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ASSESSMENT OF HBV DNA VIRAL LOAD IN SEROLOGICALLY POSITIVE INDIVIDUALS: A CROSS-SECTIONAL POPULATION STUDY OF EAST GODAVARI DISTRICT, COASTAL ANDHRA PRADESH

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ABSTRACT

Hepatitis B virus (HBV) infection continues to be a serious global health concern, particularly in developing countries. An estimated 296 million people worldwide are living with chronic HBV infection, many of whom remain undiagnosed. Serological markers such as hepatitis B surface antigen (HBsAg) and hepatitis B e antigen (HBeAg) are widely used for initial diagnosis and classification of HBV infection phases. However, these markers alone do not reflect the true replication status of the virus. Quantification of HBV DNA (Viral Load) is crucial for evaluating the stage of infection, assessing infectivity, monitoring response to therapy, and guiding clinical management decisions. This study was undertaken to evaluate the HBV viral load among serologically positive individuals and correlate it with their serological profile and clinical parameters.

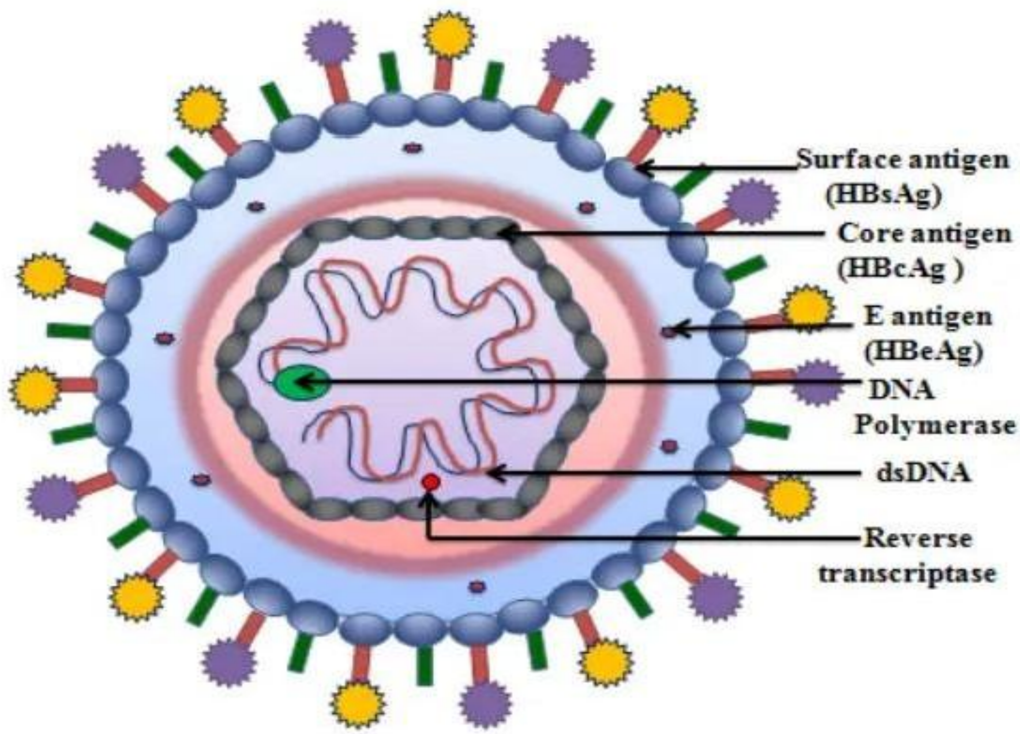
Keywords: HBV DNA Viral load, serological Positive Individuals, Cross-Sectional Population Study, East Godavari District, Coastal Andhra Pradesh.

INTRODUCTION

According to estimates from the World Health Organization, over 2 billion people have been infected with HBV globally, with around 350 million experiencing persistent infection. HBV is believed to be responsible for up to 80% of all cases of hepato cellular carcinoma worldwide, ranking second only to tobacco as a known human carcinogen. Each year, approximately 1 million individuals succumb to hepato cellular carcinoma or liver failure around the world. Therefore, effectively managing HBV infection is a crucial objective for public health in regions where it is prevalent[1-5].

The Hepatitis B virus belongs to the Hepadnaviridae family and is a double stranded DNA virus with approximately 3,200 base pairs [6-8]. In the process of viral replication, a super coiled covalently closed circular viral DNA (cccDNA) is produced, and this DNA remains in host cells as a viral mini chromosome [5,6].

