion and therefore is not found circulating freely in the serum. Antibodies to HBcAg are detectable in serum early in the course of infection and persist indefinitely. In a patient with HBsAg present in serum, the detection of IgM anti-HBc suggest acute hepatitis B, whereas the detection of IgG anti-HBc suggest chronic hepatitis B with superimposed non-B acute hepatitis.⁵⁸

Another very useful Hepatitis B serological marker is HBeAg, which usually becomes detectable in the serum when HBsAg first appears. The presence of HBeAg in serum correlates with the presence of viral replication in the liver. Antibody to HBeAg (anti-HBe) is detectable as HBeAg disappears from the serum. The presence of anti-HBe early during acute hepatitis B is associated with a high likelihood of spontaneous resolution of acute infection.⁵⁸

The adaptation of molecular techniques to HBV has proven to be of great utility in the diagnosis of infection as well as in monitoring responses during

treatment. The Polymerase chain Reaction has lent an extraordinary level of sensitivity to the detection of HBV DNA; this approach is capable of detecting as little as 10-50 genome equivalent per milliliter.⁵⁸

Pathogenesis

The HBV replication cycle is not directly cytotoxic to cells. This fact accords well with the observation that many HBV carriers are asymptomatic and have minimal liver injury, despite extensive and ongoing intrahepatic replication of the virus. It is now thought that host immune responses to viral antigens displayed on infected hepatocytes are the principal determinants of hepatocellular injury. This notion is consistent with the clinical observation that patients with immune defects who are infected with HBV often have mild acute liver injury but high rates of chronic carriage. The immune responses to HBV and their role in the pathogenesis of hepatitis B are incompletely understood. Correlative clinical studies show that in acute, self-limited hepatitis B, strong T-cell responses to many HBV antigens are readily demonstrable in the peripheral blood. These responses involve both major-histocompatibility-complex (MHC) class II-restricted, CD4⁺ helper T cells and MHC class I-restricted, CD8⁺ cytotoxic T lymphocytes. The antiviral cytotoxic T-lymphocyte response is directed against multiple epitopes within the HBV core, polymerase, and envelope proteins; strong helper T-cell responses to C and P proteins have also been demonstrated in acute infection. By contrast, in chronic carriers of HBV, such virus-specific T-cell responses are greatly attenuated, at least as assayed in

cells from the peripheral blood. However, antibody responses are vigorous and sustained in both situations (although free antibodies against HBsAg [anti-HBs antibodies] are not detectable in carriers because of the excess of circulating HBsAg). This pattern strongly suggests that T-cell responses, especially the responses of cytotoxic T lymphocytes, play a central role in viral clearance. The mechanisms by which cytotoxic T lymphocytes kills liver cells and cause viral clearance have been incisively investigated in transgenic mice that express viral antigens or contain replication-competent viral genomes in the liver.⁶¹

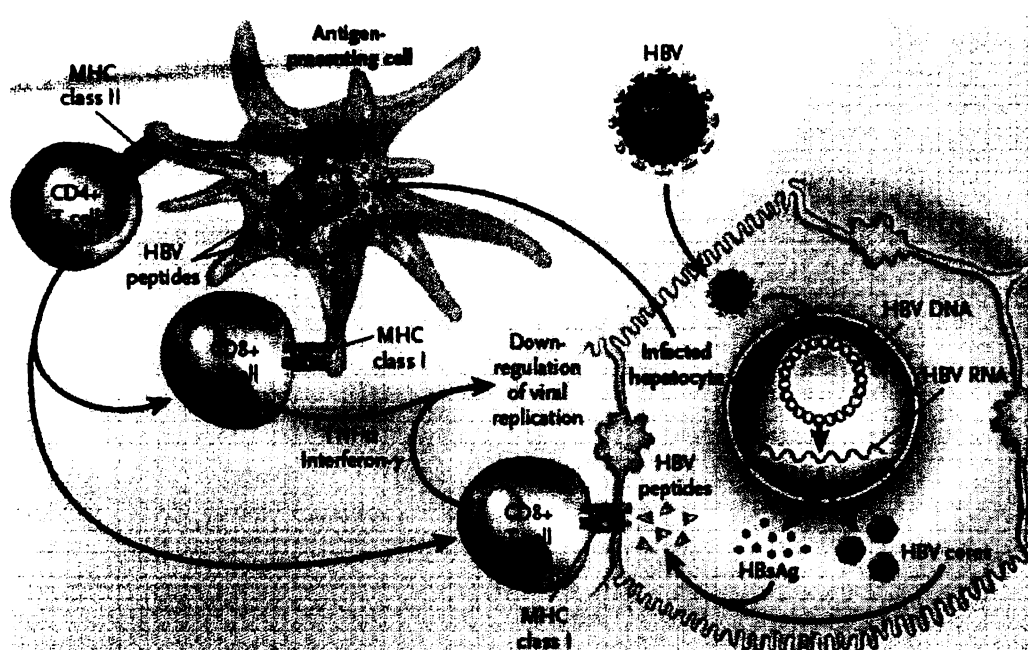


Figure no. 9: Cellular Immune Responses to Hepatitis B Virus



Clinical Manifestation

Primary HBV infection in susceptible (nonimmune) hosts can be either symptomatic or asymptomatic. The latter is more common than the former, especially in young children. Most primary infections in adults, whether symptomatic or not, are self-limited, with clearance of virus from blood and liver and the development of lasting immunity to reinfection. However, some primary infections in healthy adults (generally less than 5 percent) do not resolve but develop into persistent infections. In such cases, viral replication continues in the liver and there is continual viremia, although the titers of virus in the liver and blood are variable. Persistent HBV infection may be symptomatic or asymptomatic. People with subclinical persistent infection, normal serum aminotransferase levels, and normal or nearly normal findings on liver biopsy are termed asymptomatic chronic HBV carriers; those with abnormal liver function and histological features are classified as having chronic hepatitis B. Cirrhosis, a condition in which regenerative nodules and fibrosis coexist with severe liver injury, develops in about 20 percent of people with chronic hepatitis B. The resulting hepatic insufficiency and portal hypertension make this process one of the most feared consequences of chronic HBV infection.⁶¹

Primary Infection

In primary infection, HBsAg becomes detectable in the blood after an incubation period of 4 to 10 weeks, followed shortly by antibodies against the HBV core antigen (anti-HBc antibodies), which early in infection are mainly of the IgM isotype. Viremia is well established by the time HBsAg is detected, and titers of virus in acute infection are very high — frequently 10^9 to 10^{10} virions per milliliter. Circulating HBeAg becomes detectable in most cases, and studies of chimpanzees and other animals with primary hepadnaviral infection show that 75 to 100 percent of hepatocytes are infected when this antigen is evident. Thus, it is not surprising that epidemiologic studies consistently show high rates of both vertical and horizontal transmissibility during acute HBV infection.⁶¹

When liver injury does occur in primary infection, alanine aminotransferase levels do not increase until after viral infection is well established, reflecting the time required to generate the T-cell-mediated immune response that triggers liver injury. Once this response is under way, titers of virus in blood and liver begin to drop. The fact that infection can be cleared from virtually all hepatocytes without massive hepatic destruction (in most cases) is a testament to the extraordinary power of the non-cytolytic clearance mechanisms described above. With clearance of the infection, the viral antigens HBsAg and HBeAg disappear from the circulation, and free anti-HBs antibodies become detectable. Surprisingly, in self-limited infection, as defined by the disappearance of the viral antigens and the appearance of anti-HBs antibodies, low levels of HBV DNA in the blood may persist for many years, if not for life. It is not known

whether this DNA contains the entire HBV genome, or even whether it is contained in virions. However, inoculation of serum from three subjects with persistent HBV DNA into chimpanzees has not led to documented infectivity.⁶¹

Persistent Infection

In persistent HBV infection, the early events unfold as in self-limited infection, but HBsAg remains in the blood and virus production continues, often for life. However, levels of viremia in chronic infection are generally substantially lower than during primary infection, although they can vary considerably from person to person. High titers of HBV in the blood are often indicated by the continued presence of HBeAg. Typically, there are 10^7 to 10^9 virions per milliliter in the blood in such cases, which are highly infectious. But most people with persistent infection, especially those with anti-HBe antibodies, have somewhat lower levels of viremia. One feature of chronic HBV infection that is not widely appreciated is its dynamic natural history. Even though, in most cases, HBsAg remains detectable for life, titers of viral DNA tend to decline over time. With the passage of time, there is also a tendency for HBeAg to disappear from the blood, along with seroconversion to positivity for anti-HBe antibodies — a progression that occurs at a rate of 5 to 10 percent per year in persistently infected people.⁶¹

Often, the disappearance of HBeAg is preceded or accompanied by a transient rise in alanine aminotransferase levels, known as a flare, which suggests that the process reflects immune-mediated destruction of infected hepatocytes. Reductions in the level of viremia as great as five orders of

magnitude may accompany seroconversion to anti-HBe antibodies. Thus, the natural history of HBV persistence suggests that there is an ongoing immune attack on infected cells in the liver — an attack that is usually inadequate to eradicate infection altogether, but that does reduce the number of infected cells and thereby lowers the circulating viral load. The widely held view that circulating viral DNA disappears when anti-HBe antibodies appear is incorrect; this idea reflects the fact that, for many years, HBV DNA was measured by relatively insensitive hybridization methods with a detection limit of 10^5 to 10^6 virions per milliliter. Thanks to the advent of the polymerase-chain-reaction (PCR) method, we now know that at least 70 to 85 percent of people with anti-HBe antibodies have detectable viral DNA in the circulation, typically in the range of 10^3 to 10^5 molecules per milliliter, and sometimes higher. Although these levels of HBV DNA are relatively low, they are hardly negligible.⁶¹

Hepatocellular Carcinoma

Another feature of the natural history of HBV infection is its link to primary hepatocellular carcinoma. Chronically infected subjects have a risk of hepatocellular carcinoma that is 100 times as high as that for noncarriers; within the HBsAg-positive group, HBeAg-positive carriers have the highest risk of hepatocellular carcinoma, but even carriers with anti-HBe antibodies have a substantial risk of cancer. (Although the role of HBV in provoking hepatocellular carcinoma is undisputed, its cellular and molecular mechanisms remain incompletely understood.) Given these facts, twice a year screening of

chronically infected patients with measurements of serum alpha fetoprotein or hepatic ultrasonography, or both, is warranted. However, there is debate as to when such screening should begin. Furthermore, screening is imperfect — alpha fetoprotein screening, for example, has an excellent negative predictive value, but its positive predictive value ranges from 9 to 30 percent.⁶¹

Treatment of HBV

There are currently several treatments for chronic hepatitis B that can increase a person's chance of clearing the infection. Treatments are available in the form of antivirals such as lamivudine and adefovir and immune system modulators such as interferon alpha (Uniferon). There are several other antivirals under investigation. Roughly, all of the currently available treatments, when used alone, are about equally efficacious. However, some individuals are much more likely to respond than others. It does not appear that combination therapy offers any advantages.⁶² In general, each works by reducing the viral load by several orders of magnitude thus helping a body's immune system clear the infection.

Treatment strategies should be individualized by a doctor and patient. Considerations include the risks associated with each treatment, a person's likelihood of clearing the virus with treatment, a person's risk for developing complications of persistent infection, and development of viral resistance with some of the treatments.

On October 27, 2006 telbivudine gained FDA approval for chronic treatment of chronic hepatitis B. Its side effects profile is generally less than that of other anti-virals in comparison, especially lamivudine. It is marketed under the brand name Tyzeka in the US and Sebivo outside the US. It is already approved in Switzerland.⁶³⁰

Chronic carriers should be strongly encouraged to avoid consuming alcohol as it increases their risk for cirrhosis and hepatocellular carcinoma (liver cancer). Infants born to mothers known to carry hepatitis B can be treated with antibodies to the hepatitis B virus (hepatitis B immune globulin or HBIG). When given with the vaccine within twelve hours of birth, the risk of acquiring hepatitis B is reduced by 95%. This treatment also allows a mother to safely breastfeed her child.⁶³

An individual exposed to the virus who has never been vaccinated may be treated with HBIG immediately following the exposure. For instance, a health care worker accidentally stuck by a needle used in a hepatitis B carrier would qualify.⁶³

HEPATITIS C VIRUS

History

The story of hepatitis C began a little more than 2 decades ago, when researchers transmitted non-A, non-B hepatitis from patients with transfusion-associated hepatitis to chimpanzees, demonstrating that the disease resulted from a transmissible agent. A major breakthrough came in 1989 with the cloning of the hepatitis C virus (HCV) genome by Choo et al.⁶⁴ Shortly after the discovery of HCV, it became apparent that this newly identified virus is the principal causal agent for non-A, non-B hepatitis.

Epidemiology

Global Scenario

Hepatitis C virus (HCV) continues to be a major disease burden on the world. In 1999, the WHO estimated a worldwide prevalence of about 3% with the virus affecting 170 million people worldwide.⁶⁵ The prevalence of hepatitis C is not well documented in many countries, partly due to the expense and practical difficulties involved in the detection of HCV RNA in the serum of infected individuals. In Europe, general prevalence of HCV is about 1% but varies among the different countries.⁶⁶ In the United Kingdom, at least 200,000 adults carry HCV.⁶⁷ Most populations in the Americas, Europe, and South-East Asia have HCV prevalence rates of under 2.5%. In the Western Pacific regions and parts of South America, prevalence rates are higher - between 2.5-4.9%. Recently, HCV

prevalence studies have come out of Pakistan in the Middle East. 751 out of 16,400 patients (4.57%) were found to +HCV Ab from 1998-2002 with the largest age group from 41-50. ⁶⁸ Among male blood donors in Karachi, Pakistan, the seroprevalence of HCV was 1.8% with a trend of increasing proportion of positive donors from 1998-2002. ⁶⁹

Indian Scenario

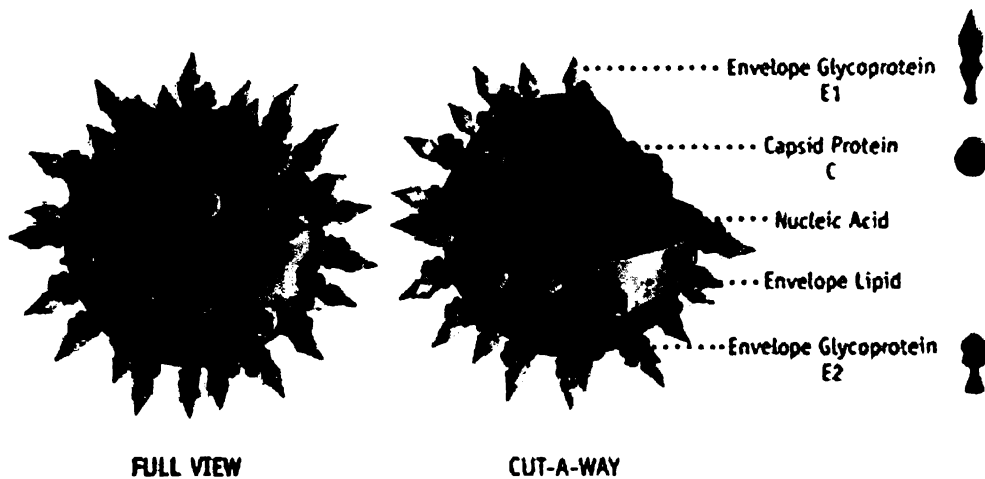
The prevalence of and risk factors for HCV infection are not well characterized in most developing countries. The World Health Organization estimates that there are 10–24 million HCV-infected persons living in India. Estimates of the prevalence of HCV infection in India vary from <1% among voluntary blood donors to 190% among injection drug users (IDUs). ⁷⁰ In a community based study in West the HCV prevalence was 0.87%. ⁷¹

Hepatitis C Virus Biology

HCV is a small virus, with an estimated diameter of 40 and 50nm and has a lipid –containing envelope. HCV virions have not yet been well characterized due to the low titer of circulating free virions and the lack of an efficient cell culture system. However, recent reports have identified viral particles by immune electron microscopy, where they appear as 55-65nm spherical particles with fine spike like structures projecting from the external envelope, as 3-nm core particles, or as 50nm particles within cytoplasmic vesicles of HCV-infected Daudi

cells, released after detergent treatment of virions purified from infectious plasma.⁷²

MODEL OF THE HUMAN HEPATITIS C VIRUS



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Published in The PRN Notebook, Volume 6, Number 1, March 2001 and The PRN Notebook Online at www.prn.org
Three-dimensional model of HCV created by Louis E. Henderson, Ph.D., Frederick Cancer Research Center

Figure no. 10: Diagrammatic representation of Hepatitis C Virus

Classification

HCV has little overall nucleotide or amino acid homology between HCV and any other known viral sequence, thus indicating that HCV is a unique viral pathogen. However, accumulated data show that HCV shares partial sequence homology and has a very similar genomic organization with members of the family Flaviviridae, which includes the human flaviviruses (i.e. yellow fever virus and dengue virus, among others) and animal pestiviruses (hog cholera virus and bovine viral diarrhoea virus). Because of these remarkable similarities, HCV has been proposed as a new third genus within the family Flaviviridae.⁷²

Viral Structure, Antigen and Genomic Organization

HCV is a positive RNA virus with a genome containing approximately 9500 nucleotides. It has an open reading frame that encodes a large polyprotein of about 3000 amino acids. When cleaved by viral and host enzymes this polyprotein produces 10 polypeptides. The genomic organization of HCV consists of a 5' untranslated region (5' UTR) of about 340 base pairs and downstream to the 5' UTR there are regions that encode the following proteins: "core", envelope 1, envelope 2, p7, non-structural region 2, non-structural region 3 (NS3), non-structural regions 4A and 4B, and non-structural regions 5A and 5B (NS5A and NS5B). Downstream to this coding region there is another untranslated region of approximately 200 nucleotides (3' UTR). The 5' UTR is highly conserved and therefore is the target for diagnostic assays. It has a

complex secondary structure and is the putative internal ribosome entry site, with an RNA sequence that directs the ribosomes to initiate translation.⁷³

The HCV core has RNA binding capacity and appears to be associated with the HCV genome. It has been suggested that this protein induces hepatic steatosis in transgenic mice and is an important factor in the development of hepatocellular carcinoma in patients with chronic HCV infection. Serum quantification of the core protein by ELISA correlates with serum levels of HCV RNA. HCV envelope 1 and envelope 2 proteins have molecular masses of approximately 31 and 70 kDa, respectively. They interact with each other and may be a heterodimer. The envelope 1 region is used for genotyping for clinical purposes. The first 81 nucleotides of the envelope 2 region are thought to be the hypervariable region 1 (HVR1) of HCV. HVR1 appears to induce the production of neutralizing antibodies and could function as a decoy to help the virus escape the immune system. HCV envelope 2 is thought to have binding sites for CD81, a protein expressed in the membranes of mammalian cells that is implicated in the entry of HCV into the hepatocyte.⁹¹⁻⁹³ NS3 protein has protease and helicase activities. Its helicase activity participates in the process of RNA unwinding during replication. The NS5A region has a particular nucleotide sequence that may determine the sensitivity of the virus to interferon therapy (interferon sensitivity determination region). Nevertheless, other investigators have reported discordant results and this issue is controversial. The NS5B region has motifs characteristic of an RNA-dependent RNA polymerase, an enzyme responsible for viral replication.⁷³

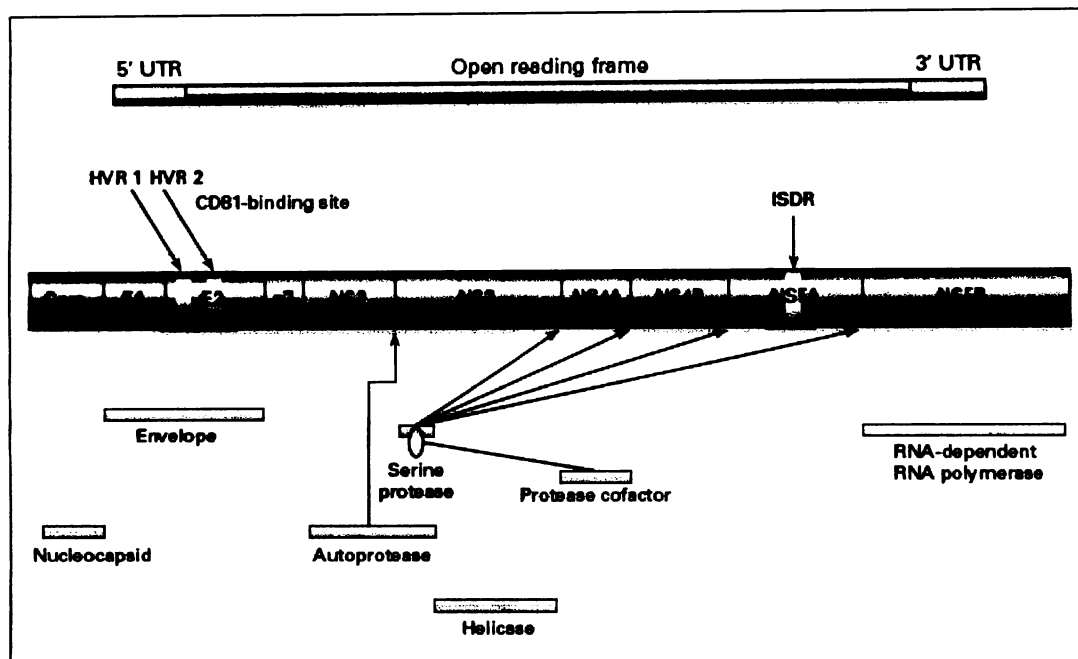


Figure no. 11: Diagrammatic representation of Hepatitis C Virus Genome

Genotypes

HCV is divided among six genotypes with numerous subtypes. These genotypes can differ up to 30% from each other in nucleotide sequence. Depending on the HCV genotype, length of treatment can differ. Genotype 1b is less responsive to alpha-interferon therapy compared to genotypes 2 and 3. It is therefore important to track the different genotypes of the HCV virus. In the NHANESIII study done in the US, 56.7% were classified as 1a, 17% as 1b, 3.5% as 2a, 11.4% as 2b, 7.4% as 3a, 0.9% as 4, 3.2% as type 6. 50% of all infections were by genotype 1 with a higher percentage of genotype 3 among the IVDU population in England. Genotype 1b was the predominant genotype (46% among blood donors) in Chile and was found in all infected patients with hepatocarcinoma in one study. This same genotype was also found in 82% of 147 chronic hepatitis C patients in Chile as well. Genotype 1b is also dominant in Japan. In Beijing, China, of 63 HCV-RNA samples, 52% were genotype 2 and 29% type 3. In Thailand, HCV 3a was the most common genotype at 50-60% with 1a, 1b, and 6 comprising the rest (10-20% each). Out of 90 patients in Estonia, 73.3% carried 1b, 20% with 3a, and 6.7% with 2a. Genotype 3 is most common on the Indian subcontinent while genotype 4 is the most common genotype in Africa and the Middle East. Genotype 5 can be found in South Africa and as mentioned above, genotype 6 can be found in South-East Asia. ⁷⁴