Fig–1 Structure of Hepatitis B Virus



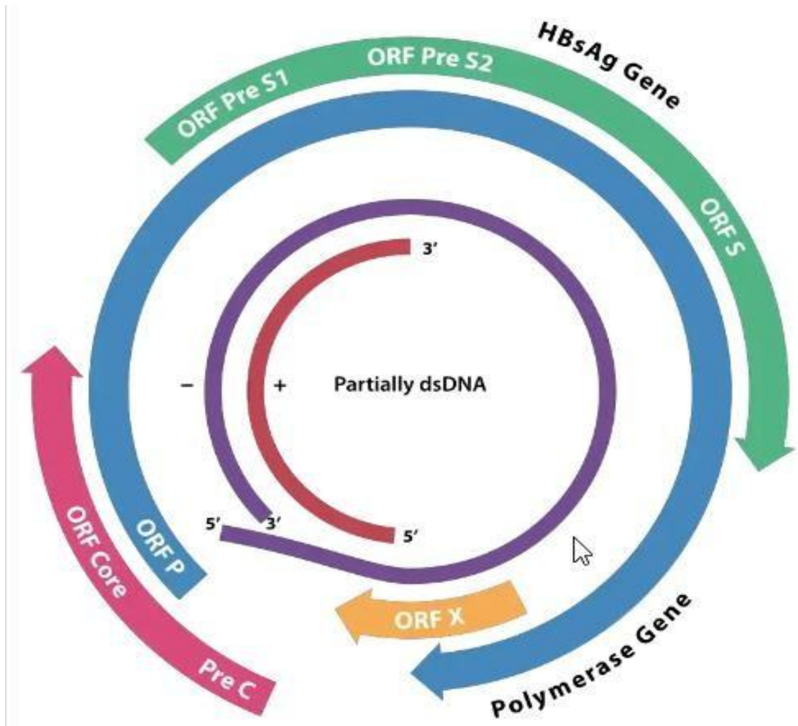
Hepatitis B virus belongs to the family of Hepadna viruses. The viral particle, also known as a Dane particle, is composed of an exterior lipid envelope and an icosahedral protein nucleo capsid core. The nucleo capsid encases viral DNA and a DNA polymerase with reverse transcriptase activity comparable to that of retroviruses. The outer envelope contains proteins that contribute to viral binding and penetration into vulnerable cells. With a virion diameter of 42 nm, the virus is one of the smallest enveloped animal viruses, yet pleomorphic forms exist, including filamentous and spherical bodies without a core. These non-infectious particles are made of the lipid and protein that form part of the virion's surface, which is known as the surface antigen (HBsAg) and is produced in excess during the virus's life cycle [7].

Components of HBV

HBs Ag (Hepatitis B surface antigen) was the first discovered hepatitis B viral protein. It's made up of small (S), medium (M), and big (L) proteins. HBc Ag (Hepatitis B core antigen) is the principal structural protein of the HBV icosahedral nucleo capsid, and It plays a role in viral replication. Capsid development is the primary factor in cell infection. It is unknown if HBc Ag must be in the capsid form in order to contribute to viral clearance invivo. Along with the pre-genomic RNA, Hepatitis B virus DNA polymerase is integrated into the nucleo capsid (pg RNA). The pg RNA undergoes reverse transcription within the capsid to produce the (-) DNA strand. The RNase activity of the polymerase simultaneously degrades the majority of the RNA template. After (+) DNA strand synthesis, the polymerase becomes covalently attached to the (-) DNA strand. After a virion has infected a new cell, the polymerase is discarded. HBe Ag (Hepatitis B envelope antigen) is located between the icosahedral nucleo capsid core and the lipid envelope; none the less, it is non particulate, is secreted, and accumulates in serum. The

HBe Ag and HBc A greading frames are identical. HB x is small,154 amino acids in length, nonstructural, and plays a crucial role in HBV r9CgKDSHhyFVbCH2zkSDduhBDhwYqLHhVm-associated liver disease. Numerous activities have been connected to HB x expression. However, the molecular processes behind the majority of these functions remain unclear. This multifunctional protein promotes cellular signaling pathways and is required for viral infection. Hepatitis D virus requires HBV envelope particles to become virulent [8].

Fig–2 Genome Organization of HBV



The HBV genome is composed of circular DNA, however it is remarkable because the DNA is not double-stranded throughout. The viral DNA polymerase is attached to one end of the full-length strand. The genome consists of 3020–3320 nucleotides (for the full-length strand) and1700–2800 nucleotides (for the shorter strand) (forth short length strand). The negative-sense, non-coding (non-coding) strand is complementary to the viral mRNA. Immediately upon infection, the viral DNA is discovered in the nucleus. The partially double-stranded DNA becomes fully double-stranded through the completion of the (+) sense strand by cellular DNA polymerases (viral DNA polymerase is used at a later stage) and the elimination of the viral polymerase protein (P) from the (-) sense strand and a short sequence of RNA from the (+) sense strand. Non-coding nucleotides are deleted from the ends of the (-) sense strand before rejoining the ends. Covalently closed circular DNA (cccDNA) serves as a template for the transcription of viral genes in the nucleus of the host cell by RNA polymerase II. In the HBV genome, two enhancers named enhancer I (EnhI) and enhancer II (EnhII) have been found. Both enhancers are more active in cells of hepatic origin, and collectively they control the expression of the entire viral transcripts. C,P,S, and X are the four known genes that the genome encodes. Gene C (HBc