Replication

HCV binds with high affinity to the large external loop of CD81, a tetraspanin found on the surface of many cell types including hepatocytes.¹⁰¹ However, CD81 is not sufficient to mediate cell entry and several cofactors appear to be required. The low-density lipoprotein receptor (LDLR)¹⁰² and scavenger receptor class B type I (SR-BI) have, among others, been proposed as components of a putative HCV receptor complex.⁷³

As in most of the positive-strand RNA viruses investigated, HCV forms a membrane associated replication complex, composed of viral proteins, replicating RNA, altered cellular membranes and additional host cell factors.⁷⁰

The viral genome of the virus functions as an mRNA in the infected cells. Viral proteins are initially translated in an HCV-infected cell from genomic RNA. Translation of an HCV polyprotein proceeds in an internal ribosome entry site-independent manner. As all the viral proteins are likely to be produced from a single genomic ORF, posttranslational regulation of viral proteins is paramount in regulating levels of specific virus proteins during replication.⁷³

During the replication process, HCV needs to synthesize a negative-stranded RNA to use as a template for generating progeny virions RNAs. The mechanism of negative-stranded RNA synthesis from a positive-stranded viral genome is not well understood. The switch to translation or assembly and the release of viral particles are still poorly understood.⁷³

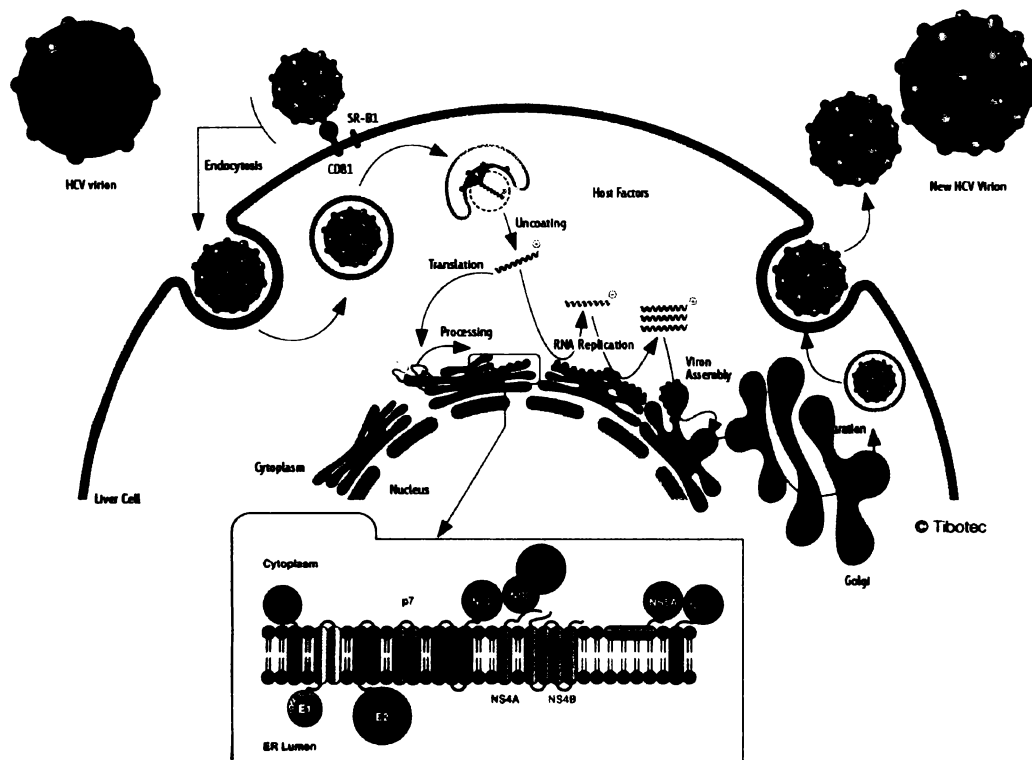
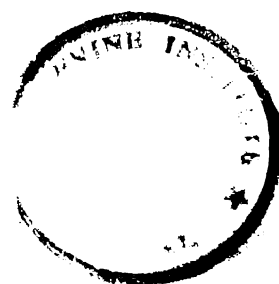


Figure no. 12: Hepatitis C Virus Life Cycle



Transmission

The greatest risk for transmission of HCV is from sharing drug-injecting equipment, which accounts for 80 per cent of the cases.

Vertical transmission has been recorded in fewer than 10 per cent of pregnant women, with the risk much higher if the mother contracts HCV during pregnancy. There is some evidence that HCV can be transmitted sexually: gay men have higher prevalence rates of HCV than blood donors. However, attempts to estimate the risks of sexual transmission are inconclusive.

Both vertical and (hetero) sexual transmission of HCV are more common in people co-infected with HIV. This might have implications for health promotion aimed at people with HIV.

Other possible routes of infection are by household routes, such as sharing razors or toothbrushes.⁷⁵

Diagnosis of HCV

HCV is diagnosed serologically by detecting specific antibodies using the enzyme-linked immunosorbent assay (ELISA). Early tests were unreliable, but second and third generation ELISAs have reduced false positives

As with HIV, there can be a three- to six-month 'window period' following HCV infection, before seroconversion occurs, and during this time people might not test positive for HCV antibodies. The ELISA test cannot determine whether or not someone is a carrier or if the disease has run its course and the antigen is no longer present.

Reverse-Transcriptase Polymerase chain reaction (RT-PCR) testing for the virus is not widely available, is expensive. It can be used for confirmation where ELISA tests are indeterminate; for early diagnosis of acute hepatitis; and for monitoring perinatal transmission and follow up of antiviral drug treatment.⁷⁶

If there is evidence of active hepatitis C, liver ultrasound and a liver biopsy are usually indicated, although these investigations are not indicated for those people with asymptomatic or non-active HCV, as they are not necessary.⁷⁷

Pathogenesis

HCV infection causes acute and chronic hepatitis. Acute hepatitis is followed by chronic hepatitis in about 50% of cases, and 20% of chronic hepatitis cases will develop cirrhosis and hepatocellular carcinoma. The incubation period before the development of acute hepatitis is about 5 to 12 weeks. A significant number of cases however, are asymptomatic. Cirrhosis may develop in chronic hepatitis patients as early as 1.5 years after infection by transfusion. Presence of HCV antibodies and hepatocellular carcinoma are strongly associated.⁷⁸

Hepatitis C virus can establish persistent infection despite an active humoral and cellular immune response that is generally targeted against all viral proteins. The virus may escape from the humoral immune response if the kinetics of infection and viral replication do not allow complete neutralization of the virus by HCV-specific antibodies after primary infection. Although virus-specific antibodies may interfere with viral entry into host cells and opsonize the virus for elimination by macrophages, they cannot eliminate HCV from infected

cells. In addition, HCV has a high mutation rate, especially in the hypervariable region of the envelope proteins that can be recognized by neutralizing antibodies (antibodies that can bind and eliminate virus). Several studies have demonstrated that the humoral immune response can select HCV variants with sequence changes that allow escape from antibody recognition. Thus, progression to persistent HCV infection is most likely a multifactorial process that depends on multiple aspects of virus– host interaction.⁷⁹

The cellular immune response probably plays an important role in the outcome of HCV infection because of its ability to recognize and eliminate virus from infected cells. Most studies have concentrated on the antigen-specific immune response that is mediated by CD4-positive helper T cells and CD8-positive cytotoxic T cells. Because chronic rather than acute infection is diagnosed in most patients, immunologic studies have been performed on patients with persistent infection who could not clear HCV. Only a few studies have analyzed the cellular immune response during the acute phase of infection. These studies suggest that the strength and quality of both helper T-cell and cytotoxic T-cell responses differ between patients who recover and those who develop chronic infection. More important, the viral sequences that are recognized most frequently and vigorously by HCV-specific T cells vary little among all the HCV genotypes.⁷⁹

Furthermore, several of these frequently recognized viral peptides bind with high affinity to many different class II MHC molecules, suggesting that they can be efficiently presented and recognized by patients with different MHC

haplotypes. Thus, these viral sequences could be explored for development of preventive or therapeutic vaccines against HCV.⁷⁹

The cellular response against HCV could be interfered with in several ways. First, HCV elicits only a weak T-cell response in patients who develop chronic infection. In the blood of patients with chronic hepatitis C, the frequency of cytotoxic T-cell precursors that are specific for individual HCV peptides is much lower than the frequency of T cells that recognize an influenza virus peptide as a recall antigen or peptides of other viruses that can be cleared, such as cytomegalovirus. The reasons for this relative weakness of the cellular immune response are not known. Certainly, general immune tolerance or immunosuppression is not the cause of persistent HCV infection, because most chronically infected patients display normal immune responses against other viral agents. The emergence of viral mutants or quasi-species with sequence variations in T-cell epitopes may contribute to the apparent ineffectiveness of cell-mediated immune response. There is also increasing evidence that several HCV proteins, such as core, E2, and NS5A, interfere with the immune response. Furthermore, infected hepatocytes, which lack co-stimulatory molecules, may be relatively inefficient in priming the immune system, and the liver has been proposed as the major site where activated T cells are destroyed.⁷⁹

Finally, the cellular immune response is a double-edged sword. An immune response that is ineffective in clearing HCV infection may be more harmful to the liver, causing chronic inflammation, hepatocellular injury, and, over several decades, liver fibrosis and cirrhosis. Progression to persistent infection

and the immunologic mechanisms of liver injury are the consequence of complicated interactions between the virus and host. Identification of immunologic correlates of viral clearance may contribute to the development of an effective vaccine and better therapy for HCV infection.⁷⁹

Clinical Manifestation

Hepatitis C infection causes acute disease that is often mild or symptomatic, but a high tendency exists for the infection to persist and lead to serious chronic liver disease. The clinical expression of HCV infection depends on the stage of liver disease (i.e. acute hepatitis, chronic hepatitis, cirrhosis or end-stage chronic liver disease). HCV may also produce certain nonhepatic disease that may be due to direct viral replication or to the immune response to the chronic viral infection.

Acute Hepatitis

Clinically acute hepatitis C resembles other forms of acute viral hepatitis with onset of malaise, nausea, and right upper quadrant pain followed by dark urine and jaundice. The mean incubation period to onset of symptoms is 7 weeks (range, 3-20 weeks). However, long before onset of symptoms, viral markers of infection appear: HCV RNA becomes detectable in serum within 1 to 2 weeks of exposure and increases to levels of 10^6 to 10^8 genomes/mL.⁶ After several weeks, serum alanine aminotransferase (ALT) levels begin to increase and shortly thereafter, clinical symptoms appear. The severity of the acute illness is

variable; virtually all infected patients have a transient elevation in ALT, and peak levels are greater than 10-fold elevated in more than 80% of patients. Yet, only a third of patients develop jaundice or symptoms; in the remainder, the disease is anicteric and subclinical. If clinically apparent, the illness generally lasts for 2 to 12 weeks. Acute hepatitis C can result in fulminant hepatitis, but this is rare. In cases of acute, self-limited disease, HCV RNA becomes undetectable within a few weeks of onset of symptoms and aminotransferase levels return to normal.⁷⁸

Chronic Infection

Acute, self-limited hepatitis is not the most common course of acute HCV infection. In the majority of patients, symptoms of acute hepatitis resolve, but ALT levels often remain elevated and HCV RNA persists. Indeed, a propensity to chronicity is the most distinguishing characteristic of hepatitis C, occurring in at least 85% of patients with acute HCV infection. The factors that lead to chronicity in hepatitis C are not well defined. The quasispecies nature of HCV and the tendency of the envelope gene of the virus to mutate rapidly may be key factors—the virus constantly escaping immune recognition by mutations in the antigenic epitopes to which any neutralizing antibody is made. The role of cellular immunity to HCV antigens in explaining why 15% of patients clear HCV infection while the majority do not may also be important. Typically, patients with chronic hepatitis C have few if any symptoms, and these are usually nonspecific, intermittent, and mild. The most common symptom is fatigue, but anorexia, nausea, right upper quadrant pain, dark urine, and itching can occur, especially if

the disease is severe or advanced. Most patients continue to have increased serum aminotransferase levels, but these typically fluctuate in the range of 1.5 to 10 times the upper limit of the normal range and may be intermittently normal.

The majority of patients with acute infection develop chronic hepatitis, the severity of the chronic liver disease can vary considerably. The major serious complication of chronic hepatitis C is the development of cirrhosis. Cirrhosis can develop rapidly, within 1 to 2 years of exposure, or slowly, within 2 to 3 decades. In studies with 10 to 20 years of follow-up, evaluation cirrhosis develops in 20% to 30% of patients. It is unclear whether the remaining patients will eventually develop cirrhosis or whether the numbers of patients developing cirrhosis will level off with time. Thus, chronic hepatitis C probably does not have one typical course; there are probably multiple typical courses, from rapidly progressive to slowly progressive to nonprogressive. The determinants of severity in chronic hepatitis C are not known. Features that have been suggested as predictive of more severe or more rapidly progressive disease are older age, immunodeficiency, concurrent alcohol abuse, and in some series, certain HCV strains and high degrees of genetic heterogeneity. Once cirrhosis develops, the symptoms of end-stage liver disease can appear, including marked fatigue, muscle weakness and wasting, fluid retention, easy bruisability, upper intestinal hemorrhage, jaundice, dark urine, and itching. Nevertheless, some patients with cirrhosis remain asymptomatic of liver disease until they have major complications of cirrhosis, such as variceal hemorrhage or ascites. With development of complications of cirrhosis, the patient is said to have end-stage

liver disease. Once end-stage liver disease has developed, the only practical means of restoring health is liver transplantation. Recurrent HCV infection of the new graft occurs in almost all patients who undergo transplantation for hepatitis C, but in many cases the recurrent infection is mild.⁷⁸

Chronic hepatitis C is also a major cause of hepatocellular carcinoma.³⁷ This tumor occurs largely in patients with long-standing disease, and the majorities have cirrhosis. Therapies for hepatocellular carcinoma are unsatisfactory, and attention must be focused on prevention of development of cirrhosis and early detection of liver cancer.⁷⁸

Extrahepatic Manifestations of Hepatitis C

The spectrum of hepatitis C also includes several nonhepatic manifestations, including arthritis, keratoconjunctivitis sicca, lichen planus, glomerulonephritis, and essential mixed cryoglobulinemia (EMC). EMC is a syndrome marked by varying combinations of fatigue, muscle and joint aches, arthritis, skin rash (hives, purpura, or vasculitis), neuropathy, and glomerulonephritis.^{38,39} Cryoglobulins are found in serum composed of immune complexes of HCV and antibody to HCV, immunoglobulins, rheumatoid factor, and complement. Hepatitis C appears to be the most common cause of EMC, a fact that was not appreciated before the availability of serological tests for HCV. Resolution of the hepatitis C is followed by resolution of the EMC.⁷⁸

Overall, this syndrome might best be called HCV-related systemic vasculitis. A final clinical manifestation of chronic hepatitis C is porphyria cutanea

tarda.⁴¹ This form of porphyria is found in several forms of chronic liver disease, often in association with iron overload.⁷⁸

Treatment

All patients should be informed of the natural progression of the disease, measures to prevent viral transmission and treatment options, including the risks and benefits of antiviral therapy.

Among the many factors that influence the decision to treat or not is the severity of the disease, likelihood of response to treatment, concomitant medical problems, age of the patient and contraindications for the use of interferon or ribavirin. Treatment with interferon and ribavirin is recommended for patients with confirmed HCV infection who are expected to benefit from antiviral therapy after a complete clinical assessment and review of the contraindications has been completed. Combination treatment should be considered for patients with elevated liver function tests who have never been treated with interferon (interferon naïve) and for those who have relapsed after an initial response to monotherapy with interferon. Patients at greatest risk for disease progression (e.g., those with evidence of moderate inflammation or fibrosis on liver biopsy) should be given treatment priority. For patients with normal liver enzymes or for those in which aminotransferase levels failed to return to normal or HCV RNA failed to clear during monotherapy with interferon (nonresponders), treatment is currently not recommended outside clinical trials. Retreatment of nonresponders with interferon monotherapy results in response rates below 15%, and

combination treatment with interferon alpha-2b and ribavirin for 6 months seems not to improve sustained response rates.⁷⁹⁻⁸³ Interferon treatment of patients with advanced cirrhosis can be associated with severe side effects and is contraindicated for patients with decompensated cirrhosis.⁸⁵⁻⁸⁶

HIV AND HEPATITIS B AND C VIRUS

Introduction

Hepatic events have emerged as a key issue in HIV-infected patients. At the beginning of the AIDS era, liver dysfunction in HIV-infected patients mainly corresponded with opportunistic infections including AIDS-related cholangitis associated with parasitic infections (cryptosporidiosis and microsporidiosis), viral infections (e.g., with CMV), or mycobacterial infections; tumors (lymphoma and Kaposi sarcoma); and drug-related hepatitis (caused by trimetoprim-sulfamethoxazole and other antibiotics). All of these events reflected the severe immunodeficiency state of patients who, at that time, had a very poor prognosis. The developments of HAART have resulted in a significant decrease in morbidity and mortality among HIV-infected patients. HAART has completely modified the pattern of hepatic events in HIV infection, and the liver is now one of the most important organs to consider when treating HIV-infected patients. Liver disease is one of the leading causes of morbidity and mortality nowadays, which reflects several nonexclusive pathogenic processes that include drug-related hepatotoxicities; liver diseases, such as steatosis or nonalcoholic steatohepatitis (NASH); and other liver diseases that are common among patients who abuse alcohol or drugs but chronic hepatitis B virus (HBV) and hepatitis C virus (HCV) infection are the prominent ones.⁸⁷

HIV, Hepatitis B virus and Hepatitis C virus share route of transmission that explain the high rate of hepatitis co-infection in HIV -infected individuals. Indeed, 10% of patients with HIV-infection reveal markers of HBV infection.⁸⁸ Around 30% of all HIV infected individuals in the western world have a concomitant HCV infection.⁸⁹ Especially high rates of HIV/HCV co-infection can be observed in intravenous drug users and hemophiliacs, in whom rates of co-infection reach 90%. HIV accelerates HBV and HCV liver disease, especially when HIV associated immunodeficiency progresses. Morbidity and mortality due to the sequelae of HBV and HCV infections now play a leading role in HIV associated opportunistic infections.

Epidemiology and Transmission Mechanisms

Hepatitis C virus (HCV), Hepatitis B Virus and Human Immunodeficiency Virus (HIV) share the same transmission mechanisms: parenteral, sexual and vertical. Thus, the prevalence of HBV and HCV in the HIV-positive population depends on risk factors involved in the transmission of HIV and HCV, respectively.

HIV-infected patients present prevalence rates for HBV and HCV that are much greater than the rates for the general population. These rates in the coinfecting population may range from 4% to 90%⁹⁰. In Europe and USA, HIV-HBV coinfection has been seen in 6%-14%^{91,92} of all patients while HIV-HCV co-infection has been variably reported ranging from 25% to almost 50%^{93,94} of

these patients. Evidence of exposure to HBV and HCV has been found in 8.7% and 7.8%, respectively, of HIV patients from Thailand ⁹⁵ in Southeast Asia.

India, with a whopping 5.2 million cases of HIV infection, has distinction of harboring the second highest number of these patients in the world. Within India also, variable co-infection rates have been reported from region to region. HIV-HBV co-infection (HBsAg positives) is reported in 6% of people from Chennai, South India ⁹⁶, 7.5% in Chandigarh, Northwest India ⁹⁷ and 16% in Mumbai, Western India. ⁹⁸ Similarly, HIV-HCV co-infection rates also vary from 4.8%-21.4% in South India ^{96,99} and 30% in Western India ⁹⁸ to as high as 92% in the North- East. ¹⁰⁰

Injection drug use has played an important role in parenteral transmission of HIV, hepatitis B virus (HBV), and hepatitis C virus (HCV). Several studies have described high prevalence of antibodies to HCV (anti-HCV) and hepatitis B core antigen (anti-HBc) among injection drug users (IDUs) in the United States and other countries¹⁰¹⁻¹⁰³ and have measured coinfection rates of HIV, HBV, and HCV. In a study M. K. Saha *et al* in Manipur a 100% HBV and 92% HCV infection was observed among the injectible drug users. Additionally according to CDC, among HIV-positive IDUs, the HCV estimate is between 50% and 90% ¹⁰⁴.