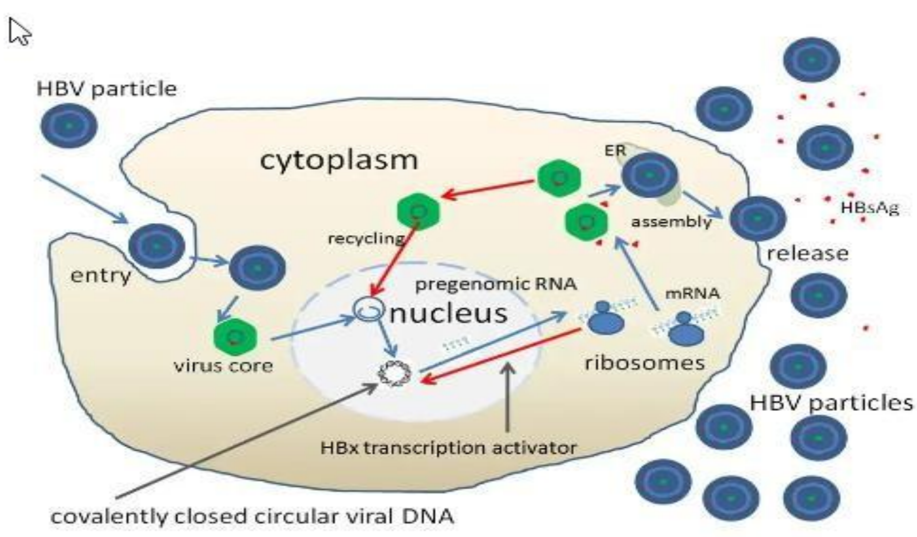
Ag) encodes the core protein, and its start codon is preceded by an upstream in-frame AUG start codon from which the pre-core protein is created. The pre-core protein is processed proteolytically to generate HBe Ag. Gene P codes for the DNA polymerase. Gene S is the gene that encodes the surface antigen (HBsAg). The HBs Ag gene is one continuous open reading frame, but It contains three “start”(ATG) codons that divide it into three portions, pre-S1, pre-S2, and S. Due to the presence of numerous Start codons, polypeptides of three distinct sizes a reproduced: large, middle, and tiny (pre-S1+pre-S2+S,pre-S2+S,orS). Some evidence suggests that the gene X-encoded protein may function as a transcriptional trans activator, although its function is not completely understood. Intriguingly, a 40k Da X- Core fusion protein is encoded by a lengthy, 3.9kb viral transcript whose function is unknown.

After these conditions round of transcription, the 3.9kb RNA is polyadenylated. The synthesis of the3.9kbRNA begins at the promoter region of the X gene. Other long pre genomic/pre-core (pg/pc) RNA species have identical characteristics. The viral transcription machinery must therefore disregard the poly(A) signal during the initial transcription round. Multiple non coding RNA elements have been discovered in the HBV genome. Included among these are HBVPRE alpha, HBVPRE beta, and HBVRNA encapsulation signal epsilon. The cccDNA, or covalently closed circular DNA, can serve as a source of renewed virus production after the immune response to the acute infection has subsided. As a result, it forms a reservoir of infectious viral particles, contributing to the development of chronic hepatitis in specific individuals [9,10]. Detection of HBV-DNA can occur approximately 21days prior to the typical appearance of HBs Ag in the serum [11]. During the window period, when HBs Ag has disappeared and anti-HBs is not yet detectable, HBV-DNA detection can be useful to detect HBV infectious status [8].It is now evident that a group of HBV infected persons may not express HBs Ag in the sera due to various factors. Several cases of post-transfusion hepatitis have also occurred after transfusion of HBs Ag-negative blood. Furthermore, nothing is known about the entity of occult HBV infection or HBs Ag-negative HBV-infected subjects in Bangladesh [12].

Life cycle of HBV

The Hepatitis B virus has a complex life cycle. Hepatitis B is one of the few non-retro viral viruses known to utilize reverse transcription in its replication process.

Fig– 3 Life cycle of HBV



1. Attachment: By attaching to receptors on the surface of the cell and entering through endocytos is mediated byeitherclathrinorcaveolin-1,the virus gains access to the cell. HBV attaches to heparin sulphate proteogly can first. The pre-S1region of the HBVL protein binds firmly to the SLC10A1 gene-encoded cell surface receptor sodiumtaurocolate Co transporting polypeptide (NTCP). The majority of NTCP is located in the sinusoidal membrane of liver cells. The tissue specificity of HBV infection correlates with the presence of NTCP in liver cells.

2. Penetration: After endocytosis, the membrane of the virus fuses with the membrane of the host cell, releasing the nucleo capsid into the cytoplasm.

3. Uncoating: Because the virus replicates through the production of RNA by a host enzyme, the viral genomic DNA must be transported to the nucleus of the host cell. Microtubules are believed to transfer the capsid to the nuclear pore. The core proteins separate from the partly double-stranded viral DNA, which is then converted into covalently closed circular DNA (cccDNA), which acts as a template for transcription of four viral mRNAs.

4. Replication: The biggest mRNA (which is longer than the viral genome) is utilized to produce new copies of the viral genome as well as the capsid core protein and viral RNA-dependent-DNA- polymerase.

5. Assembly: These four viral transcripts undergo additional processing and form progeny virions, which are either discharged from the cell or returned to then ucleus to be re-cycled.

6. **Release:** The lengthy mRNA is subsequently carried back to the cytoplasm, where the reverse transcriptase activity of the virion P protein synthesizes DNA.

Transmission of Hepatitis B: In highly endemic regions, hepatitis B is typically transmitted from mother to child at birth (perinatal transmission) or through horizontal transmission (exposure to infected blood), particularly from an infected child to an uninfected child during the first five years of life. It is typical for infants infected from their mothers or before the age of 5 to acquirea persistent infection.

Hepatitis B is also transmitted through needle stick injuries, tattooing, piercing, and exposure to infected blood and body fluids, including saliva, menstrual, vaginal, and seminal secretions. The virus can also be transmitted by the reuse of contaminated needles, syringes, or others harp objects in healthcare settings, the community, or among drug users. Sexual transmission is more common among unvaccinated individuals with several partners. In less than 5% of cases, Hepatitis B infection acquired in maturity results in chronic hepatitis, where as infection in infancy and early childhood results in chronic hepatitis in approximately 95% of cases. This is the foundation for enhancing and prioritizing baby and kid vaccinations.

The hepatitis B virus can persist for atleast seven days out side the body. During this period, the virus can still cause infection if it enters the body of an unvaccinated individual. The hepatitis B virus has an incubation period ranging from 30 to 180 days. The virus can be diagnosed 30 to 60 days after infection and can persist and develop into chronic hepatitis B, particularly when transmitted during infancy or youth.

Pathogenesis of Hepatitis B

There appear to be three pathways involved in liver cell damage during HBV infections. The initial reaction is an HLA class I-restricted cytotoxic T-cell (CTL) response against HBc Ag/HBe Ag on HBV-infected hepatocytes. The direct cytopathic effect of HBc Ag expression in infected hepatocytes is a second potential mechanism. A third proposed mechanism involves a high amount of HBs Ag expression and ineffective secretion. In the acute phase, the portal tracts exhibit symptoms of inflammation, and the infiltration is mostly lymphocytic. As they die, infected hepatocytes in the liver parenchyma exhibit ballooning and produce acidophilic (Councilman) bodies. In chronic hepatitis, damage extends beyond the portal tracts, creating the appearance of piecemeal necrosis. There is also some lobular inflammation present. As the condition advances, fibrosis and ultimately cirrhosis occur. Continued immune mediated death of hepatocytes expressing viral antigens causes chronic liver injury. In addition, autoimmune reactions may contribute to the damage, as certain liver- specific antigens induce immune responses.

Diagnosis of Hepatitis B

The goal of diagnostic laboratory tests of patients with clinical hepatitis is to, first, identify the causative hepatitis virus and, second (for HBV), differentiate between acute and chronic infections. Hepatitis is diagnosed based on clinical symptoms and biochemical testing that detect liver damage. Amino transferases, bilirubin, and prothrombin time elevations all contribute to the initial diagnosis of hepatitis. ELISA, also known as enzyme-linked immune sorbent assay, and other immunologic techniques for detecting viral antigens and antibodies are the principal way of differentiating between HAV, HBV, HCV, and HDV. In addition, the presence or lack of particular antiviral antibodies and viral antigens enables the distinction between acute and chronic HBV infections.

The most common method for diagnosing HBV is the detection of HBs Ag in the blood stream. Other routinely conducted tests include HBe Ag, HBV DNA, and anti–core antibody. IgM anti–core antibody is symptomatic of recent HBV exposure, where as IgG core antibody is present in persons with chronic infection and those who have recovered from infection. Subjects who have been effectively inoculated with HBV vaccine are positive for anti-HBs Ag.

If a liver biopsy specimen is available, immune histo chemistry using an antibody against the surface antigen can be conducted. Screening has been suggested for all pregnant women and newly arriving immigrants from countries with an HBV prevalence rate of atleast2%. Current research investigates whether pregnant women living in regions with high HBV endemicity should be screened for occult HBV infection utilizing sensitive assays for HBV DNA.

Hepatitis B is a multifaceted disease that requires a comprehensive assessment involving serological, biochemical, molecular, and histological evaluations for accurate diagnosis [13]. In recent studies, it has been demonstrated that the level of HBV-DNA in serum or plasma is closely associated with the biochemical and histological indicators of the disease, providing a more precise reflection of HBV replicative activity. As a result, the measurement of HBV-DNA in serum has emerged as a crucial tool for identifying individuals with high viral replication and form on it oring patients undergoing therapy [3]. Various quantitative assays are currently employed to measure the level of HBV-DNA load in serum samples, with real-time PCR being particularly recognized for its high sensitivity. The presence of HBV-DNA persists both during the acute and chronic phases of the disease. Its detection is more accurate than that of HBe Ag, often appearing before the biochemical evidence of hepatitis [13].

MATERIALS & METHODS

The present study includes 38 Clinical samples, known for presence of Hepatitis B virus were used for DNA isolation (Eisenach et. al.) using Huwel nucleic acid extraction kit and Quantiplus HBV quantitatives RT PCR kit. Serological assays and nucleic acid extraction of HBV DNA was done in Bio safety cabinet class II B2.

1. Serological assays

a. Qualitative HBs Ag Assay

HBs Ag is a rapid, qualitative, two sites and which immune assay for the detection of Hepatitis B surface antigen, a marker for Hepatitis B infections, in serum/plasma specimen (17-19). Blood containing the Hepatitis B Virus (HBV) is potentially infectious. Hepatitis B surface Antigen (HBsAg), earlier known as Australia antigen, is among the first serological markers that circulate in the blood of infected persons even two to three weeks prior to the appearance of clinical symptoms. The levels of HBs Ag are especially elevated during the symptomatic Phase and decline thereafter. Detection of HBV using HBs Ag as the marker to screen blood donors is essential to reduce the risk of transmission of Hepatitis B by blood transfusion. HBsAg detection is also useful for screening high risk groups for HBV and for differential diagnosis of Hepatitis infection. Sensa HepB-one step test for HBs Ag detects the presence of HBsAg in serum/plasma specimens, qualitatively, at concentrations as low as 0.3 ng/ml.

Testing Procedure

Bring the sealed pouch to room temperature, open the pouch and remove the device. Once opened, the device must be used immediately.Dispense 150µl (3drops) of serum/plasma into the sample port‘S’ using a micropipette refrigerated specimens must be brought to room temperature prior to testing.Attheend of 20 minutes read the results as follows:

b.Qualitative HBe Ag Assay

HBe Ag is a rapid, self-performing, immune chromatographic assay for the detection of HBeAg in human serum. The hepatitis B'e' antigen (HBeAg), has been found in the hepatocytes during proliferation of the hepatitis virus. Its detection is associated with pronounced viral infection and infectivity [16]. After treatment, in the recovery phase following acute Hepatitis B infection, HBeAg becomes negative. HBeAg is generally detectable at the same time as HBsAg and disappears before HBsAg disappears. The presence of HBeAg in chronic infection indicates that HBV is actively reproducing and there is a high possibility of liver damage. In acute infection, HBeAg is generally present in trace amounts and its disappearance indicates acute or chronic Hepatitis B which is in the process of healing. The assay detects the presence of HBeAg in human serum, thereby giving an assessment of the infectivity and the patient's status.