The risk of materno-fetal transmission of HCV has been assessed by different studies. On average, the materno-fetal transmission risk of HCV is estimated as 6% in HIV-negative mothers ¹⁰⁵. In HIV-HCV co-infected mothers, the risk is greater. On average, it is estimated as 14%, ranging from 5% to 36% ¹⁰⁵. Many studies have demonstrated that HCV viremia is significantly greater in

HIV co-infected patients ^{106, 107}. It is believed that the HCV viral load of the mother is related to the risk of materno-fetal transmission of the virus. Regarding vertical transmission of HBV in the presence of HIV there is no data available.

There are a number of studies in the literature relating HCV transmission and risk behavior for sexually transmitted diseases (STD) or HCV-infected sexual partners.¹⁰⁸ Thomas DL *et al* in study among individual visiting a STD clinic found STD as independent factor for the prevalence of HCV infection.¹⁰⁹ In one more similar study by Robert A *et al* a twofold HCV prevalence was observed among STD patients when compared with HCV prevalence among the general population in the study.¹¹⁰

Similarly numerous studies have reported a high HBV transmission in STD patients. In a study by A Risbud *et al* high prevalence of 10.86 per 100 person years was observed in Pune among STD positive patients.¹¹¹ The population infected with HIV generally presents high-risk behavior for the transmission of STD and frequently present HBV or HCV- infected partners. Thus, the HIV-infected population, even without parenterally exposure factors, can have a greater association with HBV or HCV infection than the non HIV-infected population.

Some authors believe that the sexual transmission of HCV can be facilitated by the presence of an HIV-infected sexual partner; however, studies in this area are controversial. Some researchers observed that sexual partners of co-infected subjects presented a higher risk of being positive to HCV than the regular partners of patients infected only with HCV. The results, however, were

not statistically significant considering the studied groups.¹⁰⁸ In a study by J Tor et al ¹¹² homosexual men had a high prevalence of HIV and hepatitis B virus infections with a low prevalence of hepatitis C virus infection, the presence of which was not related to that of the other two infections. Conversely, heterosexual partners of intravenous drug users had low prevalence of the three virus infections, but the presence of hepatitis C virus infection correlated significantly with the presence of HIV and hepatitis B infections. Thus the rate of sexual transmission of hepatitis C virus seems to be low, even in partners of people known to be seropositive for this virus.

Epidemiology and High Risk Groups

Differences in infectivity between HBV, HCV and HIV have been observed in several settings. HBV and HIV are more efficiently transmitted perinatally and sexually than HCV. In the perinatal setting, maternal coinfection with HIV facilitates the transmission of HCV to newborns.¹¹³ MSM and long-term partners of persons with chronic infection have been shown to be at extraordinarily high risk for acquiring HBV and HIV infection. Although high-risk sexual behaviour (e.g. unprotected sex with multiple partners) is a risk factor for acquiring HCV infection, MSM are at no greater risk than heterosexuals, and long-term partners of persons with chronic HCV infection are rarely infected.¹¹⁴ Reasons for these differences are unclear.

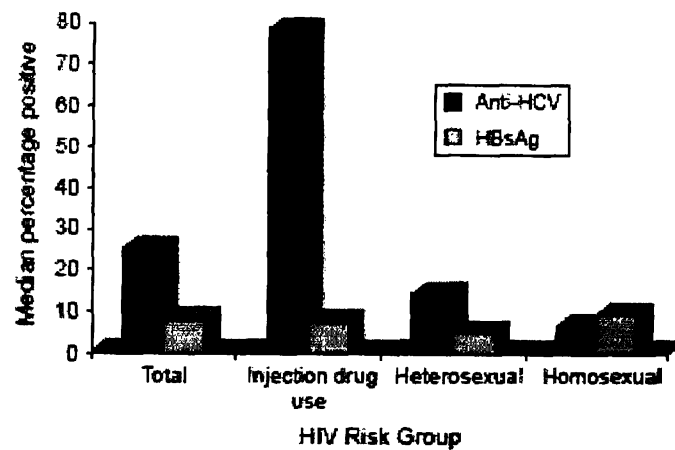


Fig. 1. Prevalence of chronic hepatitis B virus and hepatitis C virus infections in HIV positive populations by HIV risk group. 200

In the occupational setting, exposures to contaminated needle sticks among healthcare workers have demonstrated that HBV is 100 times more infectious than HIV and HCV is 10 times more infectious than HIV.¹¹⁴ In addition, experimental studies in animals have demonstrated that HBV and HCV can remain viable in dried blood on environmental surfaces at room temperature.^{115,116} Although HIV has been propagated in cell culture after drying at room temperature,¹¹⁷ it may not be infectious in humans. The difference in environmental survival capabilities of HBV and HCV compared with HIV may be one of the explanations for the more rapid acquisition of HBV and HCV infection among injection drug users, the association of HBV and HCV infections with

sharing drug preparation equipment in addition to needles and syringes, ^{118,119} and the more frequent episodes of iatrogenic transmission of HBV and HCV. ¹²⁰

HIV and HBV co-infection

Immunosuppression enhances HBV replication yet diminishes immunological hepatocyte damage; hence it is difficult to predict the effect of HIV co-infection on the course of HBV. Initial autopsy studies of co-infected patients who died of AIDS showed less severe liver injury in those with HBV than in those without HBV, implying that severe immunosuppression reduced histological injury in HBV. However, further data indicate a more complex interaction between the two viruses. ¹²¹

The majority of studies indicate that HIV infection is associated with an increased chronicity rate of acute HBV infection. HIV-HBV co-infection is also associated with an increased frequency of markers of replicative HBV infection (HBeAg, HBV DNA positivity), consistent with loss of immune control of viral replication, but a lower alanine aminotransferase (ALT) activity, consistent with evidence for the immunopathogenesis of liver injury. ¹²¹

HIV-HBV co-infection reduces the rate of spontaneous HBsAg and HBeAg seroconversion. The prevalence of HBeAg-negative chronic hepatitis B as well as the HBV inactive carrier state thus tends to be lower in HIV/HBV-co-infected individuals. Although higher serum HBV-DNA levels are seen in co-infected patients, a recent report has shown that HBV-DNA titers do not increase shortly

after HIV seroconversion in previous HBV carriers. Despite higher serum HBV-DNA levels, hepatic necroinflammation tends to be milder in HIV/HBV-coinfected individuals, which is in agreement with the postulated immune-mediated pathogenicity of HBV. However, the enhanced replication levels of HBV in HIV-co-infected patients may result paradoxically in the progression of more severe liver fibrosis.¹²¹

There are contradictory data on the activity of inflammatory liver disease in co-infected patients. Cohort studies from northern Europe and USA of men who have sex with men (MSM) showed a significantly less severe necro-inflammatory activity in HIV co-infected patients. In contrast, some studies performed in Californian and French cohorts that included injection drug users showed increased necro-inflammatory activity in HIV seropositive subjects.¹²²

There are also contradictory data on the impact of HIV on hepatitis B progression towards cirrhosis: some studies from northern Europe and USA performed in the pre-HAART era did not reveal an unfavorable impact of HIV co-infection on hepatitis B evolution while three French studies identified a more rapid progression towards cirrhosis in HIV co-infected individuals. These discrepancies could be related to differences in the prevalence of infecting HBV genotypes and of mutant HBeAg defective HBV strains or the degree of immune suppression, or to the different prevalence of co-factors associated with liver injury (alcohol, HCV, HDV) in the various cohorts.¹²²

A recent, large case-control study by Thio et al. Individuals co-infected with HIV and HBV were at a greater risk of liver-related death than those infected

by HIV or HBV alone (or not infected with either virus). Risk of liver death in individuals with HIV was associated with HBsAg reactivity independent of age, history of drug use and number of male sexual partners. These results have been confirmed by another cohort study from the USA, which demonstrated a high liver-related mortality in HIV infected people with chronic hepatitis B. HIV co-infection also leads to a worsening of hepatitis B disease with an increase of liver-related mortality. ¹²²

HAART has a major impact on HBV co-infection because of restoration of specific and non-specific immune responses and decrease of aberrant activation and dysregulation of the immune system. In addition, at least three antiretrovirals (lamivudine, tenofovir and emtricitabine) are potent inhibitors of HBV replication. HAART may therefore influence the natural history of chronic hepatitis B by

- preventing acute infection in patients using anti-retrovirals with dual activity,
- inhibiting HBV replication after the introduction of antiretrovirals with dual activity,
- reactivating HBV replication after withdrawal of antiretrovirals with dual activity or emergence of HBV resistant strains,
- restoring adaptive HBV specific immune response and innate non-specific immune response,
- and reducing aberrant activation of the immune system.

An earlier use of HAART for prevention of severe immune dysfunction and the inclusion of a drug with dual anti-HBV activity, which might slow down progression of chronic hepatitis B in HIV-infected patients, are recommended.¹²²

HIV-HCV Coinfection

HIV infection appears to adversely affect each stage in the *natural history* of HCV infection. In a recent study on HCV and HIV dynamics in co-infected subjects, the estimated HCV virion half-life was longer in the HIV-HCV co-infected group, which suggests that co-infection may contribute to a slower clearance of HCV. On average, HIV infected patients harbour 10-fold higher titres of HCV viraemia compared with HIV-negative persons. As far as factors that could be associated with the increase in HCV viraemia, some investigators report a negative correlation between HCV RNA load and CD4 cell count, whereas others have not found such correlation.¹²³

Studies on liver fibrosis have compellingly shown that coinfection with HIV worsens the histological course of HCV infection by increasing and accelerating the risk of cirrhosis. In a cross-sectional study, after 10 years of HCV infection, 15% of HIV-positive patients developed cirrhosis compared with only 3% in the HIV-negative group. The mean time from estimated onset of HCV infection to cirrhosis was significantly longer in HIV-negative (23 years) than HIV- positive patients (7 years). This higher rate of cirrhosis and shorter evolution could be explained by a significantly increased rate of the yearly progression of the fibrosis. Indeed, the median fibrosis progression rate of HIV–HCV co-infected

patients and patients infected only by HCV is 0.153 and 0.106 fibrosis units per year, respectively. At this rate of fibrosis progression, the median duration from HCV infection to cirrhosis is 26 years in HIV-infected patients and 38 years in non-HIV infected patients. These results have been confirmed by others, and it was further shown that the observed fibrosis progression in patients for whom paired liver biopsies were available, was even more rapid, with a median of only 14 years from infection to cirrhosis.¹²³

In HCV mono-infected patients, the major factors known to be associated with an increased risk of progression to cirrhosis are male gender, older age at HCV infection, and excessive alcohol consumption; viral load and genotype do not seem to influence significantly the progression rate. Although it is well established that progression of liver fibrosis is accelerated in co-infected patients, factors associated with a more severe progression of liver fibrosis have not yet been well defined in these patients. The impact of gender on the course of chronic hepatitis C seems not to be significant in co-infected patients. As in HCV mono-infected patients, older age at HCV infection was significantly associated with severe fibrosis progression; whereas the increased risk of alcohol consumption for cirrhosis was only found in some studies, but not in others. In the Hepatitis/HIV Spanish Study Group, patients with HCV infection by genotype 1 had a higher histological score, reflecting a higher stage of liver damage; however, other reports failed to confirm the association between HCV genotype and severe liver damage.¹²³

As far as HIV-related variables are concerned, an immunodepressed state, expressed by a low CD4 cell count, is systematically associated with severe fibrosis. It has been suggested that chronic use of antiretroviral therapy containing protease inhibitors, together with reduction in alcohol consumption and maintenance of high CD4 count could have a beneficial impact on liver fibrosis progression in HIV–HCV co-infected patients. However, HAART benefit on liver fibrosis was not supported by others.¹²³

In HCV mono-infected cirrhotic patients, liver cancer develops after a long period of chronic infection; thus, the age at diagnosis commonly ranges from 65 to 75 years. Garcia-Samaniego et al analyzed the clinical outcome of a small group of HIV-infected individuals diagnosed with HCV-related hepatocellular carcinoma and compared it to that of a control group, matched for age, sex, duration of HCV infection and alcohol consumption. An unusually rapid development of hepatocellular carcinoma in HIV-infected patients was observed in this study, with an age of diagnosis, for 45% of the patients, under the age of 40 years. In a more recent study, the mean time between exposure to HCV and the development of hepatocellular carcinoma was reported to be about 10 years. HIV infection has also been associated with HCV antibody loss. In HIV- infected patients, serological assays may fail to detect the presence of HCV antibodies despite active replication of HCV. Loss of reactivity to HCV antigens has also been described in other forms of immunosuppression such as transplant recipients or patients on chronic haemodialysis.¹²³

Mechanisms that could explain the accelerated progression of hepatitis C in HIV- positive patients are currently only poorly understood. The decline of cell-mediated immunity associated with HIV infection could allow greater replication of HCV. In turn, a quantitative increase in HCV viraemia may explain the higher frequency of cirrhosis among HIV-infected patients. However there is no clear correlation between the level of viraemia and the severity of liver disease. HIV infection could contribute to liver injury through immune or direct viral effects. Mechanisms that are important for tissue damage could be modified in immunocompromised patients, such as the local pattern of cytokine production, expression of adhesion molecules or release of fibrogenic factors. HIV co-infection has been shown to importantly alter the HCV-specific cytokine response towards a greater production of proinflammatory type 1 cytokines. Moreover, HIV infection itself may exert a direct cytopathic effect on liver cells independently of other cofactors since HIV-1 infects not only peripheral blood CD4 cells, but also specialized intrahepatic immune cells such as Kupffer cells, intrahepatic mononuclear cells and hepatocytes.¹²³

3. Aims & Objectives

HIV-infection rates are increasing dramatically in India. It is highly prevalent in particular groups of people. These people show high-risk behaviour, which increase their possibility of acquiring HIV. One of such high-risk group is the migrant workers. Significant migration exists between states in India. Migration in support of work for extended periods takes migrants away from the social environment provided by their families and community. This leaves them outside the usual normal constraints and thus more likely to engage in high-risk behaviour and increase their probability of getting infected with HIV. The infection with HIV results in immunosuppression that is the result of both quantitative and qualitative abnormalities of the helper/inducer subset of T lymphocytes. This results in an array of opportunistic pathogens and malignancies thereby increasing the HIV related mortality.

Morbidity and mortality associated with HIV infection have decreased with the introduction of highly active antiretroviral therapy. But of recent concern is the increase of opportunistic infections; particularly with hepatitis B virus and hepatitis C virus in HIV infected population. Because of the shared route of transmission, a significant number of HIV-infected patients are coinfectd with hepatitis B or C virus. There is a lack of data regarding HBV and HCV prevalence among HIV infected migrant workers .Our study will help us to asses mediating factors in behavioral, biological and social agents accountable to the prevalence of these infections. This study may in turn also assist to implement proper intervention against the potential spread of these infections in view of the return

migration of these migrants, which in sequence may spread the infections among innocent rural people.

- To study sociodemographic factors and the high-risk behavior exhibited by the HIV seropositive Migrant Workers visiting Mathadi Kamgar Hospital, Koperkhairne, and Vashi.
- To study the immunological status by determining the CD4 cell count.
- To study the prevalence of HBV and HCV infections in HIV seropositive migrant workers with the help of following assay.

HBV

- a) ELISA for Detection of HBsAg, HBeAg and Anti-HBeAg
- b) HBV DNA detection by PCR

HCV

- a) ELISA for Detection of Anti-HCV
 - b) HCV RNA detection by RT-PCR
- To study the association of HBV and HCV infection with the sociodemographic factors and the high-risk behavior exhibited by the study group.

4. Materials & Methods

1. Study Population

The study population comprised of 100 HIV seropositive Migrant Workers. All the selected individuals were attending the OPD or admitted to Mathadi Kamgar Hospital, Koperkhairne, Vashi. The individuals were selective irrespective of their age and sex.

The control group comprised of 50 healthy individuals visiting Haffkine Institute for their annual medical checkup.

All the individuals selected (Study population and Control population) were provided with brief information about the project and the samples were only collected after their consent. The individuals in the study were subjected to a structured questionnaire, which included data on demographics, STD, medical history and sexual behaviour. Presumptive diagnosis of sexually transmitted disease was based on the reports and medical history provided by the hospital.

Ethical committee approval for the project from the Mathadi Kamgar Hospital was taken prior to sample collection.

2. Blood Collection

Ten ml of blood was drawn by vein puncture from all the individuals. Two ml of the blood collected was added in K3-EDTA and the rest was collected in a plain bulb. The blood in the plain was allowed to clot at room temperature and serum was separated within 3hrs from the time of collection. The serum was aliquoted in vials and stored at -20°C till further use.

3. HIV Serostatus Confirmation

Sera samples were tested for the presence of HIV1 and 2 antibodies by commercially available ELISA Kit (Sandwich ELISA SD HIV 1/2 ELISA, Ver.3, Standard Diagnostic Inc., and Indirect Elisa, Micro Lisa, J. Mitra and Co.)

In sandwich ELISA the microplate was coated with recombinant HIV1/2 antigens (gp41, p24 and gp36) on the well. During the first incubation, anti-HIV in patients serum was bound to the recombinant HIV1/2 antigens. Following this, all unbound material was removed by aspiration and washing. The recombinant HIV1/2 antigens-enzyme conjugate (gp41, p24, gp36) bound to anti-HIV 1/2, thus forming a sandwich. After a subsequent incubation the unbound material was removed by aspiration and washing. The residual activity found in the wells was directly proportional to the anti-HIV1/2 concentration in the patient's serum. After incubation with the Tetramethylbenzidine (TMB) substrate solution, the plate is read using an ELISA reader at 450nm.

In the indirect ELISA, the recombinant proteins representing the immunodominant epitopes (gp41, C-terminus of gp120 and gp36) of HIV 1/2, were coated onto the microtitre wells. Samples were added to these wells and incubated. Antibodies to HIV1/2, if present in the specimen, will bind to the specific antigens adsorbed onto the surface of the wells. The plate was then washed to remove unbound material. Horse Raddish Peroxidase (HRP) conjugated anti-human IgG was added to each well. This conjugate binds to the HIV antigen-antibody complex present. Finally, a substrate solution containing

chromogen and hydrogen peroxide was added to the wells and incubated. After incubation, the colour development is read using an ELISA reader at 450nm.

4. CD4-CD8 Counts

The CD4-CD8 cell count was measured using BD FACSCount system. It employed a direct two-color immunofluorescence method for enumerating absolute lymphocyte counts (cells/ μ L whole blood) of the following mature human lymphocyte subsets: CD3+ T-lymphocytes, CD3+CD4+ T lymphocytes and CD3+CD8+ T-lymphocytes. In addition, the CD4:CD8 ratio was provided.

Principle

The BD FACSCount system used was designed to analyze unlysed whole blood, collected in liquid EDTA. When whole blood (50 μ l) was added to the tubes of a sample reagent pair (CD4 tube and CD8 tube), the fluorochrome-labeled antibodies in the tubes bind specifically to antigens on the surface of lymphocytes. The BD FACSCount instrument detected two fluorescent colors and measured relative cell size. CD3 cells fluoresced red and CD4 and CD8 cells fluoresced yellow when analyzed on the BD FACSCount instrument. A known number of reference beads were present in each reagent tube and functioned as fluorescence and quantitation standard for calculating the absolute counts for CD4+, CD8+, and CD3+ T lymphocytes. Fixative solution was added to the stained samples prior to analysis to preserve the integrity of the antibody binding. No lysing was necessary.

Procedure

100µl of blood was drawn from K3-EDTA containing bulb. 50µl each was added to the reagent pair tubes for the estimation of CD4/CD3 and CD8/CD3. After incubation in the dark for 90 minutes, the fixative solution was added to these tubes and the cells were counted using the BD FACSCount instrument. The sorting and analysis of the cells was fully automated.

5. Detection of Hepatitis B Virus

5a.HBsAg ELISA

The HBsAg detection was performed using a commercially available “Direct Sandwich” ELISA based on the use of polystyrene microwells coated with *mouse monoclonal antibodies* to HBsAg, procured from Diasorin [Diasorin **ETI-MAK-4**].

In assay procedure the patient sample was incubated in the microwell coated with mouse monoclonal antibodies to HBsAg. If HBsAg was present in the sample, it binds to the antibody coated on the microwell. Excess sample was removed by a washing step, and the enzyme tracer (anti-HBs antibody (sheep) conjugated to horseradish peroxidase (HRP)) was then added to the microwells and allowed to incubate. The enzyme tracer binds to any antigen-antibody complexes present in the microwells. Excess enzyme tracer was removed by a washing step, and a chromogen/substrate solution was added to the microwells and allowed to incubate. If a sample contains HBsAg, the bound enzyme

(horseradish peroxidase) chemically reduces the substrate peroxide, which concurrently oxidizes the chromogen tetramethylbenzidine (TMB) to a blue colour (650 nm). The blue colour turns to yellow (450 nm) after addition of the stop solution. If a sample does not contain HBsAg, the microwell will be colorless after the chromogen/substrate solution was added and will remain colourless after the stop solution was added. Colour intensity, which was measured spectrophotometrically, was indicative of the presence of HBsAg. Absorbance value readings for patient specimens were compared to a cut-off value determined from the mean absorbance of the calibrator.

5b. HBeAg ELISA

The HBeAg detection assay was performed using a commercially available direct, non-competitive test based on the use of polystyrene microwells coated with mouse monoclonal antibodies to hepatitis B e antigen (HBeAg), procured from Diasorin [Diasorin **ETI-EBK PLUS**]. An enzyme tracer containing horseradish peroxidase-labelled mouse monoclonal antibody to HBeAg detects any captured HBeAg.