Testing Procedure

Bring the kit components of INSIGHT HBeAg device to room temperature before testing. Open a foil pouch by tearing along the“ notch”. Remove the testing device and the sample applicator.

Check the colour of the desiccant pouch. It should be blue. If the desiccant has turned colour less or pink, discard the test device and use another device. Once opened, the device must be used immediately. Label the device with specimen identity. Place the testing device on a flat horizontal surface. Holding the sample applicator vertically, carefully dispense exactly 2 drops of the serum specimen into the specimen port(S). Alternatively, using a micropipette, carefully dispense exactly100µl of the serum specimen into the specimen port (S). Start the stopwatch. Read the result swithin10minutes.Do not interpret the results beyond 15 minutes.

Negative Result: Only one pink/purple coloured band appears at the Control Region(C).This indicates absence of HBeAg in the specimen.

Positive Result: Two pink/purple coloured bands appear at the Control Region(C) and Test Region (T).This indicates that the specimen contains detectable amount of HBeAg.

Invalid Result: The test result is invalid if no band appears either at the Control Region (C) or Test Region (T). In such cases, verify the test procedure and repeat the test with a new INSIGHT HBeAg device.

C. Qualitative Anti HBe Assay

HBeAb is a rapid, competitive, self-performing, immunochromatographic assay for the detection of HBeAb in human serum [15-16]. After treatment, in the recovery phase following acute Hepatitis B infection, HBeAg becomes negative. Anti bodies to the 'e 'antigen (HBeAb) normally appears a few weeks after HBeAg is no longer detectable. The presence of HBeAb generally indicates that the patient is recovering from the acute stage of the illness. INSIGHT HBeAb detects the presence of HBeAb in human serum and helps determine whether the HBV infection is resolved. Presence of HBeAb suggest slow or missing infectivity and serves as a measure of patient infectivity. In monitoring the cause of accute and chronic HBV infections, the occurence of HBeAb and disappearance of HBeAg are of prognostic value [17].

Testing Procedure

Bring the kit components of INSIGHT HBeAb device to room temperature before testing. Open a foil pouch by tearing along the“notch”. .Remove the testing device and the sample applicator. Check the colour of the desiccant pouch. It should be blue. If the desiccant has turned colour less or pink, discard the test device and use another device. Once opened, the device must be used immediately..Label the device with specimen identity. Place the testing device on a flat horizontal surface. Holding the sample applicator vertically, carefully dispense exactly 2 drops of the serum specimen into the specimen port(S). Alternatively, using a micropipette, carefully dispense exactly 100µl of the serum specimen into the specimen port(S). Start the stopwatch. Read the resultswithin10minutes.Donotinterpret the results beyond 15 minutes.

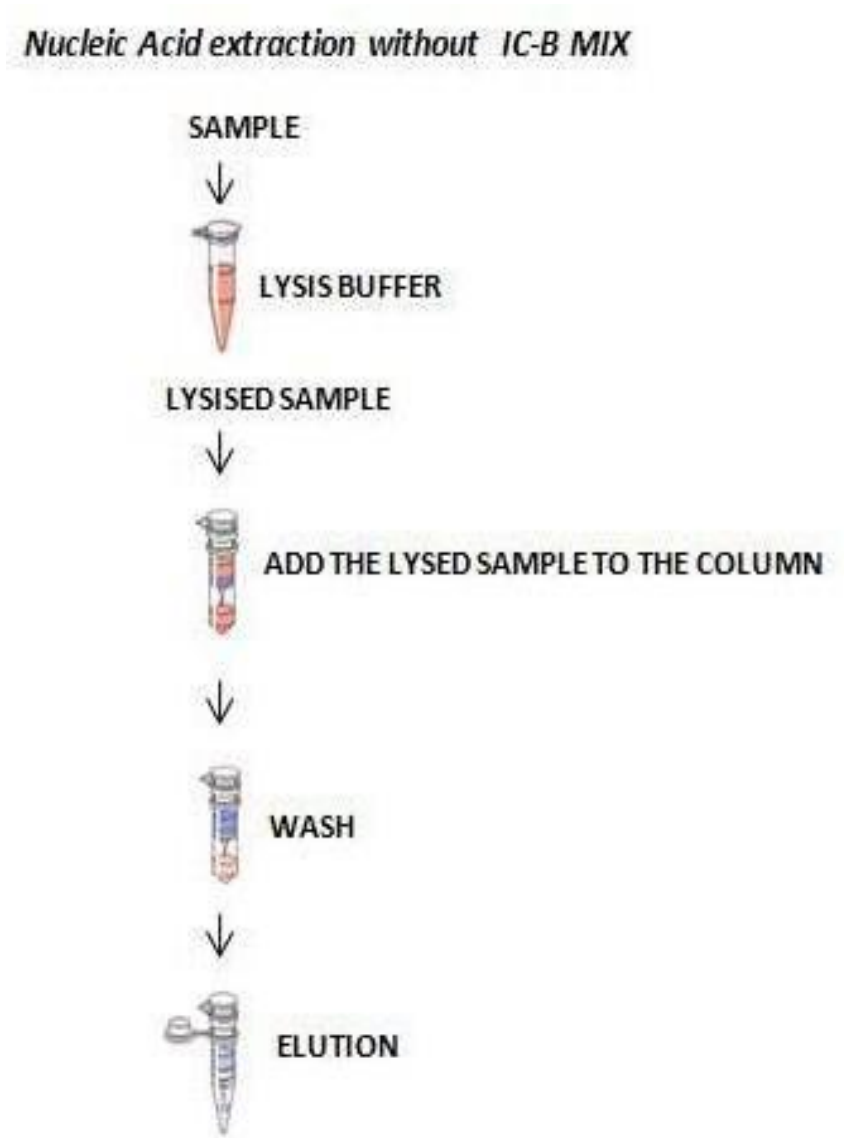
Interpretation of Results

Negative Result: Two pink/purple coloured bands appear at the Control Region (C) and Test Region (T). This indicates absence of HBeAb in the specimen.

Positive Result: Only one pink/purple coloured band appears at the Control Region(C). This indicates that the specimen contains detectable amount of HBeAb. This concludes that the active stage of HBeAb infection is almost over and the risk of being contagious is greatly reduced.

Invalid Result: The test result is invalid if no band appears either at the Control Region(C) or Test Region (T). In such cases, verify the test procedure and repeat the test with a new INSIGHT HBeAb device.

2. Molecular assay
HBV DNA Extraction from serum using Huwel nucleic acid extraction kit
Fig-4 Nucleic Acid Extraction an over view



DNA Extraction procedure
To 1.5mL micro centrifuge tube added 500 µl of lysis buffer, 150 µl serum sample and 10 µl of proteinase K added, voetexed for few sec. and incubated at room temperature for 10 minutes. At the end of the incubation, transfer the lysate solution into the spin column tube and centrifuged at 8000rpm for 1 minute. Changed the collection tube and added 500µl of wash buffer-1and centrifuged at 8000rpm for 1 minute.

Changed the collection tube and added 500µl of wash buffer-2 and centrifuged at 8000rpm for 1 minute. Changed the collection tube and centrifuged at 12000 rpm for1minute to remove the traces of wash buffer-2. Transferred the spin column to a fresh1.5ml tube.Added50µl of elution buffer and incubated 5minutes at room temperature. At the end of the incubation, spin column tube was centrifuged at 8000rpm for1 minute. The elutes were stored at-20°C and used for q PCR reactions q PCR Protocol

Preparation of Reaction Master Mix

Assay procedure with Quantiplus HBV quantitation RT PCR Kit involves1step q PCR. It is recommended that Positive and negative control (PW should be used as negative control) are required to be included in a single run for acquiring proper results.

Setup a real time single step RT PCR reaction as follows

q PCR reaction mix composition without Internal Control (WhenIC-BMixis added during Nucleic Acid extraction). q PCR reaction mix composition with Internal Control (When IC-B Mix is not added during Nucleic Acid extraction).

Table–1 qPCR reaction mix composition

Components	**Volume per reaction (µL)** **(for final vol.of 25 µL)**
Quantiplus HBV Ready Mix	15.0
Internal Control (IC)	1.0
Extracted HBV DNA/PC/PW	10.0

RT PCR Cycling conditions

1. **Configure the following program in the machine.**

Table–2 RT PCR Cycling parameters

Steps	**No. of cycles**	**Temperature (°C)**	**Time**
1. (Initial denaturation)	1	95	15 min
2. (PCRcycling)	40	95	15 min
		60	60 min
Plate Read/Data Acquisition in FAM, and Yakima Yellow/VIC channel			

Data Analysis

Analyze the data after completion of their uncheck the Rn/Cycle amplification plot and ΔRn/Cycle amplification plot to observe the amplification signal generated by different samples in the run.

Setting the threshold for the PCR Data analysis

The threshold should be set either automatically (by the machine itself)/ or manually just above the background signal of the negative controls and negative samples by referring to Rn/Cycle amplification plot. The mean threshold value calculated from these experiments will most likely work for the majority of future runs, but the user should never the less review the generated threshold value at regular intervals.

RESULTS

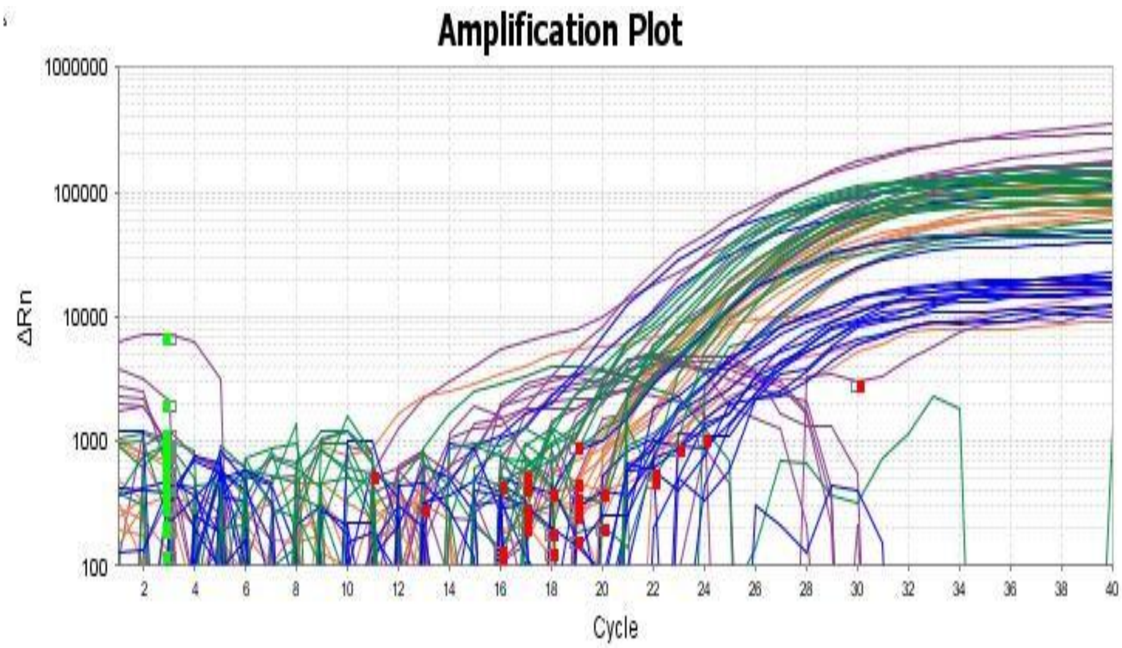
Among 28 HBV-DNA positive patients, 21 patients (75%) were male and 7 patients (25%) were female. Mean age of these 28 patients was 34±14 years.