In the assay procedure, patient sample were incubated with incubation buffer in antibody-coated microwells. If HBeAg was present in the sample, it binds to the antibody coated on the microwell. Excess sample was removed by a washing step, and the enzyme tracer was then added to the microwells and allowed to incubate. The enzyme tracer binds to any antigen-antibody complexes present in the microwells. Excess enzyme tracer was removed by a washing

step, and a chromogen/substrate solution was added to the microwells and allowed to incubate. If a sample contains HBeAg, the bound enzyme (horseradish peroxidase) chemically reduces the substrate peroxide, which concurrently oxidizes the chromogen tetramethylbenzidine (TMB) to a blue colour (650 nm). The blue colour turns to yellow (450 nm) after addition of the stop solution. If a sample does not contain HBeAg, the microwell will be colourless after the chromogen/substrate solution was added and will remain colourless after the stop solution was added. Colour intensity, which was measured spectrophotometrically, was indicative of the presence of HBeAg. Absorbance value readings for patient specimens were compared to a cut-off value determined from the mean absorbance of the calibrator.

5c. Anti-HBeAg ELISA

The Anti-HBeAg detection was performed with a commercially available competitive test based on the use of polystyrene microwells coated with mouse monoclonal antibodies to hepatitis B e antigen (HBeAg), procured from Diasorin [Diasorin **ETI-AB-EBK PLUS**]. An enzyme tracer containing horseradish peroxidase-labelled mouse monoclonal antibody to HBeAg detects any captured HBeAg/anti-HBe complexes.

In the assay procedure, patient sample and recombinant HBeAg contained in the neutralizing solution were incubated with incubation buffer in antibody-coated microwells. If anti-HBe was present in the sample, it competes with the antibody coated on the microwell for the recombinant HBeAg. Excess

sample and recombinant HBeAg were removed by a washing step, and the enzyme tracer was then added to the microwells and allowed to incubate. The enzyme tracer binds to any antigen-antibody complexes present in the microwells. The quantity of enzyme tracer that binds to the solid phase via recombinant HBeAg and the consequent enzyme activity were inversely indicative of the anti-HBe present in the sample. Excess enzyme tracer was removed by a washing step, and a chromogen/substrate solution was added to the microwells and allowed to incubate. If a sample does not contain anti-HBe, the bound enzyme (horseradish peroxidase) chemically reduces the substrate peroxide, which concurrently oxidizes the chromogen tetramethylbenzidine (TMB) to a blue colour (650 nm). The blue colour turns to yellow (450 nm) after addition of the stop solution. If a sample contains anti-HBe, the microwell will be colourless after the chromogen/substrate solution was added and will remain colourless after the stop solution is added. Colour intensity, which was measured spectrophotometrically, was inversely indicative of the presence of anti-HBe. Absorbance value readings for patient specimens are compared to a cut-off value determined from the mean absorbance of the calibrator.

5d. Detection of HBV DNA Detection by Polymerase Chain Reaction

5d.i Extraction of DNA

DNA was extracted from the serum using a DNAZOL BD method. 250ul of serum sample was used for the extraction of DNA.

The sample was first mixed in 1ml of DNAZOL BD (Molecular Research Center Inc) and 4ul of Poly acryl carrier molecule (Molecular Research Centre Inc). The mixture was vortexed and stored at room temperature for 5 minutes for lysis. After the incubation 500ul of isopropanol (SRL Pvt. Ltd.) was added and the mixture was again vortexed and incubated at room temperature for 5 minutes for precipitation. The mixture was then centrifuged at 6000g for 6 minutes. The DNA pellet obtained was then washed with 0.5 ml of DNAZOL BD and centrifuged at 6000g for 5 minutes. The DNA pellet obtained was again washed with 1ml of 75% ethanol and centrifuged at 6000g for 5 minutes. The DNA pellet was further air dried in the laminar hood for 30minutes. The DNA pellet was then redissolved in 200ul of D/W and stored at -20°C till further use.

Spectrophotometer Evaluation of DNA

The extracted DNA sample was checked for its purity using UV spectrophotometer. The sample was checked using a 2mM Na₂HPO₄ buffer at pH 8.5.¹²⁴ The samples having A_{260/280} ratio between 1.8-1.9 were considered excellent for PCR.

5d.ii HBV DNA Detection by Polymerase Chain Reaction

The HBV DNA detection by PCR was performed by amplifying a sequence from the highly conserved region of the surface gene from the HBV genome.¹²⁵ The amplified sequence was 597 base pairs long. The details of the sequence are as follows.

Sequence (190-787) : gctcgtgttacaggcggggttttctgttgacaagaatcctcacaataccac
agagtctagactcgtggaggacttctcaattttctagggggagcaccac
gtgtcctggccaaaattcgagtcaccaacccaacccaatcactcaccaacctt
tgtcctccaacttgtcctggctatcgctggatgtgtctgcggcggtttatcatatt
cctcttcacctgctgctatgcctcatcttctgttggttctctggactaccaagg
tatgttgcccggttgtcctctactccaggaacatcaactaccagcacggga
ccatgcagaacctgcacgattcctgctcaaggaacctctatgtttccctctgt
tgctgtacaaaaccttcggacggaaactgcactgtattcccatcccatcat
cctgggctttcgcaagattcctatgggagtgaggcctcagtcggttctcctggc
tcagtttactagtgccattgttcagtggttcgtagggctttccccactgtttgg
cttcagctatatggatgatgtggtattgggggccaagtctgtacaacatctt
gagtccc

Length : 598 base pairs

Melting Temperature : 84.28

GC content : 50.0 %

The primers selected for the PCR were as follows

a) The forward primer was HBV 2 F ¹²⁵

Sequence of 11F : 5'gctcgtgttacaggcggggttttctt 3'

Length : 24 base pairs

Melting Temperature : 61.26°C

GC content : 51.85%

b) The Reverse Primer was HBV 2 R ¹²⁵

Sequence of 18R : 5'gggactcaagatgtgtacagacttgg 3'

Length : 27 base pairs

Melting Temperature : 59.74°C

GC content : 48.15 %

The reagents required for PCR were procured from Fermentas Lifesciences. The concentration and amount of reagents used in each reaction are mentioned in Table no.1. The PCR was performed taking proper precautions in order to avoid any contamination. The Cycling parameters for the PCR are mentioned in Table no. 2.

Table no. 1 Reaction Components for PCR

Component	Volume	Final Concentration
Master Mix		
DNase-RNase Free water	33.5 µl	-
10 X PCR Buffer	5.0 µl	1X
25mM MgCl ₂	3µl	1.5mM
dNTP Mix (containing 10mM of each dNTP)	1.0 µl	200µm of each dNTP
Primer HBV 2 F	1.0 µl	25 pmole
Primer HBV 2 R	1.0 µl	25 pmole
Taq DNA Polymerase 1unit/µl	0.5 µl	2.5 units
Template RNA	3.0 µl	-
Total Volume	50µl	

Table No. 2 Thermal Cycler Parameters

Parameter	Time	Temperature
Initial Denaturation	5 minutes	94 °C
3-step Cycling		
Denaturation	30 seconds	94 °C
Annealing	30 seconds	59 °C
Extension	1 minute	72 °C
Number of Cycles: 40 Cycles		
Final Extension	10 minutes	72 °C

Analysis of PCR Product

The PCR product was analyzed using agarose gel electrophoresis. A 1.5% low electro-osmotic agarose (Bangalore GENEI) gel was used. The gel was made in Tris Borate EDTA buffer (TBE) pH 8.0. Ethidium Bromide was added at a concentration of 0.5 µg/ml of the gel. The PCR product was mixed with a loading buffer containing Bromophenol blue and Xylene Cyanol as tracking dye and Sucrose to increase the density and loaded in the gel. A voltage of 4 volt/cm was applied during the run. A 50 base pair DNA ladder (Biomol Prod.No. 56576) was also run along with the samples for reference. The bands were visualized under a UV Transilluminator. The photographs of the gel were taken with the help of Gel-Doc (Gel Documentation System, Biotron).

Note: The primers and the reagent mentioned in the synopsis got degraded due to the freezer malfunction, thus a fresh new set of primers and reagents was ordered and used.

6. Detection of Hepatitis C Virus

6a. Anti-HCV ELISA

The Anti-HCV detection assay was done with a commercially available ELISA test based on the use of polystyrene microwells coated with recombinant antigens representing epitopes to HCV: Core, NS3 NS4 and NS5 procured from Biokit, Spain [Bioelisa HCV].

In the assay procedure the serum samples were added to the wells. If antibodies specific for HCV were present in the sample they will form stable complexes with the HCV antigen on the well. The unbound and extra material was removed by a washing step. A rabbit anti- human IgG labelled with horseradish peroxidase (conjugate) was added and if the antigen/antibody complex was present, the conjugate will bind to the complex. A second washing was given to remove excess material. An enzyme substrate solution containing a chromogen [tetramethylbenzidine (TMB)] was then added. The solution would turn blue if the sample solution was positive. The blue colour changed to yellow after blocking the reaction with sulphuric acid. The intensity of the colour was proportional to anti-HCV antibodies in the sample. Wells containing negative samples remained colourless.

6b. Detection of HCV RNA by Reverse Transcriptase PCR (RT-PCR)

6b. i Extraction of RNA

The RNA extraction was carried out from 250µl serum using Proteinase K (SRL Pvt. Ltd.) and SDS (SRL Pvt. Ltd.).¹²⁶ The 250µl of serum sample was first mixed and incubated with the complete extraction buffer (50 mmol/L Tris-HCl (pH 8.4), 10 mmol/L EDTA, 0.2 mol/L NaCl, 2% SDS, and 1 mg/mL Proteinase K) at 60°C for 30 minutes. After the incubation, the sample was centrifuged for 5 seconds and 500µl of phenol: chloroform (prepared by dissolving 100gm ultra pure phenol from SRL Pvt. Ltd. in 100ml of molecular biological grade chloroform from SRL Pvt. Ltd. stored at 4°C) was added. The sample was then mixed

thoroughly to get an emulsion and centrifuged at 12,000rpm for 5 min. After the centrifugation the upper aqueous layer was removed and transferred to a fresh microcentrifuge tube containing 500µl of chloroform (SRL Pvt. Ltd.). The sample was then centrifuged at 12,000 rpm for 5 min. From the upper aqueous layer 315µl of the sample was removed and added to a fresh microcentrifuge tube containing 1µl of Glycogen (Hi Media) concentration: 20mg/ml. To this mixture 315µl isopropanol was added and the whole mixture was incubated at room temperature for 10 minutes. After incubation the sample was centrifuged at 14,000 rpm for 30 minutes at 20°C. Carefully the supernatant was removed and the white pellet was washed with 1ml of 70% ethanol. The pellet was then air dried in the laminar hood for 30 minutes and then redissolved in 20µl DNase RNase free water.

Spectrophotometric evaluation of RNA

The extracted RNA sample was checked for its purity using UV spectrophotometer. The sample was checked using a 2mM Na₂HPO₄ buffer pH 8.5.¹²⁴ The samples having A_{260/280} ratio between 1.9-2.0 were considered excellent for RT-PCR. The RNA sample was also checked for phenol contamination by examining the absorbance at 230 nm.

6b.ii HCV RNA detection by Reverse Transcriptase Polymerase Chain Reaction

The amplification of the hepatitis C virus (HCV) RNA was carried out by one-step RT-PCR (Qiagen® One-step RT-PCR Kit). Reverse transcription and PCR was carried out sequentially in the same tube.

The sequence selected for detection of HCV is 323bp long (18-340) sequence from the 5'NC region of the HCV virus. ¹²⁶

The Sequence details are as follows

Sequence (18-340) : ggcgacactccacatgaatcactcccctgtgaggaactactgtcttcac
gcagaaagcgtctagccatggcgtagtatgagtgctgcagcctccag
gacccccctcccgggagagccatagtggtctgcggaaccggtgagtac
accggaattgccaggacgaccgggtccttcttgataaaccgctcaatg
cctggagattggcgctgccccgcaagactgctagccgagtagtggtgg
gtcgcgaaaggccttggtactgcctgatagggtgctgcgagtgcccc
gggaggtctcgtagaccgtgcac

Length : 323 base pairs

Melting Temperature : 81.316

GC content : 59.75%

The primers selected for the RT-PCR were as follows

a) The forward primer was 11F ¹²⁶

Sequence of 11F(18-42) : 5'ggcgacactccaccatagatcact 3'

Length : 24 base pairs

Melting Temperature : 66.28 °C

GC content : 54.17%

b) The Reverse Primer was 18R ¹²⁶

Sequence of 18R (340-319) : 5'ggcgacactccaccatagatcact 3'

Length : 21 base pairs

Melting Temperature : 66.47 °C

GC content : 61.90 %

The amplification of the hepatitis C virus (HCV) RNA was carried out by one-step RT-PCR (Qiagen® One-step RT-PCR Kit). Reverse transcription and PCR was carried out sequentially in the same tube. All components required for both reactions were added during the setup.

Protocol

1. RNA, primer solutions, dNTP Mix, 5x QIAGEN OneStep RT-PCR Buffer, and RNase-free water were first thawed and then placed on ice. It is important to mix the solutions completely before use to avoid localized differences in salt concentration.
2. Master mix was prepared according to Table no.3. The master mix contained

all the components required for RT-PCR except the template RNA. A volume of master mix 10% greater than that required for the total number of reactions was prepared. A negative control (without template RNA) was included in every experiment.

3. Master Mix was mixed thoroughly, and dispensed in appropriate volumes into PCR tubes.
4. Template RNA ($\leq 2 \mu\text{g}/\text{reaction}$) was added to individual PCR tubes.
5. Since the thermal cycler (Techne Genius) used during the experiment had a heating lid hence mineral oil was not used.
6. The Thermal Cycler was programmed according to the outline mentioned in Tableno.4. The program included steps for both Reverse Transcription and PCR. The PCR amplification segment was started with an initial heating step at 95°C for 15 min to activate HotStar Taq DNA Polymerase.
7. The RT-PCR program was started while PCR tubes were still on ice. The PCR tubes were only placed in the Thermal Cycler when it reached a temperature of 50°C .
8. After amplification, samples were run on 1.5% Agarose gel.

Table no. 3 Reaction Components for One-Step RT-PCR

Component	Volume	Final Concentration
Master Mix		
RNase- Free water	22.0 µl	-
5X QIAGEN OneStep RT-PCR Buffer*	10.0 µl	1X
dNTP Mix (containing 10mM of each dNTP)	2.0 µl	400µm of each dNTP
Primer 11F	1.0 µl	25 pmole
Primer 18R	1.0 µl	25 pmole
QIAGEN OneStep RT-PCR Enzyme Mix	2.0 µl	-
Template RNA	2.0 µl	-
Total Volume	50µl	

Table No. 4 Thermal Cycler conditions

Parameter	Time	Temperature
Reverse Transcription	30 minutes	50°C
Initial PCR activation step	15 minutes	95°C
3-step Cycling		
Denaturation	30 seconds	94°C
Annealing	30 seconds	61°C
Extension	45 seconds	72°C
Number of Cycles : 40 Cycles		
Final Extension	10 minutes	72°C

Analysis of RT-PCR Product

The RT-PCR product was analyzed using agarose gel electrophoresis. A 1.5% low electro-endo-osmotic agarose (Banglore GENEI) gel was used. The gel was made in Tris Borate EDTA buffer (TBE) pH 8.0. Ethidium Bromide was added at concentration of 0.5ug/ml of the gel. The RT-PCR product was mixed with a loading buffer containing Bromophenol blue and Xylene Cyanol as tracking dye and Sucrose to increase the density and the loaded in the gel. A voltage of 4 volt/cm was applied during the run. A 50 base pair ladder (Biomol Prod.No. 56576) was also run along with the samples as a reference. The bands were visualized under a UV Transilluminator. The photographs of the gel were taken with the help of Gel-Doc (Gel Documentation System, Biotron).

5. Results

In the present study 100 known HIV seropositive asymptomatic migrant workers were screened for the prevalence of HBV and HCV infections from Mathadi Kamgar Hospital, Koperkhairne, Vashi. A HIV-1/2 ELISA confirmed the serostatus of all the migrant workers and the control group. The control group comprised of 50 healthy individuals visiting Haffkine Institute for their annual medical checkup. All the individuals were subjected to a structured questionnaire, which included data on sociodemographics, STDs, clinical history and sexual activities. Ethical Committee approval was obtained from the Mathadi Kamgar Hospital, Koperkhairne, Vashi prior to sample collection.

Distribution of Sociodemographic factors and High-risk behaviors in the HIV infected study group and the control group

The sociodemographic characteristics of the study group and the control group are compared in the table no.1. In the present study, the study group comprised of 53 (53%) males and 47 (47%) females. The control group had 29 (58%) males and 21 (42%) females.

In the study group, 10 (10%) individuals belonged to the age group of 18-24yrs and 35 (35%) individuals belonged to the age group of 25-34yrs. In the study group among the age group of 35-44yrs, 38 (38%) individuals were present and in the age group of 45-44yrs there were 14 (14%) individuals. In the study group 3(3%) individuals belonged to the age group of ≥ 55 yrs.

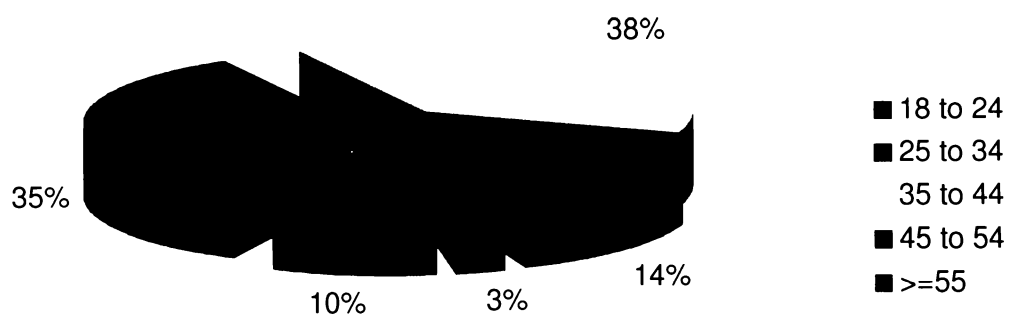
In the control group 5 (10%) individuals belonged to the age group of 18-24yrs and 16 (32%) individuals belonged to the age group of 25-34yrs. In the

control group among the age group of 35-44yrs, 14 (28%) individuals were present and in the age group of 45-44yrs there were 12 (24%) individuals. In the control group 3 (6%) individuals belonged to the age group of ≥ 55 yrs.

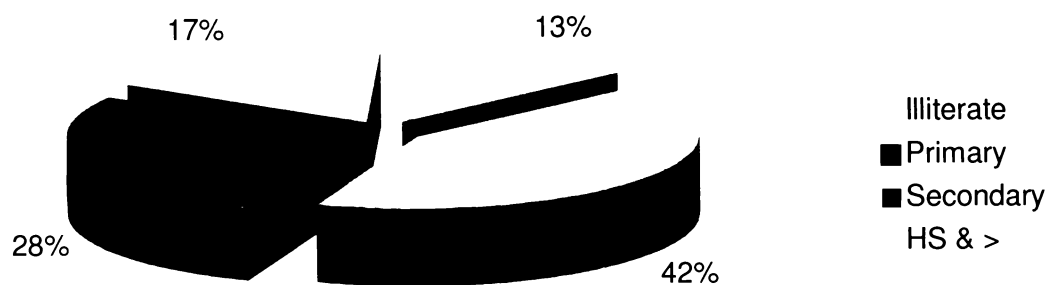
Table no.1 Distribution of sociodemographic factors among the study and the control group

		Study Group (n=100)	Control Group (n=50)	p-value
Sex	Female	47(47)	21 (42)	0.605
	Male	53 (53)	29 (58)	Not significant (F)
Age (years)	18 to 24	10 (10)	5 (10)	
	25 to 34	35 (35)	16 (32)	
	35 to 44	38 (38)	14 (28)	
	45 to 54	14 (14)	12 (24)	0.445
	≥ 55	3 (3)	3 (6)	Not significant (P)
Educational Status	Illiterate	42 (42)	0 (0)	
	Primary	28 (28)	0 (0)	
	Secondary	17 (17)	36 (72)	9.41×10^{-19}
	HS & >	13 (13)	14 (28)	Significant (L)
Religion	Hindu	85 (85)	50 (100)	
	Christian	7 (7)	0 (0)	0.002
	Muslim	8 (8)	0 (0)	Significant (L)
Mother Tongue	Hindi	40 (40)	9 (18)	
	Marathi	52 (52)	41 (82)	0.00025
	Other	8 (8)	0 (0)	Significant (L)
Marital Status	Yes	93 (93)	47 (94)	1.0
	No	7(7)	3 (6)	Not significant (F)
Living with Family	Yes	55 (55)	50 (100)	1.64×10^{-10}
	No	45 (45)	0 (0)	Significant (F)
Knowledge of AIDS	Yes	29 (29)	50 (100)	2.05×10^{-14}
	No	71 (71)	0 (0)	Significant (F)

Note: P = Pearson's Chi-square test, C = Continuity Correction, L = Likelihood Ratio, F = Fisher's Exact Test; Figures in parenthesis indicate percentage



Graph no.1 Age wise Distribution of HIV infection in the study group



Graph no.2 Education wise distribution of HIV infection in the study group.

Among the 100 individuals in the study group 42 (42%) individuals were illiterate. Among the 58% literate individuals in the study group, 28 (28%) had primary education, 17 (17%) had secondary education and 14 (14%) had higher secondary education, whereas the control group had 100% literacy level in which 36 (72%) individuals had a secondary education and 14 (28%) had higher secondary education.

Out of the 100 individuals in the study group 85 (85%) individuals were Hindu, 7(7%) individuals were Christian and 8 (8%) individuals were Muslim. In the control group all the 50 individuals were Hindu.

In the study group out of 100 individuals mother tongue of 40(40%) individuals was Hindi, 52 (52%) individuals was Marathi and the remaining 8 (8%) individuals was Tamil or Telugu. In the control group, out of 50 individuals, mother tongue of 9 (12%) individuals was Hindi and the remaining 41 (82%) individuals was Marathi.

Out of 100 individuals in the study group 93 (93%) individuals were married and none of the individuals was a widow or widower neither any one of the study group was a divorcee. In the control group out of 50 individuals 47 (94%) individuals were married and none of the individuals was a widow or widower neither any one of the control group was a divorcee.

In the study, out of 100 individuals in the study group, 45 (45%) individuals stayed away from their family whereas in control group all individuals stayed with their families. In the study group 71 (71%) individuals had no knowledge of

HIV/AIDS whereas all the individuals in the control group were aware of information regarding HIV/AIDS.

Table no.2 Distribution of high-risk behaviour among the study and the control group

		Study group (n=100)	Control Group (n=50)	P-value
HRF-Extramarital affair/CSW	Yes	59 (59)	0 (0)	3.30×10^{-14}
	No	41 (41)	50 (100)	Significant (F)
Use of condom	Yes	9 (9)	50 (100)	6.60×10^{-15}
	No	91 (91)	0 (0)	Significant (F)
Blood transfusion Self	Yes	3 (3)	0 (0)	0.551
	No	97 (97)	50 (100)	Not significant (F)
History of past or Current STD	Yes	37 (37)	0 (0)	2.94×10^{-8}
	No	63 (63)	50 (100)	Significant (F)
Alcohol Consumption	Yes	47(47)	5 (10)	3.67×10^{-6}
	No	53 (53)	45 (90)	Significant (F)

Note: P = Pearson's Chi-square test, C = Continuity Correction, L = Likelihood Ratio, F = Fisher's Exact Test; Figures in parenthesis indicate percentage

As shown in the table no.2 in the study group, 59 (59%) individuals had extramarital affair / frequent visit to CSWs, whereas none of the individuals in the controls reported the same. Out of 100 individuals, in the study group 91(91%) individuals showed unprotected sex practices i.e. infrequent or no use of condom, whereas all the individuals in the control group had protected sex practices. Three (3%) individuals in the study group had a blood transfusion. In the control group none of the individuals had blood transfusion. In the study group, 37 (37%) individuals had history of past or current STD infection (Herpes

infection) whereas in the control group had no individuals with history or past current STD. Alcohol consumption was observed in 47 (47%) individuals in the study group and 5 (10%) individuals in the control group.

Prevalence of Hepatitis B Virus and Hepatitis C Virus

As shown in table no.3, the overall HBV infection was found in 24 (24%) individuals in the study group (Odds Ratio [OR]= 1.579 95% CI= 1.343-1.856).

As shown in table no.4 HBsAg was positive in 9 (9%) individuals of the study population. Five individuals in the study population were positive for HBeAg. Among the 9 HBsAg positive individuals 4 (44.4 %) individuals were positive for HBeAg. Eighteen (18%) individuals in the study population were positive for Anti-HBeAg. Among the eighteen Anti-HBeAg positive individuals in the study population 4 (22.2 %) were positive for HBsAg. HBV DNA PCR was performed for detection of HBV DNA from the HBV surface gene of the HBV virus. HBV DNA PCR was positive for 10 individuals in the study population. HBV DNA was detected in all the HBsAg and HBeAg positive and 4 anti-HBeAg positive individuals by PCR. One individual in the study group showed presence of HBV DNA and HBeAg in the absence of HBsAg. The HBV prevalence was 2% (n=1) in the control group.

As shown in table no.3 the HCV infection was found in 4 (4%) individuals in the study population (OR= 1.521 95%CI= 1.353-1.71). All the 4 individuals were positive for anti-HCV. HCV RNA was also detected by RT-PCR. The presence of HCV RNA was detected in 4 individuals in the study population. All

the individuals showing presence of anti-HCV were positive for HCV RNA RT-PCR. In the control group none of the individuals showed no presence of any markers for HCV infection.

Table no.3 Prevalence of HIV, HBV and HCV in the Study group and the control group

	Study group (n=100)	Control Group (n=50)
HIV Prevalence	100 (4)	Nil
HBV Prevalence	24 (24)	1(2)
HCV Prevalence	4(4)	Nil

Figures in parenthesis indicate percentage

Table no.4 Distribution of HBV makers

	HBsAg	HBeAg	Anti-HBeAg	HBV DNA
Study group (n=100)	9 (9)	5 (5)	18 (18)	10 (10)
Control group (n = 50)	1 (2)	Nil	Nil	Nil

Figures in parenthesis indicate percentage

Table no.5 Distribution of HCV makers

	Anti-HCV	HCV RNA
Study group (n=100)	4 (4)	4 (4)
Control group (n = 50)	Nil	Nil

Figures in parenthesis indicate percentage

Table no. 6 Distribution of CD4 cell counts in the study group

	CD4 cells/mm ³		
	>=500	200 to 499	< 200
HIV	13 (13)	39(39)	48 (48)
HBV	4 (16.70)	6 (25)	14 (58.3)
HCV	Nil	1(25)	3 (75)

Figures in parenthesis indicate percentage

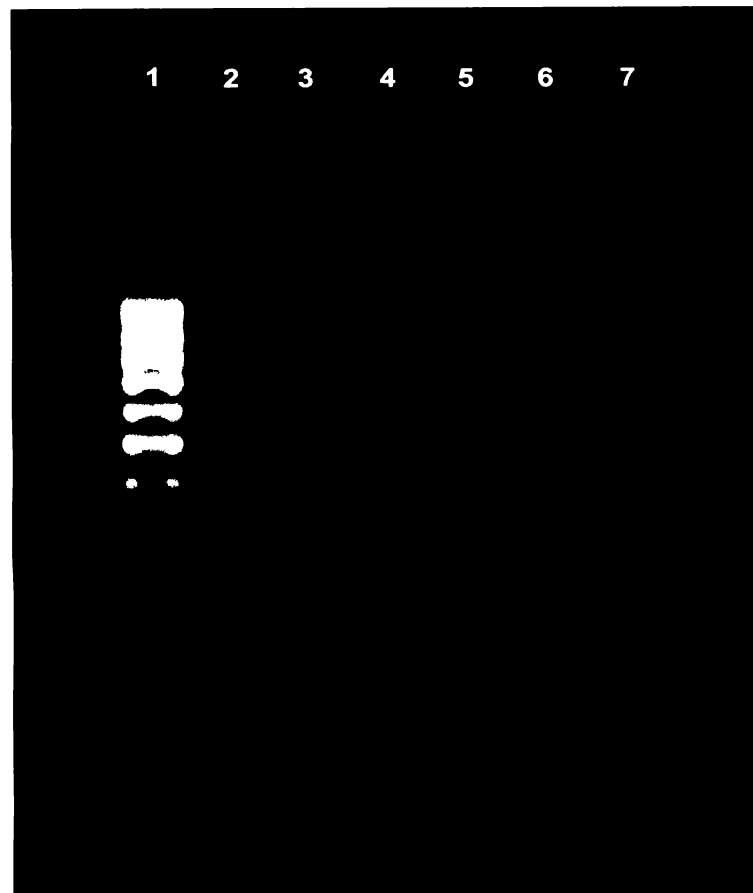


Figure no.1: Amplification of HCV RNA by RT-PCR

The 1.5% agarose gel shows bands of HCV positive samples in lane 4 and 6. The amplified bands are of 323base pairs. Lane 1 shows the 50 base pair DNA ladder.

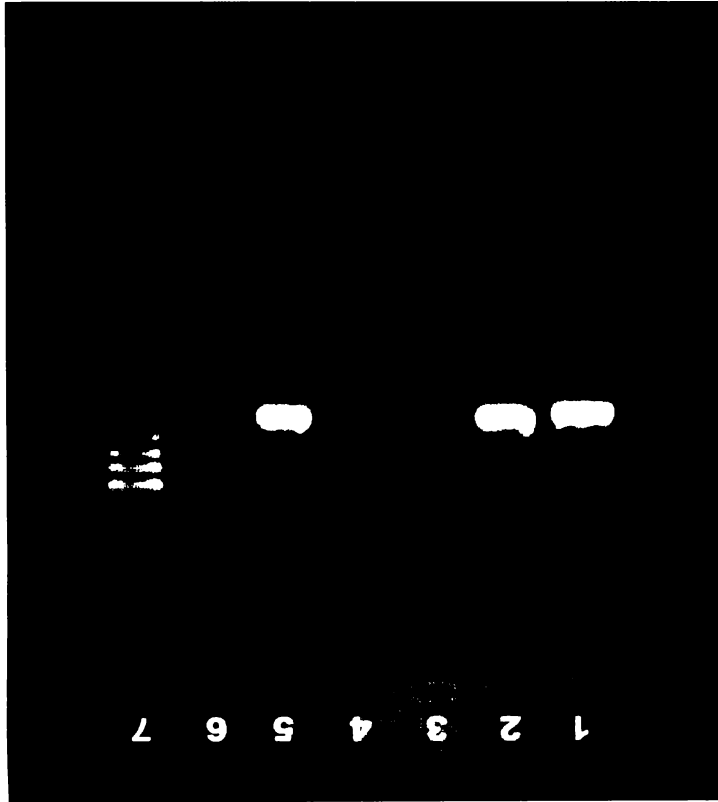
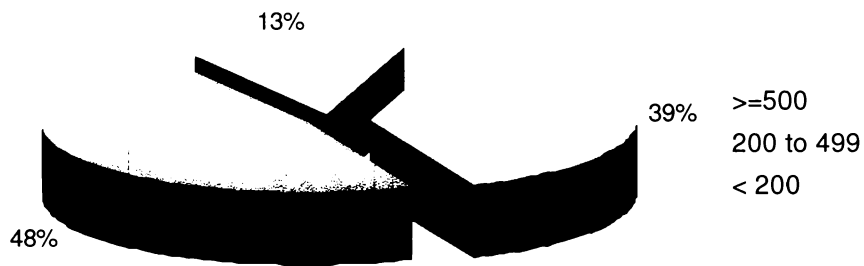
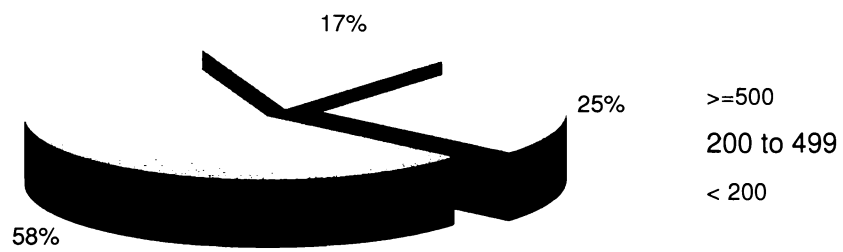


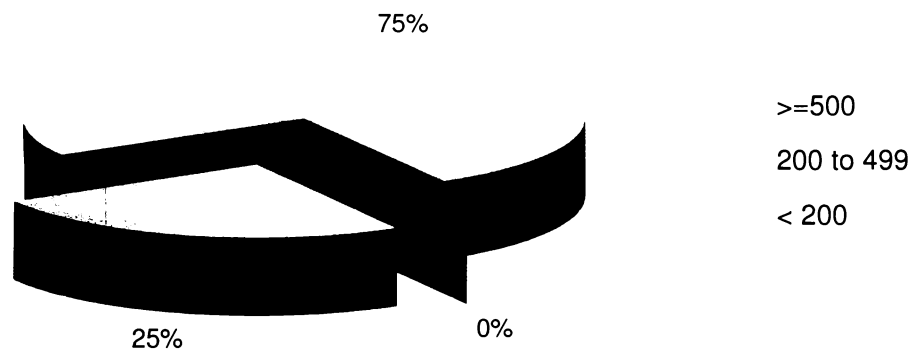
Figure no.2: Amplification of HBV RNA by PCR
The 1.5% agarose gel shows bands of HBV positive samples in
lane 1, 2 and 5. The amplified bands are of 597 base pairs.
Lane 6 shows the 50 base pair DNA ladder.



Graph no.3 Distribution of CD4 cells/mm³ in the study group



Graph no.4 Distribution of CD4 cells/mm³ in the HIV-HBV infected individuals in the study group



Graph no.5 Distribution of CD4 cells/mm³ in the HIV-HCV infected individuals in the study group

As shown in table no. 6, 13 (13%) individuals had CD4 cell counts above 500cells/mm³ from the study group, 39 (39%) individuals had CD4 cell counts between 200 to 499cells/ mm³ and forty-eight (48%) individuals had CD4 cell counts lower than 200cells/ mm³.

In the study group among out of twenty-four HBV infected individuals 4 (16.7%) individuals had CD4 cell counts above 500cells/mm³, 6 (25%) individuals had CD4 cell counts between 200 to 499 cells/ mm³ and 14 (58.3%) individuals had CD4 cell counts lower than 200cells/ mm³.

In the study group out of four HCV infected individuals, one (25%) individual had CD4 cell counts between 200 to 499 cells/ mm³ and 3 (75%) individuals had CD4 cell counts lower than 200cells/ mm³.

Distribution of sociodemographic factors in the HBV and HCV infected individuals in the study group

As shown in table no.7, in the present study, in the study group HBV infection was found in 11 (45.8 %) males and 13 (54.2 %) females and HCV infection was observed in 3(75%) males and 1(25%) female.

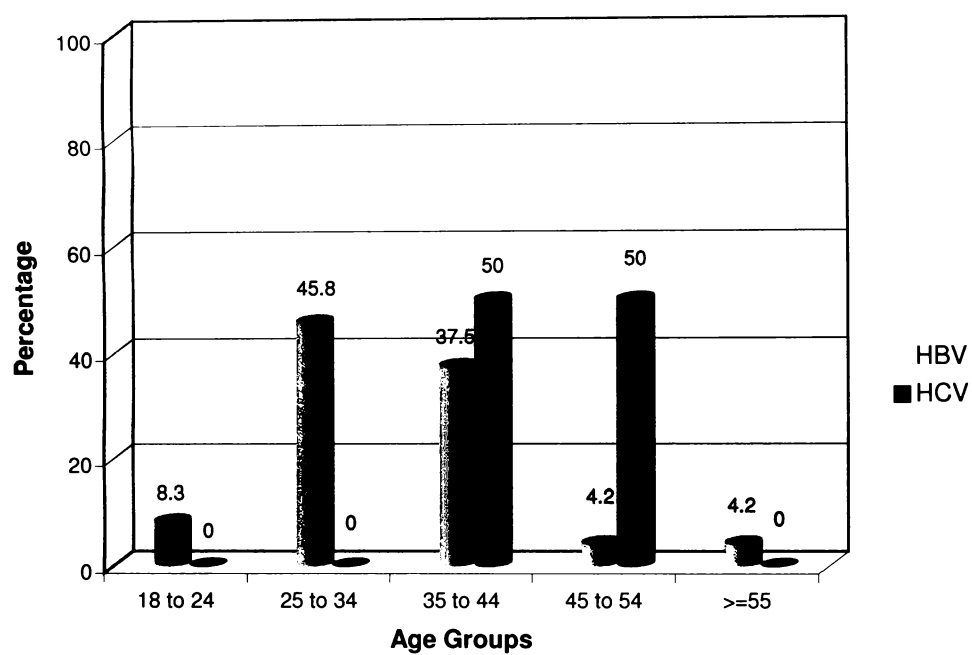
In the age wise distribution, 11 (45.8%) individuals from the age group of 25-34 yrs and 9 (37.5%) individuals from the age group of 35-44yrs were HBV infected in the study group. In the youngest age group of 18-24yrs the HBV infection was found in 2 (8.3%) individuals and in the 45-54yrs and 55yrs and above age group the HBV infection was found in 1 (4.2%) individual in each

group. In age wise distribution of the HCV infection, 2(50%) individuals were infected in the each age group of 35 to 44yrs and 45 to 54yrs.

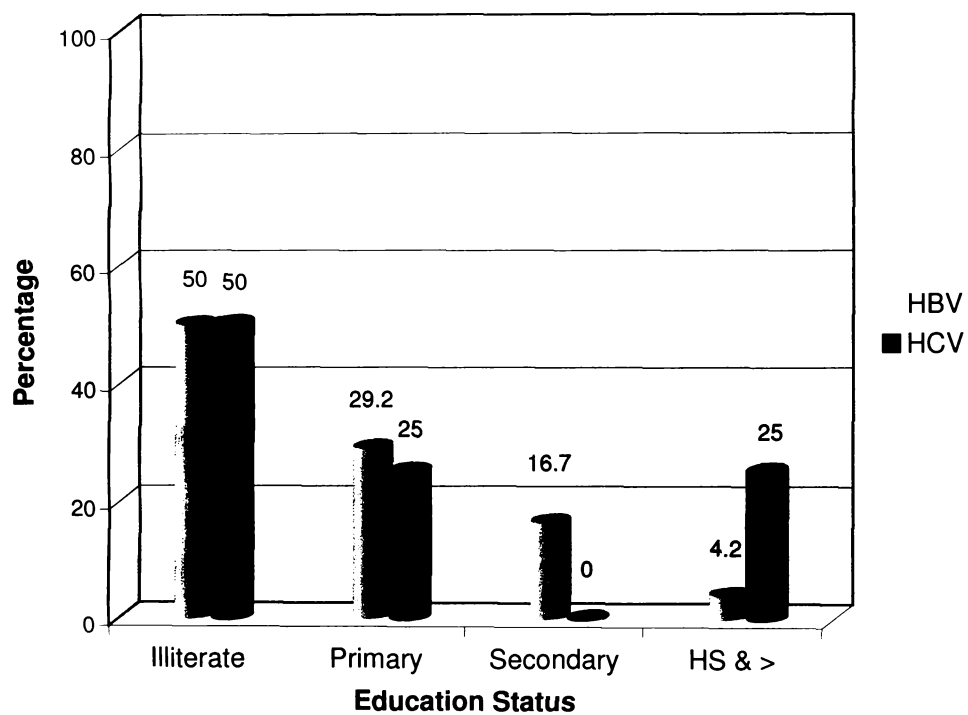
Table no.7 Distribution of Sociodemographic factors in the HBV and HCV infected individuals in the study group

		HBV infected individuals (n =24)	HCV infected individuals (n =4))
Sex	Female	13 (54.20)	1 (25)
	Male	11 (45.80)	3(75)
Age (years)	18 to 24	2 (8.30)	0
	25 to 34	11(45.8)	0
	35 to 44	9 (37.5)	2 (50)
	45 to 54	1 (4.2)	2 (50)
	>=55	1 (4.2)	0
Educational Status	Illiterate	12 (50)	2 (50)
	Primary	7 (29.2)	1 (25)
	Secondary	4 (16.7)	0
	HS & >	1 (4.2)	1 (25)
Religion	Hindu	20 (83.3)	3 (75)
	Christian	2 (8.3)	0
	Muslim	2 (8.3)	1 (25)
Mother Tongue	Hindi	8 (33.30)	3 (75)
	Marathi	14 (58.3)	1(25)
	Other	2 (28.3)	0
Marital Status	Yes	20 (83.3)	4 (100)
	No	4 (16.7)	0
Living with Family	Yes	15 (62.5)	1 (25)
	No	9 (37.5)	3 (75)
Knowledge of AIDS	Yes	5 (20.8)	1 (255)
	No	19 (79.2)	3 (75)

Figures in parenthesis indicate percentage



Graph no. 6 Age wise Distribution of HBV and HCV infection in the study group



Graph no. 7 Education status wise distribution of HBV and HCV infection

As shown in table no.7 in the present study, among the HBV infected individuals in the study group 12 (50%) individuals were illiterate, 7(29.2%) individuals had primary education, 4(16.7%) individuals had secondary, and 1(4.2%) individual had higher secondary education. In the present study, out of 4 HCV infected individuals in the study group 2(50%) individuals were illiterate, 1(25%) individual had primary education and 1(25%) individual had higher secondary education.

As shown in Table no.7 in the present study, out of 24 the HBV infected individuals in the study group, 20 (83.3%) individuals were Hindu, and 2 (8.3%) individuals were Christian and Muslim. In the present study, out of 4 HCV infected individuals in the study group, 3 (75%) individuals were Hindu, and 1 (25%) individual was Muslim.

In the present study, out of 24 HBV infected individuals, in the study group, mother tongue of 8(33.30%) individuals was Hindi, 14(58.3%) individuals was Marathi and the remaining 2(8.3%) individuals was Tamil or Telugu. In the present study, out of 4 the HCV infected individuals in the study group, mother tongue of 3 (75%) individuals was Hindi and 1 (25%) individual was Marathi.

Out of the total 24 HBV infected individuals, in the study group 20 (83.30%) individuals were married. In the present study, out of the total 4 HCV infected individuals in the study group all individuals were married.

In the present study, out of 24 HBV infected individuals in the study group, 15(62.5%) individuals used stay with their family and 9 (37.5 %) individuals had no knowledge of HIV/AIDS. Out of 4 HCV infected individuals in the study group,

1 (25%) individual stayed with his family and 3 (75 %) individuals had no knowledge of HIV/AIDS.