I. Distribution of sex in HBV DNA positive patients.

Table–3 shows HBV DNA Positive in patients.

Sex	Total(n=38)	Percentage
Male	N= 21	75%
Female	N=7	25%

Fig:5 Show the Amplification of HBVDNA.



HBV DNA positive patients

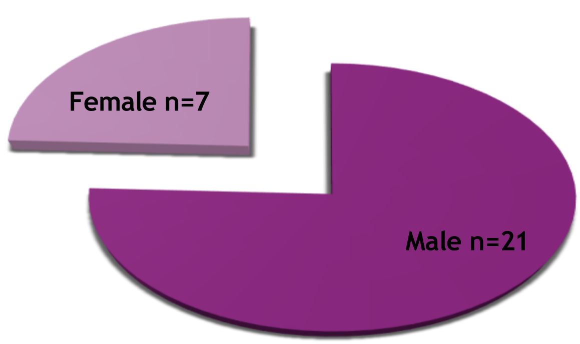


Fig-6. Pic image of patients males vs females.

II. Detection of HBV-DNA by RT PCR among HBs Ag positive patients (n=60).

Total patients	HBsAg positive	HBV-DNA Detected	HBV-DNA Not Detected
N=38	38(100%)	28(73.7%)	10(26.3%)

Table–4.shows HBV DNA vs HBeAg.

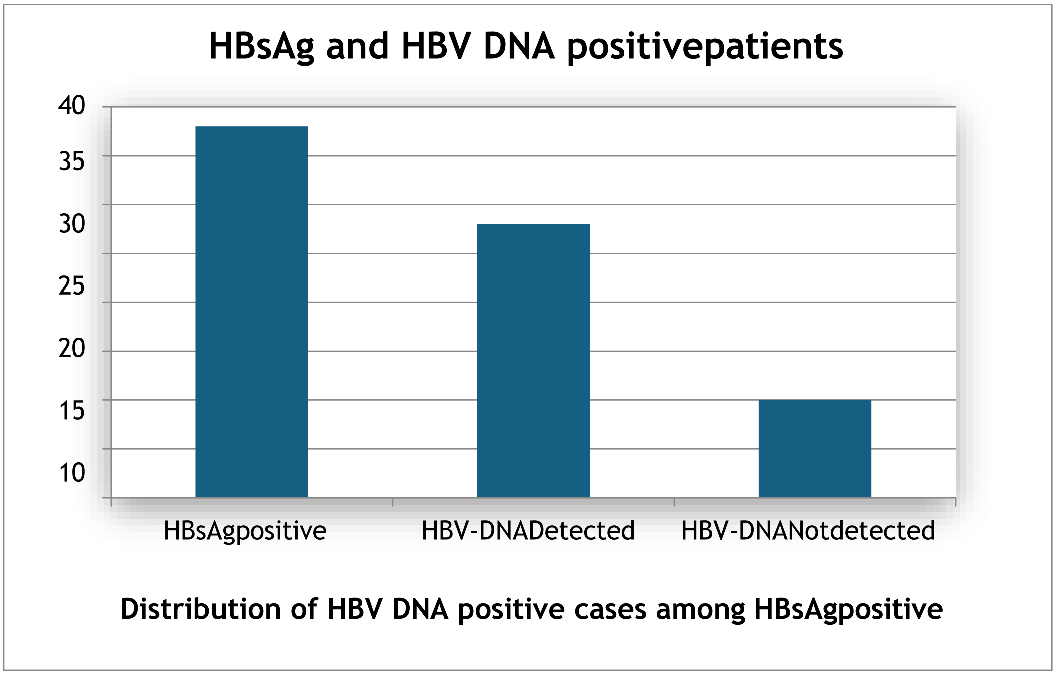


Fig-7.shows the graphical representation of HBsAg and HBV DNA

III. Detection of HBV-DNA by RT-PCR and detection of HBeAg, anti-HBe by Rapid in HBsAg positive patients (n=38).

Table–5. Shows HBV DNA vs HBeAg vs Anti HBe

Detection of HBVDNA	**HBeAg positive**	**Anti-HBe Positive**	**Both HBeAg & anti-HBe negative**
Detected (28)	12 (42.8%)	7(25%)	13(46.4%)
Not Detected (10)	0 (0%)	3 (10.7%)	7(25%)
Total	12 (31.5%)	10(26.8%)	20(52.6%)

IV. Table- 6. Hepatitis B DNA levels and its relation with HBeAg reactivity (According to the American Association for the Study of Liver Disease (AASLD Practice Guidelines)

	HBVDNA levels (<2000IU/ml)	**HBVDNA levels (2000-20000 IU/ml)**	**HBVDNA levels (>20000IU/ml)**
HBe Ag Positive (n=12)	6 (50%)	2 (16.7%)	4 (33.3%)
HBe Ag Negative and HBV DNA detected n=16/28)	15 (93.7%)	0	1 (6.25%)

DISCUSSION

In the present study **t**he cross-sectional research was conducted at GSL Medical College and General Hospital, Rajahmundry. The study took place from May to June 2025 and focused on HBsAg positive patients of both sexes with an age limit of up to 60 years. A total of 38 HBsAg positive patients were in from GSL Medical College and General Hospital, Rajahmundry for the study. Relevant medical history was obtained from the patients. Following written consent from the patients, the study involved two primary procedures: Firstly, HBV-DNA was detected and quantified using RT-PCR in the molecular biology laboratory. Secondly, serum HBeAg and anti-HBe were detected using a rapid method. The presence of viral DNA in the serum provides compelling evidence for the existence of infectious virions. HBV DNA levels become detectable within 30 days of infection, typically peaking during acute hepatitis, and subsequently declining until resolution of the infection occurs spontaneously.

In this present study, out of a total of 38 patients who tested positive for HBsAg, RT PCR identified HBV-DNA in 73.7% of individuals, while 26.3% tested negative for HBV-DNA. This outcome aligns with a study conducted by Rahman W et al 2014 [2] among HBsAg positive patients, where HBV-DNA was detected in the serum of 60.7% of patients and 39.3% tested negative for HBV-DNA. Among 28 (73.7%) HBV-DNA positive patients, 12 (42.8%) were HBeAg positive which indicates that the patients may be in immune tolerance phase and 7 (25%) patients were anti- HBe positive which indicates that the patients may be in immune clearance phase (Table -6). These findings correlate with the studies by Dantaetalandyun-fanetal..Again, among these 28HBV-DNA positive patients,16(57.1%) patients were negative for HBeAg. This correlates with the study by [23, 26] which showed that HBV-DNA was detected in 77.0% of HBeAg negative patients and all HBeAg positive patients.

On the other hand, among 10 (26.3%) HBV-DNA negative patients, none were HBeAg positiveand10 (26.3%) patients were anti-HBe positive which indicates that the patients may be of silent infection which correlates with the study of MRMdetal. 13 (46.4%) patients were negative by both the tests (HBeAg and anti-HBe) which indicates that the patients may be of pre core mutant variety or carriers which correlate with the study of Yun-Fan et al.

Among 28 HBV-DNA positive patients, 21 (75%) were male and 7 (25%) were female, thus depicting male predominance. Lef4VBGAccRtrpb6bamiXkVBUUgmz6PMbB±14years.These findings correlate with the study by Azita Ganjietal and Rahman Wetal2014[20,21], which also showed male predominance (75%) and mean age of these subjects was 37±11 years. The detection and quantitation of HBV-DNA play an important role in diagnosis as well as monitoring HBV infection to assess therapeutic response to antiviral in chronic Hepatitis B patients [25-28]. Asymptomatic HBsAg positive carriers should not be considered as having inactive disease [29]. They should be followed up every 3 to 6 months to know the activity of the disease and development of complications. Majority of these carriers suffer from chronic hepatitis rather than acute hepatitis probably contracting the disease in prenatal period or early childhood [30].

The development of liver carcinoma may be related to persistent HBsAg positivity and HBeAg seropositivity. As per the American Association for the Study of Liver Disease (AASLD Practice Guidelines) attire of 20,000IU inpatients positive for HBeAg may be an independent risk factor for liver carcinoma [14].In HBeAg negative patients, those patients who have compensated liver disease and attire of over 2000 require treatment and if de compensated, any detectable DNA level is considered significant. However, there is no cut-off to distinguish HBeAg negative patients with chronic infection and chronic Hepatitis B carriers [14, 24-29]. In our study, 6 HBeAg positive patients had titres less than 20000 but 2 out of 12 had values above 2000 IU/ml and 4 showed HBeAg positive in >20000 IU/ml . However, among the HBeAg negative group with detectable DNA levels, i.e. in 15 out of 28 patients, 1 patients had attire of more than 20000IU/ml, 0patients were in 2000-20000 range and the other 15 patients had titres less than 2000 IU/ml [31-33]. The Present study highlights the discrepancy in the number of patients with Hepatitis B who underwent only serological testing and those who underwent molecular testing for viral load monitoring. Hence, though molecular assays are themostreliable to monitor the viral load, other serological parameters are still being preferred for financial reasons.

CONCLUSION

The present study highlights that a significant number of serologically positive individuals in the East Godavari region carry detectable levels of HBV DNA, indicating active viral replication. The presence of viral DNA even in HBeAg- negative individuals emphasizes the limitation of relying solely on serological markers for disease monitoring. Quantification of HBV DNA is therefore essential for accurate assessment of disease activity, guiding treatment decisions, and preventing complications such as cirrhosis and hepatocellular carcinoma. These findings support the need for integrating molecular testing into HBV screening programs, especially in high-prevalence areas like East Godavari, to improve disease management and public health outcomes.

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