Table no.8 Distribution of High risk behaviours in the HBV and HCV infected individuals in the study group

		HBV infected individuals (n =24)	HCV infected individuals (n =4))
HRF-Extramarital affair/CSW	Yes	14 (58.3)	3 (75)
	No	10(41.7)	1(25)
Use of condom	Yes	0(0)	1(25)
	No	24(100)	3(75)
Blood transfusion Self	Yes	0(0)	0(0)
	No	24(100)	4(100)
History of past or Current STD	Yes	12(50)	2(50)
	No	12(50)	2(50)
Alcohol	Yes	11(45.8)	3(75)
	No	13(54.2)	1(25)

Figures in parenthesis indicate percentage

As shown in table no.8 in the present study, out of the 24 HBV infected individuals, in the study group, 14(58.30 %) had extramarital affairs/ frequent visit to CSWs. Out of the 4 HCV infected individuals, in the study group, 3 (75 %) individuals had extramarital affairs/ frequent visit to CSWs.

In the HBV infected individuals, in the study population, none of the HBV infected individuals in the study group had protected sex i.e. use of condom while having a sexual intercourse. In the HCV infected individuals, in the study population, 1(25%) individual in the study group practiced use of condom.

Out of the 24 HBV infected and 4 HCV infected individuals in the study group, none of the individuals had history of blood transfusion.

History of past or current STD infection were seen in 12(50%) HBV infected individuals in the study group. Out of 4 HCV infected individuals 2 (50%) individuals had a history of past or current STD infection.

Alcohol consumption was found in 45.8% of the HBV infected individuals in the study group. In HCV infected individuals in the present study 3(75%) individuals reported of alcohol consumption in the study group.

To see whether any of demographic and high-risk behavior variables like extramarital affairs/ frequent visit to CSWs, no safe sex practices, marital status, blood transfusion, history/current STD infection, alcohol addiction, knowledge of HIV/AIDS, living with family are the potential predictors of the HBV infection in the study population logistic regression analysis was performed but none of the variables were found significant predictor of the of the HBV infection.

Regression analysis to find the potential predictor of HCV infection among the HIV infected migrant workers was not possible due to the small number of the HCV infected population.

6. Discussion

Migration is one of many social factors that have contributed to the AIDS epidemic.^{127,128} Several studies have shown that people who are more mobile or *who have recently changed residence* tend to be at higher risk for HIV and other sexually transmitted diseases (STDs) than people in more stable living arrangements.^{127,129,130} In Uganda, people who had moved within the last 5 years were 3 times more likely to be infected with HIV than those who had lived in the same place for more than 10 years,¹³¹ and in South Africa, people who had recently changed their residence were 3 times more likely to be infected with HIV than those who had not.¹³²

Further HIV and Hepatitis B and Hepatitis C virus share routes of transmission, which leads to high rate of hepatitis co-infection in HIV, infected individuals. About, 10% of individuals with HIV-infection reveal presence of markers for chronic hepatitis B infection¹³³ and around 30% of all HIV-infected individuals in the western industrialized countries have a concomitant HCV infection.¹³⁴ The prevalence of the co-infection varies from place to place and is dependent on the high-risk behaviour exhibited by the HIV-infected individuals. HIV accelerates HBV and HCV liver disease, especially when HIV-associated immunodeficiency progresses. Morbidity and mortality due to sequela of HBV and HCV infections plays leading role in HIV -associated opportunistic infections.^{135,136}

Despite the elevated risk of HIV/AIDS in migrant workers, and reported elevation of infectious hepatitis in HIV infected patients, there is a dearth of published information on the prevalence of HBV and HCV among HIV infected *migrant workers*.

Thus in the present study we tried to determine the prevalence of HBV and HCV infection in HIV infected migrant workers, and to relate the demographic factors and high-risk behaviour exhibited by the migrant workers to the hepatic infection.

The study group, in the present study, included 100 asymptomatic HIV seropositive migrant workers admitted or visiting the OPD section of the Mathadi Kamgar Hospital Koperkhairne, Vashi. The control group included 50 healthy volunteers visiting Haffkine Institute for Annual Medical check up.

In the present study, the study group and the control group included almost equal number of males and females in the each group. Table no. 1.

Age distribution of HIV seropositive individuals shows that in the present study most of the individuals in the study group were in the age group of 25 –44 years. This might be because; this age group is the more sexually active, and have higher rates of multiple sexual partners.¹³⁷

Other researchers have reported similar results. In a hospital based study in western in Bhairahawa by Chander A et al a high prevalence of HIV (9%) was reported in the male population among the age group of 30-44yrs.¹³⁸ In Zaire peak seroprevalence for HIV positives was reported between 16-29 years.¹³⁹ Mohanty and colleagues observed that the largest number of HIV positives were

to be in the age group of 21-30 years, followed by 31-40 years for both the sexes. In a study conducted by Talib V H et al maximum number of HIV positive (75%) cases were reported in the age group of 20-30years.¹⁴⁰ In a study, among high risk group in Upper Assam, Nazarika et al observed that 48.4% of HIV infected cases belonged to the age group of 21-30 years followed by 31.3% in the age group of 31-40 years.¹⁴¹

Low level of education status is observed to be associated with high prevalence of HIV infection.^{142, 143} In the present study among the HIV seropositive study group 42% were illiterate and 28% had primary education whereas the remaining had secondary and higher secondary education.

In a study by Kristen C et al to detect HIV prevalence, risk behaviors, health care use, and mental health status of transgender persons a similar result was observed. In his study the low education status of the transgender population was significantly associated with the HIV prevalence.¹⁴²

Kelley A et al reported a similar significant association of low education status with the high prevalence of HIV in a study among a cohort of sex workers in Cameroon.¹⁴³

A study by Nunn AJ et al in Uganda, reported a higher HIV prevalence in people who had moved within the last 5 years were 3 times¹³¹ In one more study by Abdool KQ et al, in South Africa a similar result was reported. In his study, people who had recently changed their residence were 3 times more likely to be infected with HIV than those who had not.¹³²

In the present study 55(55%) individuals from the study group had moved away with their families and were 45(45%) staying alone or with colleague at work. In the control group all the individuals stayed with their families.

India's AIDS case surveillance system attributes that 86% of HIV infections to sexual risks.¹⁴⁴ In the present study, in the study group all the individuals reported of acquiring HIV through heterosexual contact. Though three individuals had a history of blood transfusion but these individuals acknowledged heterosexual contact as source of transmission of HIV in them. None of the individuals reported of intravenous drug use activity.

Multiple sexual partners (extramarital affairs) and frequent visits to the commercial sex workers were observed 59% of the individuals in the study group.

In a study by Elia JM et al in Kilimanjaro region of Tanzania, a similar high prevalence of previous history of multiple sex partners was observed in the HIV infected individuals.¹⁴⁵ VL Gilbert et al, studied the factors associated with heterosexual transmission of HIV to individuals without a major risk within England, Wales, and Northern Ireland. He and his colleagues also reported that the HIV positive individuals in their study had a significant association with a previous history of multiple sex partners.¹⁴⁶ In a study by Kirsten C et al, among transgender persons, the HIV prevalence among male to female transgender transmission persons was also significantly associated with the multiple sex partners.¹⁴²

None of the individuals in the control group had a past or present history of multiple sexual partners (extramarital affairs) and frequent visits to the commercial sex workers.

The proper and consistent use of latex condoms when engaging in sexual intercourse--vaginal, anal, or oral--can greatly reduce a person's risk of acquiring HIV infection. In the present study, among the HIV infected individuals in the study group only 9 individuals reported of condom use regularly, the rest 91 individuals, used condom infrequently or never while casual sex. This increases the probability of acquiring HIV infection; it also increases the possibility for the transmission of the virus to the partner.

A study by Elia JM et al, also reported high prevalence of unprotected casual sex among the HIV infected individuals in Kilimanjaro region of Tanzania.¹⁴⁵ VL Gilbert et al, in his study among HIV infected individuals without high risk behaviour, similarly reported a high prevalence of no condom use while casual sex in HIV infected individuals.¹⁴⁶

Enormous evidence indicating that both ulcerative and inflammatory STDs increase the risk of HIV infection has become available in the last two decades. In study by Pattnaik K et al presence of HIV antibody was highest in persons attending the STD clinic with symptoms of sexually transmitted diseases like urethral or cervical discharge and genital ulcer.¹⁴⁷ R. C. Bollinger et al, also reported, high percentage (79%) of active genital ulcer in the newly acquired HIV infections among the individuals visiting the STD Clinic.¹⁴⁸

But in the present study only 37% of the HIV infected individuals had a history of past or current STD infection.

Alcohol is a central nervous depressant, and in moderate quantities impairs judgement. Alcohol can therefore increase risk by diminishing personal control, increasing risk taking, thinning perception of risk from unprotected sex, or by simply increasing sexual activity. In a study by Elia JM et al Alcohol consumption was reported significantly associated with the HIV prevalence in Kilimanjaro region of Tanzania.¹⁴⁵

In the present study, among the study alcohol consumption was observed in almost 50% of the individuals. In the control alcohol consumption was observed in 10% of the individuals.

HBV surface antigen (HBsAg) is one of the first serum markers to appear during the course of HBV infection and can be detected 2 to 8 weeks before biochemical evidence of liver dysfunction and the onset of jaundice. The a determinant of HBsAg, a predicted double-loop structure projecting from the surface of the HBV particle ¹⁴⁹, is the major neutralizing epitope. Antibodies to the a determinant confer protection to all the common subtypes of HBV.

HBsAg is cleared within a few months in self-limiting illness. If HBsAg persists for more than 6 months, spontaneous clearance is very unlikely and the infected individual is considered to be a chronic HBV carrier.

Among the many commercially licensed HBsAg assays offered, enzyme-linked immunosorbent assays (ELISA) are the most commonly used.

There are three possibilities of false-negative results in commercial assays. In chronic HBV carriers, the HBsAg level may be below the detection limit.¹⁵⁰ Another explanation is that virus variants yield sequences that are not recognized by the antibodies employed in the assays.

The third reason is that in different geographical locations, vaccine-escape mutants are emerging under the selective pressure of active immunization. Vaccine-escape mutants within the *a* determinant of the S gene are not as effectively recognized by conventional diagnostic tests as wild-type particle.¹⁵⁰ A further explanation is that there are variants in other parts of the genome that down-regulate the production of HBsAg.¹⁵⁰

In the present study, among the study group nine individuals were positive for HBsAg by ELISA. In the control group only one individual showed presence of HBsAg by ELISA.

Hepatitis B e antigen (HBeAg) is a precore protein. It usually becomes detectable in the serum when HBsAg first appears. The presence of HBeAg in serum correlates with the presence of viral replication in the liver. During a typical acute infection, HBeAg is detectable early in the course of disease and disappears within several weeks, as acute hepatitis resolves. Persistence of serum HBeAg for more than 3-4 months indicates a progression to chronic hepatitis. In chronic hepatitis B, HBeAg may remain detectable for many months and usually years, signifying active replication of the virus. HBeAg is an important marker of viral replication, infectivity and ongoing liver injury.⁵⁸

In HIV infected individuals the spontaneous seroconversion of HBeAg is reduced. The prevalence of HBeAg-negative chronic hepatitis B as well as the HBV inactive carrier state thus tends to be lower in HBV/HIV-co-infected individuals.¹⁵¹

In present study, among the study group five individuals showed *presence of HBeAg* by ELISA. Thus five individuals had an active viral replication of HBV and thus individuals would be highly infectious.

Antibody to HBeAg (anti-HBeAg) is detectable as HBeAg disappears from the serum. The presence of anti-HBeAg early during acute hepatitis B is associated with a high likelihood of spontaneous resolution of acute infection. In chronic hepatitis B, the loss of anti-HBeAg tends to be associated with biochemical and histological improvement.⁵⁸

In the present study, among the study group eighteen individuals showed presence of Anti-HBeAg. Thus indicating a resolving infection in the eighteen individuals. Among the eighteen individuals four individuals were positive for HBsAg indicating a carrier stage.

The presence of hepatitis B virus surface antigen (HBsAg) in serum or plasma indicates hepatitis B virus (HBV) infection, but the detection of HBsAg does not provide information on the replicative activity of the virus. ELISA also obtains false negative results in detection of HBsAg due certain variation in the HBsAg protein structure this limitation can be overcome by detecting HBV DNA.

Further, the presence or absence of HBeAg is assumed to represent a high or low replicative state of HBV, respectively. However, precore mutant HBV

that do not produce HBeAg, irrespective of their rate of replication, have been described.¹⁵² Thus HBV DNA in serum would probably reflect more accurately the replicative activity of HBV than HBeAg.

In the past, two methods were available for detecting the presence of hepatitis B virus (HBV) DNA in serum, the endogenous DNA polymerase reaction and dot or slot blot hybridization analysis. Of the two techniques, slot blot hybridization analysis was demonstrated to be the more sensitive and has been shown to be able to detect HBV DNA in amounts as low as approximately 0.1 pg, which corresponds to approximately 3×10^4 virus genomes. However, the polymerase chain reaction (PCR) assay has been shown to detect 10^5 pg of HBV DNA. Thus, PCR is the most sensitive assay currently available for detecting serum HBV DNA.¹⁵³

In the present study HBV DNA was detected by amplifying a 598 bp long sequence from the highly conserved region of the Surface gene from the HBV genome.

In the present study, in the study group ten individuals were positive for HBV DNA by PCR. Out of the ten individuals, five were positive for HBeAg and HBsAg both by ELISA indicating an actively replicating viral infection. Four individuals were positive for HBsAg only, indicating a HBV precore mutation leading to the absence of HBeAg. But this mutation can only be confirmed by sequence analyses. Sequencing of viral genome in these patients would demonstrate a presence of an HBV variant with a G to A mutation at the nucleotide 1896 in the precore region just upstream of the core start codon if the

mutation is present.⁵⁸ The mutation results in the generation of a stop codon in the precore open reading frame. Consequently, this mutated open reading frame cannot be transcribed and HBeAg cannot be translated, accounting for HBeAg-minus serology.⁵⁸

In the present study among the study group, one individual showed presence of HBV DNA by PCR, but in the absence of HBsAg. This is typically observed in occult infections. Occult hepatitis B is defined by the detection of low levels of HBV DNA in serum samples and/or within the liver in people who are HBsAg negative.¹⁵⁴ In many instances occult hepatitis B is associated with positivity for hepatitis B core antibody (HBcAb) and/or hepatitis B surface antibody (HBsAb). However, in a study no HBcAb or HbsAb could be detected in a significant proportion of individuals.¹⁵⁴ In a study by Hofer M et al 89.5% of the 57 HIV infected individuals showed occult HBV infection.¹⁵⁵ However, in the present study one more sensitive HBV DNA PCR from the core region followed by sequencing of the amplified HBV genomes of the surface and the core region should confirm the individuals for the occult HBV infection.¹⁵⁴

The overall HBV infection in the study population was 24%, which is much higher than the HBV infection in the control population (2%).

A similar HBV prevalence of 23% was observed by Tankhiwale SS et al in HIV infected indoor and outdoor patients from Indira Gandhi Medical College and Mayo General Hospital, Nagpur exhibiting history of high-risk heterosexual behaviour.¹⁵⁶

The HBV prevalence in the present study is much higher than the HBV prevalence in healthy blood donors observed by Kothari et al. In the study by Kothari et al in 200 healthy blood donors the HBsAg prevalence was 3%.¹⁵⁷

While comparing the prevalence of HIV, HBV co-infection, the prevalence of co-infection varies from place to place. The prevalence of HIV and HBV co-infection in a study conducted by Rogers MC et al in Chennai among HIV positive men and women, showed a comparatively low prevalence of 4%.¹⁵⁸ A similar HBV prevalence of 3.5% was observed in another study by Shazia MA et al, in Mumbai among 200 HIV infected hospital admitted patients.¹⁵⁹ One more study by Swati Gupta and Sarman Singh from North India also observed a lower HBV infection of 5.3% in HIV infected patients when compared with the present study.¹⁶⁰

This low prevalence observed in these studies as compared to the present study might be because only one marker (HBsAg) was analyzed in all the studies whereas in the present study along with HBsAg, HBeAg, Anti-HBeAg and HBV DNA PCR was performed to assess the HBV prevalence. Further in all of these studies none of the individuals mentioned were reported of exhibiting high-risk behaviours whereas in the present study all the individuals were migrant workers, exhibiting high-risk sexual behaviour.

In a study conducted by S. M. Ruta et al in Romania a very HBV prevalence of 78% was observed among the HIV infected Individuals as compared to the present study. This high prevalence particularly, in this study is because their

control population of HIV negative individuals itself had a very high HBV prevalence of 32%.¹⁶¹

Detection of antibodies to HCV (anti-HCV) remains the most practical means of diagnosis of HCV infection. The first generation anti-HCV assay detected antibodies to a single recombinant antigen (c-100-3), corresponding to small protein NS3 and most NS4 gene products, in ELISA format (EIA-1). EIA-1 however, lacked sensitivity (particularly for genotypes other than 1) and gave 30-70% false positive results among blood donors.⁷²

Second generation ELISA test (EIA-2) included the c100-3 and recombinant proteins from the core (c22-3) and NS3 (c33c). In some EIA-2 (c22-3) and (c33c) are combined into a single recombinant protein (c200).⁷²

The EIA-2 tests have proven to be more sensitive and specific than EIA-1 test and has significantly shorten the window period of infectivity.

The third generation test incorporates NS5 as an additional recombinant protein, while the core and NS4 proteins are present as either recombinant proteins or as synthetic peptides, depending on the manufacturer. The EIA-3 test are even more sensitive and specific than EIA-2. The increased sensitivity is due to optimization of the antigens already present in EIA-2 and not to the inclusion of NS5.⁷²

In the present study 4 individuals were positive for the antibodies to HCV by 3rd generation ELISA (EAI-3) for anti-HCV. In the control group none of the individuals were positive for the antibodies to HCV.

Antibody detection methods are of restricted use because there is a mean window period of 22 weeks between infection and seroconversion of HCV.¹⁶²

Further the detection of anti-HCV by enzyme immunoassay to screen HCV infection in HIV-1infected individuals may yield false negative results, especially in patients with advanced immunosuppression. The HCV viremia and no detectable HCV antibodies by third-generation immunoassay were found in individuals with a CD4 count of <100 cells/mm³. Thus detection of HCV RNA should be done especially in HIV–infected individuals to avoid any false negative results and to detect infection within the window period.¹⁶³

In a study by Inmaculada C et al various nucleotide sequences from the 5'untranslated region, CORE region, NS3 region, NS3/NS4 region and NS5 of the HCV genome were analyzed for detection of HCV by Reverse-Transcriptase PCR (RT-PCR). In his study it was finally reported that the amplification of the 5' Untranslated region of the HCV genome, is the best method for detecting serum HCV RNA by RT-PCR.¹⁶⁴

In the present study the HCV RNA detection was carried by amplifying a 323 base pair long sequence by RT-PCR from the most conserved 5'untranslated region of the HCV genome.

Among the study group, in the present study, HCV RNA was detected in 4 individuals in the study group by RT-PCR. In the control group HCV RNA was detected in none of the individuals.

In a study by Sarah L. et al, the HCV infection rate among patients HIV positive patients without HCV antibodies was 6.0%.¹⁶⁵ This was also observed

by a study by Rossi et al., in his study 5 of 13 patients infected with HCV failed to develop antibodies to HCV, despite prolonged HCV viremia.¹⁶⁶

However, in the present study all the individuals positive for HCV RNA by RT-PCR showed the presence of anti-bodies to HCV, thus indicating no false positive results by the HCV ELISA and no window period infection of HCV.

Thus the overall HCV prevalence in our study group was 4% lower than other studies from Western countries and other parts of India. This can be related to the type of risk-groups in this study. In Argentina, Fainboim et al¹⁶⁷ studied 484 HIV-infected patients and observed that 58.5% of them were co-infected with HCV. Moreover, it was observed that 234 (48.3%) of these patients were intravenous drug users (IDU). Saha et al¹⁶⁸ reported an HCV co-infection rate of 92% in intravenous drug-users from Northeast India. However, in our study group there were no IVDUs while the majority of HIV infections as reported by the participants were heterosexually transmitted.

The low HCV prevalence in our data could be attributed to the mode of transmission of the virus as in the present study the reported route of virus transmission was through heterosexual contact. While epidemiological evidence indicates sexual transmission of HCV, occurs much less efficiently than HBV or HIV.

In northern Europe or North America, cross sectional studies suggest that the probability of the sexual partner of an HCV infected patient being found to be infected is of the order of 0–3%. Rates in other regions, such as southern Europe and the Far East, are higher and reflect the higher background prevalence in

these countries. Other factors that have been suggested which may affect the rate of transmission include the stage of disease and HIV status, as well as the duration of the period of exposure.¹⁶⁹

Substantiating this a study by J Tor et al observed that HCV transmission by heterosexual route was very low and was strikingly related to the prevalence of HIV ¹⁷⁰, in this study only 11% of sexual partners of unknown hepatitis C virus antibody state acquired the infection although a 73% seropositive for hepatitis C virus could be expected.

The titer of HCV RNA and HCV genotype do not appear to influence the risk of HCV transmission, but high-quality studies to assess these virological factors are lacking. The stage or clinical status of liver disease of the HCV-infected individual is also not predictive of transmission risk. However, studies to date have focused only on individuals with chronic disease; whether individuals with acute hepatitis represent a subgroup at particular risk for HCV transmission is unknown.

Statistical analysis showed that none of the high-risk behaviours and sociodemographic factors like extramarital affairs/ frequent visit to CSWs, no safe sex practices, marital status, blood transfusion, history/current STD infection, alcohol addiction, knowledge of HIV/AIDS, living with family were found to be significant predictors of HBV infection.

Lower CD4 cell counts are positively associated with development of the Hepatitis B chronic carrier state.¹⁷¹ Studies suggest that a greater degree of immunosuppression, as indicated by CD4 count, correlates with worsening

outcome due to HCV. Rockstroh et al found that liver failure was observed only in HIV-HCV co-infected patients whose CD4 count fell from 230 to 50, and not in any patients with stable CD4 counts.¹⁷²

In the present study, among the study group, in the HIV-HBV co-infected individuals 14 (58.3%) individuals had CD4 cell counts less than 200cells /mm³ which indicates a future risk of liver cirrhosis. In the HIV-HCV co-infected individuals, in the present study 3(75%) individuals had CD4 cell counts less than 200cells/mm³. Thus increasing the chances of chronic carrier state and liver failure.

In the present study the HBV and HCV prevalence in the HIV infected migrant workers was higher as compared to general population. Even though the statistically association with any of the sociodemographic and high-risk behaviour exhibited by the study population between the hepatic viral infections was insignificant, it gives us the large disease burden of the hepatic viral infections in the study population. The study will help us to carry future intervention and to educate the migrant workers. Further, looking at the high HBV prevalence in the migrant workers all the HIV infected migrant workers should be vaccinated for HBV.

7. Summary and Conclusion

The present study was undertaken to determine the HBV and HCV prevalence in the HIV infected migrant workers. The HBV prevalence was analyzed by detecting the presence of HBsAg, HBeAg, and Anti-HBeAg by ELISA and HBV DNA by Polymerase Chain Reaction. The HCV prevalence was analyzed by detecting the presence of Anti-HCV by ELISA and HCV RNA by Reverse-Transcriptase Polymerase Chain Reaction.

In the present study, hundred HIV seropositive asymptomatic migrant workers were included in the study group, admitted or visiting the OPD of Mathadi Kamgar Hospital, Koperkhairne, Vashi. The control group comprised of 50 healthy volunteers visiting for annual medical checkup at Haffkine Institute.

Seventy three percent of the individuals in study were in the sexually active age group of 25-44yrs. In the study group 68% of the individuals had low education status i.e. illiterate or primary education. These factors can have a significant impact on the prevalence of HIV as the individuals in the sexually active age group of 25-44yrs have been found to high number of multiple sex partners. Further low level of education also leads misinterpretation of the risk aversive behaviors.

In the study group, 59% of the individuals had a history of frequent visits and had multiple sex partners and 91% of the individuals had unprotected sex. Alcohol consumption was observed in almost 50% of the individuals in the study group.

In the study, among the study group the overall HBV prevalence was higher (24%) as compared to the control population (2%) in the study and the

prevalence observed in the other studies among the healthy blood donors in India and population without any high-risk behaviours as exhibited by the migrant workers.

Among the HBV infected individuals five showed the presence of HBsAg, HBeAg and HBV DNA indicating a HBV infection with actively replicating virus. Thus indicating a highly infectious state of the infection, which would enhance the transmission of HBV. Four individuals in the study population showed presence of HBsAg and HBV DNA in the absence of HBeAg, indicating a precore mutation. This should be confirmed further by sequence analysis. In general, the long-term prognosis of HBeAg-negative Hepatitis B is poor. It has been found to develop cirrhosis over a mean period of 6 years. Further mortality and development of hepatocellular carcinoma is within 4 years from presentation, which is much higher compared with a group of patients with HBeAg- positive Hepatitis B.

One individual in the study group showed presence of HBV DNA with no detectable HBsAg, this is typical of occult Hepatitis B infection. This occult infection should be confirmed further by performing by one more PCR from the precore region and sequence analysis of both the surface and precore gene. The pathogenic role of occult HBV infection is unclear. It might also be a false negative result.

Eighteen individuals in the study group showed presence of antibodies to HBeAg indicating a resolving HBV infection.

In the study, among the study group the overall HCV prevalence was 4%. There were no false negative test observed, and all the individuals showing

presence of anti-HCV had HCV RNA detected by RT-PCR. None of the individuals in the control group were infected with HCV. The prevalence of HCV observed in the study is high as compared to the general population, however was low compared to high-risk populations such as injectible drug users.

The high rate of HBV, HCV, among the HIV seropositive, not only puts these infected patients at greater risk for complications such as end stage liver disease, but it also makes them a potential source of transmission of these viruses to the surrounding population.

Further studies are required to determine specific risk factors for transmission of hepatitis viruses in the migrant workers and a larger data preferably multicentric is urgently needed to clarify the picture.

To diminish the risk of contracting the viruses, in the migrant workers, short-term education programme on HBV/HCV and HIV/AIDS prevention and effective interventions should be implemented. Aggressive intervention programs that include key components such as behavior change communication, STD treatment, and increased access to condoms, and increased access to testing should be implemented. Mass media outreach, counseling, and peer education can also be used to increase awareness and life skills among the migrant workers, those with high-risk behaviors. Such prevention activities must then be evaluated. Preventive measures, such as vaccination for HBV, can thus be appropriately tailored to these hard to reach high-risk group of migrant workers, thus reducing the emerging impact.

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9. Appendix

1. 1M Tris –Cl (pH 7.4)
 Final volume: 10ml in Distilled Water
 Tris-Cl 1.57 gm

2. 0.5 M EDTA
 Final volume: 10ml in Distilled Water
 EDTA 1.86gm

3. Tris EDTA (TE) Buffer
 Final volume: 1000ml in Distilled water
 1M Tris –Cl (pH 7.4) 10ml
 0.5 M EDTA (pH 8.0) 2ml

4. Tris Borate EDTA (TBE) Buffer
 Final Volume: 1000ml in Distilled water
 Strength: 5X
 Tris Base 54gm
 Boric Acid 27.5gm
 0.5M EDTA (pH 8.0) 20ml

5. Gel Loading Buffer

Final Volume: 10ml

Strength: 6X

Bromophenol Blue	25mg
Xylene Cyanol	25gm
Sucrose	4gm

6. DNA Ladder Loading Mix

DNA Ladder	1µl
Loading Buffer	1µl
Deionized Water or DNase-RNase free Water	4µl

7. Incomplete RNA Extraction Buffer (without Proteinase K)

Final Volume: 50ml in DNase-RNase Free water

Strength: 2X

		Final Concentration
Tris-Cl	0.785gm	100mM
EDTA	0.372gm	20mM
NaCl	1.1688gm	400mM
SDS	2gm	4%

8. Proteinase K Stock 20mg/ml
Final volume: 5ml in Distilled water
Proteinase K 100mg

9. Complete extraction buffer
Final volume: 1.5ml for 5 extraction reactions
Incomplete Extraction Buffer 1.35ml
Proteinase K stock (20mg/ml) 0.15ml

10. Glycogen solution (20mg/ml)
Final Volume: 2ml in DNase-RNase Free water
Glycogen 40mg

11. Ethidium Bromide stock (10mg/ml)
Final Volume: 5ml in Distilled water
Ethidium Bromide 50mg

12. Agarose Gel (1.5%)

Preparation: Dissolve 0.45gm of agarose (Banglore Genei) in 30ml of Tris Borate EDTA buffer by heating. Add 3 μ l of Ethidium Bromide stock solution (10mg/ml) when the agarose solution is luke warm and mix it gently avoiding any air bubbles. Pour the solution in the gel caster with the comb properly placed at one end. Leave the gel caster undisturbed for 1hr for forming the gel.

10. Synopsis

**A SYNOPSIS OF THE THESIS
TO BE SUBMITTED TO THE UNIVERSITY OF MUMBAI
FOR
THE DEGREE OF MASTER OF SCIENCE
IN MICROBIOLOGY**

Title of the thesis	Prevalence of HBV and HCV in HIV infected Migrant Workers in Mumbai.
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PREVALENCE OF HBV AND HCV IN HIV INFECTED MIGRANT WORKERS IN MUMBAI

Acquired Immune Deficiency Syndrome (AIDS) is the name of a life threatening condition caused by the Human Immunodeficiency Virus (HIV). It is a serious disorder of immune system in which body's normal defense against infection breaks down, leaving it vulnerable to a host of life threatening infections including malignancies. The HIV/AIDS global epidemic has greatly exceeded earlier predictions and it is now clear that it has the potential to affect all countries and all population groups. The total number of people living with the Human Immunodeficiency Virus (HIV) in 2005 has reached 40.3 million [36.7–45.3 million] ¹. Five million people were newly infected with the virus in 2005¹.

In India, since the detection of HIV infection in a female commercial sex worker (CSW) from Tamil Nadu in 1986, there has been a steady increase in the number of HIV infected individuals. In 1999, the number of HIV infected individual was 3.5 million, which has increased to 5.21 million in 2005 ². HIV/AIDS has emerged as one of the most serious public health problems in the country.

Our country has an unbalanced development, which leads to internal migration of large populations. While some regions and sectors fall behind in their capacity to support populations, others move ahead and people migrate to access these emerging opportunities. Industrialization widens the gap between rural and urban areas, inducing a shift of the workforce towards industrializing areas. Moreover, the process of migration is influenced by social, cultural and economic factors and outcomes can be vastly different for men and women, for different groups and different locations. It is also very

difficult to monitor and control these internal migrations. As this population is floating, it is difficult for them to reach with education and preventive health, and they also tend to be deprived to access health care. Because of their lifestyles, language and culture of they are not exposed to the educative media. Hence HIV prevention messages, which impact their interpretation and utilization of risk aversive behaviour goes unattended. In fact the culture of migrancy itself affects the extent of risky behaviour practiced for example the separation from families may drive them to seek commercial sex or casual sex. Thus, migrant workers are more vulnerable to HIV infection.

Once the individual is infected, the virus breaks down the immune system and makes the host vulnerable to multiple opportunistic infections (OIs) caused by various pathogens. These opportunistic infections remain principally responsible for the morbidity and mortality associated with HIV disease.

As improvements in HIV treatment have reduced the number of deaths due to other opportunistic illnesses, liver disease due viral hepatitis infection has become a leading cause of hospitalization and death in people with HIV/AIDS ⁴⁸. Infections with the hepatitis B or C viruses are the commonest coinfection in people with HIV as they have similar routes of transmission. It is categorized as an HIV-related opportunistic illness ⁴⁸. Complications related to HIV/HCV or HBV coinfection are becoming an important medical issue.

Hepatitis B Virus is the most common cause of end-stage liver disease worldwide and is highly endemic in areas of the world with high incidence rates of HIV infection. Coinfection with hepatitis B virus (HBV) and HIV is common in 70-90% of HIV-infected individuals. ³⁻⁵

Individuals infected with HIV are approximately three to six times more likely to become chronic carriers of HBV than those who are HIV-negative ^{6,7}. Lower CD4 cell counts are positively associated with development of the chronic carrier state ⁶.

The outcome of HBV monoinfection depends on the kinetics of virus–host interactions and in particular on the strength and quality of the innate and adaptive humoral and cellular immune response ⁸. In persistently infected patients who fail to downregulate or clear HBV, the continued expression of inflammatory cytokines and recruitment of activated lymphomononuclear cells to the liver ultimately results in fibrosis, cirrhosis and hepatocellular carcinoma ⁸. HIV establishes a chronic and latent infection that induces extensive damage of the immune system through virus-related and indirect pathogenic mechanisms ⁹. Fibrosing cholestatic hepatitis, a severe and rapidly progressive form of HBV infection thought to be due to a direct cytopathic effect of the virus, has anecdotally been described in HIV positive patients ¹⁰ and in other groups of immunosuppressed patients. However, in most cases with an absent host defense, HBV replication in the liver is not cytotoxic or not-cytopathic ⁸. In HIV-infected patients, host defenses are not ‘absent’ even in the most advanced stages and are often aberrantly activated ⁹. In such cases, the quality of HIV-induced dysregulation of a still active anti-HBV immune response may be crucial in determining the quality of necro-inflammation and of the consequent ‘quantity’ of fibrogenesis. It is conceivable that HIV co-infection influences the course and natural history of HBV infection through its heavy modification of the immune response. But studies comparing anti-HBV innate and adaptive immune responses of HIV-infected and non-infected patients at various stages of the disease and during anti-retroviral therapy are still lacking.

Studies have shown disparate results with regard to the influence of HBV infection on the course of HIV. Most studies have not detected any difference in HIV disease progression ¹¹⁻¹³.

Hepatitis C virus (HCV) infects an estimated 3% of the world population. The prevalence of HIV–HCV co-infection varies markedly depending on the route of HIV infection. Although both HIV and HCV share the same routes of transmission, the relative efficiency of transmission of these two viruses varies. HCV is approximately ten times more infectious than HIV by percutaneous blood exposure to small volumes of blood. HCV is transmitted by 15–30 of every 1000 accidental needle- stick injuries ¹⁴, compared with 3 per 1000 for HIV ¹⁵. In contrast, HIV is more transmissible than HCV between heterosexual partners and from a mother to her infant ¹⁶⁻¹⁹.

HIV infection appears to affect each stage in the natural history of HCV infection. HIV is associated with higher HCV RNA levels; accelerated progression of HCV- related liver disease particularly with CD4 cell counts below 100 per microlitre, and earlier onset of hepatocellular carcinoma ²⁰⁻²³. But mechanisms that could explain the accelerated progression of Hepatitis C in HIV- positive patients are currently only poorly understood.

The decline of cell-mediated immunity associated with HIV infection allows greater replication of HCV. In turn, a quantitative increase in HCV viraemia may explain the higher frequency of cirrhosis among HIV-infected patients. However there is no clear correlation between the level of viraemia and the severity of liver disease ²⁴. HIV infection could contribute to liver injury through immune or direct viral effects. Mechanisms that are important for tissue damage could be modified in immunocompromised patients, such as the local pattern of cytokine production,

expression of adhesion molecules or release of fibrogenic factors ²⁵. HIV co-infection has been shown to importantly alter the HCV-specific cytokine response towards a greater production of proinflammatory type 1 cytokines ²⁶. Moreover, HIV infection itself may exert a direct cytopathic effect on liver cells independently of other cofactors ²⁷ since HIV-1 infects not only peripheral blood CD4 cells, but also specialized intrahepatic immune cells such as Kupffer cells, intrahepatic mononuclear cells and hepatocytes²⁸.

In the era prior to HAART, initial cross-sectional data appeared to indicate that the presence of HCV infection did not influence surrogate markers of HIV infection (such as CD4 count, p24 antigen, etc.) ²⁹, and there was no apparent impact on the progression of HIV disease ³⁰. However, the fact that all of the studies were conducted prior to the introduction of HAART, and may have been confounded by other factors that accelerated HIV disease, it is possible that the high mortality associated with AIDS and opportunistic infections during that era may have hidden any contributory effect of HCV to HIV progression. It will therefore be of interest to determine whether HCV infection prospectively influences HIV disease progression in the HAART era.

As the proper HIV and Hepatitis B or C therapy is concerned, it is advocated to start treatment of hepatitis B or C preceding HIV. Since Hepatitis B and C is widely regarded as an opportunistic infection in HIV-infected individuals and the risk of hepatotoxicity is higher in coinfecting patients than in HIV mono-infected patients. Treatment should be provided to patients with CD4 cell counts greater than 350 cells/mm³, relatively low plasma HIV- RNA levels and no active consumption of illegal drugs or high alcohol intake⁵⁰.

Despite the elevated risk of HIV/AIDS in migrant workers, and reported elevation of infectious hepatitis in HIV infected patients, there is a dearth of published information on the prevalence of HBV and HCV among HIV infected migrant workers.

In the present study, we determine the cross sectional prevalence of HBV and HCV in HIV infected migrant workers from Mumbai and sought to identify potential risk factors predisposing to the infections.

Rationale

HIV-infection rates are increasing dramatically in India. It is highly prevalent in particular groups of people. These people show high-risk behaviour, which increase their possibility of acquiring HIV. One of such high-risk group is the migrant workers. Significant migration exists between states in India. Migration in support of work for extended periods takes migrants away from the social environment provided by their families and community. This leaves them outside the usual normal constraints and thus more likely to engage in high-risk behaviour and increase their probability of getting infected with HIV. The infection with HIV results in immunosuppression that is the result of both quantitative and qualitative abnormalities of the helper/inducer subset of T lymphocytes. This results in an array of opportunistic pathogens and malignancies thereby increasing the HIV related mortality.

Morbidity and mortality associated with HIV infection have decreased with the introduction of highly active antiretroviral therapy. But of recent concern is the increase of opportunistic infections; particularly hepatitis B virus and hepatitis C virus in HIV infected population. Because of the shared route of transmission, a significant number of HIV-infected patients are coinfectd with hepatitis B or C virus. There is a lack of data regarding HBV and HCV prevalence among HIV infected migrant workers .Our study will help us to asses mediating factors in behavioral, biological and social agents accountable to these infections. This study may in turn also assist to implement proper intervention against the potential spread of these infections in view of the transmigration of these migrants, which in sequence may spread the infections among innocent rural people.

Aims and Objectives:

- To study sociodemographic factors and the high-risk behavior exhibited by the HIV seropositive Migrant Workers visiting Mathadi Kamgar Hospital, Koperkhairne, and Vashi.
- To study the immunological status by determining the CD4 cell count.
- To study the prevalence of HBV and HCV infections in HIV seropositive migrant workers with the help of following assay.

HBV

- a) ELISA for Detection of HBsAg, HBeAg and Anti-HBeAg
- b) HBV DNA detection by PCR

HCV

- a) ELISA for Detection of Anti-HCV
 - b) HCV RNA detection by RT-PCR
- To study the association of HBV and HCV infection with the sociodemographic factors and the high-risk behavior exhibited by the study population.

Materials and Methods

1. Study Population

The study population comprised of 100 HIV seropositive Migrant Workers irrespective of age and sex attending the OPD or admitted to Mathadi Kamgar Hospital, Koperkhairne.

The control group comprised of 50 healthy individuals visiting Haffkine Institute for their annual medical checkup. Ethical committees approval was taken for the project.

Samples were collected after informed consent. All the individuals were subjected to a structured questionnaire, which included data on demographics, history of STD, detail clinical history and sexual behaviour. Presumptive diagnosis of sexually transmitted disease was based on the reports and medical history provided by the hospital.

2. Blood Collection

Ten ml of blood was drawn by venipuncture from all the individuals. The blood was aliquoted appropriately in K3-EDTA and plain bulb. The serum was separated from the plain bulb, aliquoted and stored at -20°C till further use. The whole blood was used for evaluating the CD4-CD8 count.

3. HIV Serostatus Confirmation

Confirmation of the HIV serostatus was done by HIV1 and HIV2 ELISA as per the protocol provided by the manufacturer for the study population and control group. (Sandwich ELISA SD HIV 1/2 ELISA, Ver.3, Standard Diagnostic Inc., and Indirect Elisa, Micro Lisa, J. Mitra and Co.)

4. CD4-CD8 Counts

100µl of blood was drawn from K3-EDTA containing bulb. 50µl each was added to the reagent pair tubes for the estimation of CD4/CD3 and CD8/CD3. After incubation in the dark for 90 minutes, the fixative solution was added to these tubes the CD4+ T cells were counted using FACScount (Becton Dickinson). The sorting and analysis of the cells was fully automated.

5. HBV

5a.HBsAg ELISA

HBsAg is the first serological marker to appear in the circulation, well before clinical symptoms, and is the viral component usually found in the highest concentration in the serum of HBV-infected patients.

Principle: It was a third generation ELISA tests for the detection of HBsAg in human serum. The ELISA adapted the sandwich principle (antibody-antigen-antibody) as the basis of the assay. When anti-HBS+HRPO (horseradish peroxidase) conjugate was incubated with the specimens containing HBsAg, (antibody)-(antigen)-(antibody + HRPO) complexes were formed on the wells. After washing, the activity of the peroxidase on the wells reflected the presence of HBsAg in the specimen. (**Diasorin ETI-MAK-4**)

5b. HBeAg ELISA

The presence of HBeAg in serum indicates active replication of HBV; this may occur in persons with acute hepatitis B or in persons who have become chronic HBV carriers, with persistence of HBsAg.

Principle: The assay was a direct, non-competitive test based on the use of polystyrene micro wells coated with mouse monoclonal antibodies to hepatitis B e antigen (HBeAg). An enzyme tracer containing horseradish peroxidase-labelled mouse monoclonal antibody to HBeAg detected any captured HBeAg. [Diasorin **ETI-EBK PLUS**]

5c. Anti-HBeAg ELISA

Anti-HBe usually appears in the serum after HBeAg is no longer detectable and may persist for several years after the patient's recovery from acute HBV infection.

Principle: The assay was a competitive test based on the use of polystyrene micro wells coated with mouse monoclonal antibodies to hepatitis B e antigen (HBeAg). An enzyme tracer containing horseradish peroxidase-labelled mouse monoclonal antibody to HBeAg detected any captured HBeAg/ anti-HBe complexes. [Diasorin **ETI-AB-EBK PLUS**]

5d. Detection of HBV DNA Detection by PCR (Results will be added in the thesis)

5d.i Extraction of DNA

DNA was extracted from 250 µL of serum using DNAzol B (Molecular Research Center, USA) according to the manufacturer's guidelines. Serum samples were mixed with DNAzol BD and DNA is precipitated from the resulting lysate with isopropanol. The DNA

pellet was washed successively with DNAzol BD and ethanol, and dissolved in 50 µL of NaOH (8 mM) and used for amplification of HBV DNA.

5d.ii PCR

The PCR reaction will be performed in a volume of 50 µL PCR reaction mixture (Bioron Ltd.). Primer sequences used were forward primer, 5'- TCG, GAA, ATA, CAC, CTC, CTT, TCC, ATG, G-3' (HBV genome 1 353-1 377); reverse primer, 5'-GCC, TCA, AGG, TCG, GTC, GTT, GAC, A-3' (HBV genome 1 702-1 681) were used ³¹. The condition of each cycle will be, denaturation at 94°C for 30 sec, primer annealing at 55°C for 30 sec, and elongation at 72°C for 30 sec, followed by a final 10-minute elongation at 72°C. The length of PCR product was 350 bp.

6. HCV

6a. Anti-HCV ELISA

The assay was an immunoenzymatic method in which the wells of the microtitre plate are coated with the recombinant antigens representing epitopes of HCV: Core, NS3, NS4 and NS5. Antibodies specific for HCV present in the sample, will form a stable complex with the HCV antigen on the well. After washing to remove the unbound material a rabbit anti-human IgG labeled with horseradish peroxidase was added which develops a color with the help of a chromogen bound with the antigen-antibody complex.

6b.Detection of HCV RNA by Reverse Transcriptase PCR (RT-PCR)

6b.i Extraction of RNA

The RNA was extracted from 250µl serum, with 50 mmol/L Tris-HCl (pH 8.4), 10 mmol/L EDTA, 0.2 mol/L NaCl, 2% SDS, and 1 mg/mL Proteinase K by incubating at 60°C for 30 minutes. The samples were then extracted once with phenol-chloroform and once with chloroform. The RNA was precipitated at room temperature using isopropanol and 20 mg of glycogen as carrier. The precipitated RNA was washed with 70% ethanol; air dried, and redissolved in 15 µL of DEPC treated milli Q water^{32,33}.

6b.ii RT-PCR

The amplification of the hepatitis C virus (HCV) RNA was carried out by one-step RT-PCR (Qiagen® One-step RT-PCR Kit). Reverse transcription and PCR was carried out sequentially in the same tube. All components required for both reactions were added during the setup. The RT-PCR was carried out in a 50µL reaction mix. Twenty-five pmol of reverse primer 18R (5'-GTG, CAC, GGT,CTA, CGA, GAC, CTC-3') and forward primer 11F (5'-GGC, GAC, ACT, CCA, CCA, TAG, ATC, ACT-3') derived from the 5' NCR region of the HCV genome were used. Reverse transcription reaction was carried out at 50 °C for 30 minutes. Then a initial PCR activation step was carried at 94 °C for 2 minutes and further the 40 cycles of PCR were run as follows; denaturation at 94°C for 30sec, primer annealing at 61°C for 30 sec and extension at 68 °C for 45sec³⁴.

Results

In the present study 100 known HIV seropositive migrant workers were screened for the prevalence of HBV and HCV infections from Mathadi Kamgar Hospital, Koperkhairne, Vashi. A HIV-1/2 ELISA confirmed the serostatus of all the migrant workers and the control group. The control group comprised of 50 healthy individuals visiting Haffkine Institute for their annual medical checkup. All the individuals were subjected to a structured questionnaire, which included data on sociodemographics, STDs, clinical history and sexual activities.

The sociodemographic characteristics of the study group and the control group are compared in the table no.1a and 1b. The study group comprised of 53 (53%) males and 47 (47%) females. The control group had 29 (58%) males and 21 (42%) females. In the study population 73 (73%) individuals belonged to the sexual active age group of 25-44yrs. In the control group 30 (60%) individuals belong to the sexual active age group of 25-44yrs. Among the study group 42 (42%) individuals were illiterate, 28 (28%) had primary education, 17 (17%) had secondary education and 14 (14%) had higher secondary education, whereas the control group had 100% literacy level in which 36 (72%) individuals had a secondary education and 14 (28%) had higher secondary education. Out of 100 individuals in the study group 45 (45%) stayed away from their family whereas in control group all stayed with their families. In the study group 71 (71%) individuals had no knowledge of HIV/AIDS whereas all the individuals in the control group were aware of information regarding HIV/AIDS.

Table no.1a Distribution of demographic variables between cases and controls

Variables		Group				Total	
		Case		Control			
		(n=100)	%	(n=50)	%	(n=150)	%
Sex	Female	47	47.00	21	42.00	68	45.30
	Male	53	53.00	29	58.00	82	54.70
Age (years)	18 to 24	10	10.00	5	10.00	15	10.00
	25 to 34	35	35.00	16	32.00	51	34.00
	35 to 44	38	38.00	14	28.00	52	34.70
	45 to 54	14	14.00	12	24.00	26	17.30
	>=55	3	3.00	3	6.00	6	4.00
Educational Status	Illiterate	42	42.00	0	0.00	42	28.00
	Primary	28	28.00	0	0.00	28	18.70
	Secondary	17	17.00	36	72.00	53	35.30
	HS & >	13	13.00	14	28.00	27	18.00

Table no.1b Distribution of demographic variables between cases and controls

Variables		Group				Total	
		Case		Control			
		(n=100)	%	(n=50)	%	(n=150)	%
Religion	Hindu	85	85.00	50	100.00	135	90.00
	Christian	7	7.00	0	0.00	7	4.70
	Muslim	8	8.00	0	0.00	8	5.30
Language Spoken	Hindi	40	40.00	9	18.00	49	32.70
	Marathi	52	52.00	41	82.00	93	62.00
	Other	8	8.00	0	0.00	8	5.30
Married	Yes	93	93.00	47	94.00	140	93.30
	No	7	7.00	3	6.00	10	6.70
Living with Family	Yes	55	55.00	50	100.00	105	70.00
	No	45	45.00	0	0.00	45	30.00
Knowledge of AIDS	Yes	29	29.00	50	100.00	79	52.70
	No	71	71.00	0	0.00	71	47.30

Table no. 2 Distribution of high-risk behavior variables migrant workers and controls

Variables		Group				Total		Chi-square tests	
		HBV +ve		Control				Value	p-value
		(n=24)	%	(n=50)	%	(n=74)	%		
Extramartial affair/CSW	Yes	14	58.30	0	0.00	14	18.90		4.30×10^{-09}
	No	10	41.70	50	100.00	60	81.10		
								Significant (F)	
Use of condom	Yes	0	0.00	50	100.00	50	67.60		5.70×10^{-20}
	No	24	100.00	0	0.00	24	32.40		
								Significant (F)	
History/Current STD infection	Yes	12	50.00	0	0.00	12	16.20		1.23×10^{-07}
	No	12	50.00	50	100.00	62	83.80		
								Significant (F)	
Alcohol	Yes	11	45.80	5	10.00	16	21.60		0.002
	No	13	54.20	45	90.00	58	78.40		
								Significant (F)	
CD 4 No.s	≥ 500	4	16.70	50	100.00	54	73.00	64.735	8.77×10^{-15}
	499 to 200	6	25.00	0	0.00	6	8.10		
	< 200	14	58.30	0	0.00	14	18.90		
								Significant (L)	
Knowledge of previous HBV infection	Yes	0	0.00	50	100.00	50	67.60		5.70×10^{-20}
	No	24	100.00	0	0.00	24	32.40		
								Significant (F)	

Note: P = Pearson's Chi-square test, C = Continuity Correction, L = Likelihood Ratio, F = Fisher's Exact Test

The high-risk behaviour observed in the study group and the control group is compared in table no. 2. In the study group 59 (59%) individuals had extramarital sex/frequent visit to CSWs ($p = 3.30 \times 10^{-14}$). Out of 100 individuals in the study group 91(91%) showed no safe sex practices ($p \text{ value} = 6.60 \times 10^{-15}$), whereas all the individuals in the control group followed safe sex practices. Three (3%) individuals in the study group had a blood transfusion. In the control group none of the individuals had blood transfusion. In the study group 37 (37%) individuals had history/current STD infection ($p \text{ value} = 2.94 \times 10^{-08}$) whereas in the control group no individuals had history/current STD. Alcohol addiction was observed in 47 (47%) individuals in the study group ($p \text{ value} = 2.94 \times 10^{-08}$) and 5 (10%) individuals in the control group.

The overall HIV-HBV co-infection was found in 24 (24%) individuals in the study group (Odds Ratio [OR]= 1.579 95% CI= 1.343-1.856). Out of the total HBV infected individuals 14 were in a recovering stage of HBV infection, as they showed presence of anti-HBeAg antibodies in their serum. Four individuals were in the carrier stage of the HBV infection as they showed presence of both HBsAg and Anti-HBeAg in their serum. Six individuals were in incubation or carrier stage of infection as they showed presence of HBsAg and HBeAg antigen in their serum. The HBV DNA PCR results will be updated in the thesis, which will further substantiate the staging of HBV infection. The HBV prevalence was 0.5% (n=1) in the control group.

Table no. 3 Interpretation of serological Test for Hepatitis B Virus

HBsAg	HBeAg	Anti-HBeAg	Interpretation
+	+	-	Incubation or carrier Stage of infection.
+	-	+	Persistent carrier stage of infection.
-	-	+	Recovering stage of infection.

The HCV infection was found in 4 (4%) individuals in the study population (OR= 1.521 95%CI= 1.353-1.71). All the 4 individuals positive for anti-HCV showed presence of HCV RNA by RT-PCR. In the control group none of the individuals showed any presence of markers for HCV infection.

The statistical association of demographic and high-risk behavior variables between in HIV-HBV co-infected individuals and control is shown in Table no. 4a, 4b and 5. Fifty percent of the HIV-HBV co-infected individuals were illiterate (**p value= 6.18×10^{-13}**). CD4 cell count lower than 200cells/ μ l were seen in 14 (58.3%) HIV-HBV co-infected individuals. Out of 24 HIV-HBV co-infected individuals 19 (62.5%) had no knowledge of HIV/AIDS (**p value = 1.98×10^{-13}**). None of the HIV-HBV co-infected individuals followed safe sex practices. In all, 14 (58.30%) HIV-HBV co-infected individuals had extramarital affairs/ frequent visit to CSWs (**p value = 4.30×10^{-09}**) and 12 (50%) individuals had history/current STD infection (**p value = 1.23×10^{-07}**). None of the HIV-HBV and HIV-HCV coinfecting individuals had a record of previous HBV or HCV infection.

Table no.4a Distribution of demographic variables of HIV-HBV co-infected migrant workers and controls

Variables		Group				Total		Chi-square tests	
		HBV +ve		Control				Value	p-value
		(n=24)	%	(n=50)	%	(n=74)	%		
Sex	Female	13	54.20	21	42.00	34	45.90		0.455
	Male	11	45.80	29	58.00	40	54.10	Not significant (F)	
Age (years)	18 to 24	2	8.30	5	10.00	7	9.50	5.101	0.277
	25 to 34	11	45.80	16	32.00	27	36.50	Not significant (P)	
	35 to 44	9	37.50	14	28.00	23	31.10		
	45 to 54	1	4.20	12	24.00	13	17.60		
	>=55	1	4.20	3	6.00	4	5.40		
Educational Status	Illiterate	12	50.00	0	0.00	12	16.20	59.898	6.18x10 ⁻¹³
	Primary	7	29.20	0	0.00	7	9.50	Significant (L)	
	Secondary	4	16.70	36	72.00	40	54.10		
	HS & >	1	4.20	14	28.00	15	20.30		

Note: P = Pearson's Chi-square test, C = Continuity Correction, L = Likelihood Ratio, F = Fisher's Exact Test

Table no.4b Distribution of demographic variables of HIV-HBV co-infected migrant workers and controls

Variables		Group				Total		Chi-square tests	
		HBV +ve		Control				Value	p-value
		(n=24)	%	(n=50)	%	(n=74)	%		
Language spoken	Hindi	8	33.30	9	18.00	17	23.00	7.344	0.025
	Marathi	14	58.30	41	82.00	55	74.30	Significant (L)	
	Other	2	8.30	0	0.00	2	2.70		
Religion	Hindu	20	83.30	50	100.00	70	94.60	9.495	0.009
	Christian	2	8.30	0	0.00	2	2.70	Significant (L)	
	Muslim	2	8.30	0	0.00	2	2.70		
Married	Yes	20	83.30	47	94.00	67	90.50		0.204
	No	4	16.70	3	6.00	7	9.50	Not significant (F)	
Living with Family	Yes	15	62.50	50	100.00	65	87.80		1.18x10 ⁻⁰⁵
	No	9	37.50	0	0.00	9	12.20	Significant (F)	
Knowledge of AIDS	Yes	5	20.80	50	100.00	55	74.30		1.98x10 ⁻¹³
	No	19	79.20	0	0.00	19	25.70	Significant (F)	

Note: P = Pearson's Chi-square test, C = Continuity Correction, L = Likelihood Ratio, F = Fisher's Exact Test

Table no. 5 Distribution of high-risk behavior variables of HIV-HBV co-infected migrant workers and controls

Variables		Group				Total		Chi-square tests	
		HBV +ve		Control				Value	p-value
		(n=24)	%	(n=50)	%	(n=74)	%		
Extramarital affair/CSW	Yes	14	58.30	0	0.00	14	18.90		4.30×10^{-09}
	No	10	41.70	50	100.00	60	81.10	Significant (F)	
Use of condom	Yes	0	0.00	50	100.00	50	67.60		5.70×10^{-20}
	No	24	100.00	0	0.00	24	32.40	Significant (F)	
History/Current STD infection	Yes	12	50.00	0	0.00	12	16.20		1.23×10^{-07}
	No	12	50.00	50	100.00	62	83.80	Significant (F)	
Alcohol	Yes	11	45.80	5	10.00	16	21.60		0.002
	No	13	54.20	45	90.00	58	78.40	Significant (F)	
CD 4 No.s	≥ 500	4	16.70	50	100.00	54	73.00	64.735	8.77×10^{-15}
	499 to 200	6	25.00	0	0.00	6	8.10	Significant (L)	
Knowledge of previous HBV infection	< 200	14	58.30	0	0.00	14	18.90		
	Yes	0	0.00	50	100.00	50	67.60		5.70×10^{-20}
	No	24	100.00	0	0.00	24	32.40	Significant (F)	

Note: P = Pearson's Chi-square test, C = Continuity Correction, L = Likelihood Ratio, F = Fisher's Exact Test

No significance was found when demographic and high-risk behavior variables of the HBV infected population were compared within the study population itself.

The statistical analysis of demographic and high-risk behavior variables between HIV-HCV infected individuals and control was found to be non significant as the study sample was too small (n=4).

To see whether any of demographic and high-risk behavior variables like extramarital affairs/ frequent visit to CSWs, no safe sex practices, marital status, blood transfusion, history/current STD infection, alcohol addiction, knowledge of HIV/AIDS, living with family are the potential predictors of the HBV infection in the study population

logistic regression analysis was performed but none of the variables were found significant predictor of the of the HBV infection.

Table 6a. Distribution of Sociodemographic factors among HIV-HCV co-infected migrant workers

Variables		HCV positive	
		(N=4)	(%)
Sex	Female	1	25.00
	Male	3	75.00
Age (years)	18 to 24	0	0.00
	25 to 34	0	0.00
	35 to 44	2	50.00
	45 to 54	2	50.00
	>=55	0	0.00
Educational Status	Illiterate	2	50.00
	Primary	1	25.00
	Secondary	0	0.00
	HS & >	1	25.00

Table 6b. Distribution of Sociodemographic factors among HIV-HCV co-infected migrant workers

Variables		HCV positive	
		(N=4)	(%)
Religion	Hindu	3	75.00
	Christian	1	25.00
	Muslim	0	0.00
Language Spoken	Hindi	3	75.00
	Marathi	0	0.00
	Other	1	25.00
Married	Yes	4	100.00
	No	0	0.00
Living with Family	Yes	1	25.00
	No	3	75.00
Knowledge of AIDS	Yes	1	25.00
	No	3	75.00

Table 7. Distribution of high-risk behavior variables of HIV-HBV co-infected migrant workers and controls

Variables		HCV positive	
		(n=4)	%
Extramarital affair/CSW	Yes	3	75.00
	No	1	25.00
Use of condom	Yes	1	25.00
	No	3	75.00
History/Current STD infection	Yes	0	0.00
	No	4	100.00
Alcohol	Yes	2	50.00
	No	2	50.00
CD 4 No.s	≥ 500	0	0.00
	499 to 200	1	25.00
Knowledge of previous HCV infection	< 200	3	75.00
	Yes	0	0.00
	No	4	100.00

Discussion

Co-infection in HIV with Hepatitis B Virus (HBV) or Hepatitis C Virus (HCV) is further escalating the public health concern. These diseases are spread in similar ways notably through shared use of needles to inject drugs, sexual intercourse and blood transfusion. Many people are co-infected with HIV and HCV, HIV and HBV, or even all three viruses.

Co-infection with Hepatitis B virus (HBV) or Hepatitis C virus (HCV), in HIV infected patients complicates the clinical course, management and may also adversely affect therapy for HIV infection.

In the present study HBV infection was found to be 24% (OR=1.579 CI=1.343-1.856) and HCV infection was found to be 4% (OR=1.521 CI=1.353-1.71) in the HIV infected migrant workers. None of the individuals were co-infected with all three viruses together. In the control group the HBV infection was found to be 0.5% (OR=0.102 CI=0.015-0.705). There was no HCV infection found in the control group. In a study from Maharashtra by Tankhiwale S. S. *et al*, amongst 110 HIV seropositive hospitalized patients, 30.4% and 7.27% of patients were positive for HBV and HCV respectively ³⁵. Whereas, a study from Manipur by Saha M. K. *et al*, reported a very high prevalence of HBV (100%) and HCV (92%) infection, amongst HIV seropositive intravenous drug users ³⁶. The prevalence of hepatitis co-infection with HIV has been observed varying across different risk groups, because of the risk behaviour and geographic location of the study population.

Among the HIV-HBV co-infected individuals in the study group according to the serological profile, 14 individuals were in recovery stage as they showed presence of anti-HBeAg antibodies in their serum. Four individuals were in the carrier stage as they showed presence of both HBsAg and Anti-HBeAg and 6 individuals were in incubation or carrier stage as they showed presence of HBsAg and HBeAg antigen in their serum. HBV DNA PCR, which will be done, will determine the exact staging and the results will be updated in the thesis.

Educational status was associated with the HBV infection in the HIV-HBV coinfecting migrant workers ($p= 6.18 \times 10^{-13}$). The HBV infection was found decreasing as the educational status increased. This was also observed in a study conducted by A. Risbud *et al* among individuals attending STD clinic in Pune ⁵¹. Educational status directly impacts the health literacy. Low educational status leads to low health literacy, which contributes to misconception about the viruses and its transmission. More importantly it leads to misinterpretation of the risk averse behaviour related to infections further posing a threat to the surrounding population.

The route of transmission for HBV infection was through sexual contact, as none of the HIV-HBV co-infected individuals in the study group had a history intravenous drug use and blood transfusion. Sexually transmitted diseases (STD) such as Herpes zoster, syphilis were observed in HIV-HBV co-infected infected individuals (OR=5.617 CI=3.108-8.588). Robert S. *et al* observed a significant association between the HBV prevalence and STD's among homosexuals ⁵². The prevalence of STD indicates the high-risk sexual behaviour practiced such as multiple sexual partners and unsafe sex by

the HIV-HBV coinfecting migrant workers, which is creditworthy to the high prevalence of these infections in this study group.

Alcohol consumption was seen in 45.8% of HIV-HBV coinfecting individuals and 50% in HCV-HIV co-infected individuals. Alcohol consumption is a marker of a personality type given to a risk taking deviant behaviour. It enhances risk of infection by increasing sexual activity or number of sexual partners or modifying the willingness to have sex with or without using condoms. More importantly it is responsible for faster progression to end stage liver cirrhosis in HBV and HCV infected individuals ⁴⁹. It also complicates the management of HIV-HBV and HIV-HCV treatment ⁵⁰.

In the present study HCV infection was transmitted through sexual contact as none of the HIV-HCV coinfecting individuals had a record of intravenous drug use or blood transfusion. Transmission of HCV via heterosexual contact is far less efficiently than HIV ³⁹⁻⁴². But the presence of HIV co-infection results in a doubling of the likelihood that HCV would be transmitted to partners of HCV-positive sexually promiscuous individuals ⁴³. More importantly there is also evidence that this route is of greater importance among individuals with multiple sexual partners, who sustain STIs and who practice unprotected sexual intercourse ^{44,45} as observed in the HIV-HCV co-infected migrant workers.

In present study 75% of HIV-HCV co-infected individuals had CD4 counts <200. CD4 counts <200 in HIV-HCV coinfection correlates with significant increase in HCV viral load ⁴⁶. Rockstroh et al. found more rapid liver failure only in co-infected patients whose CD4 count below 230, and not in patients with stable CD4 counts ⁴⁷. Thus HIV-HCV coinfecting patients have a higher risk of proceeding to liver failure.

In the present study none of the HIV-HBV and HIV-HCV co-infected migrant workers had been previously diagnosed for HBV or HCV infections. HIV increases the severity of liver disease caused by chronic hepatitis group of viruses like HBV or HCV this accounts for significant morbidity and mortality in HIV-infected individuals who under HAART are living longer and symptom free lives. It is important to screen for HBV and HCV in all HIV-infected patients before starting any anti-HIV therapy. Thus it should be done mandatory to screen all HIV infected migrant workers for HBV and HCV infections irrespective of HIV treatment status so as to avoid future complications if HAART is administered.

In conclusion our study illustrates that HIV infected migrant workers have a high risk of infection with HBV, and to a lesser degree with HCV. Infections with both HBV and HCV are significantly higher in HIV-positive migrant workers than the control population.



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