

A Study Comparing Serological And Molecular Methods For Hepatitis B Virus Diagnosis

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ABSTRACT

Hepatitis B, a potentially life-threatening liver infection caused by the hepatitis B virus (HBV), is a major global health problem with a high risk of cirrhosis and liver cancer. India has 10-15% of the world's HBV carriers, with an estimated 40 million carriers. About 15-25% of HBsAg carriers are likely to suffer from cirrhosis and liver cancer and may die prematurely. HBV belongs to the hepadnaviridae family and consists of partially double-stranded, relaxed-circular DNA (RC-DNA). Laboratory diagnosis is based on serological and virological markers. HBsAg can be detected in the serum 30 to 60 days after exposure and persists for variable periods. Molecular methods are mainly used to detect nucleic acids. In a study, 2 patients were tested positive for HBsAg by Meriscreen HBsAg card test and 4 were tested positive for HBV DNA by PCR. The most common age group affected was 21-30 years, with 75% of HBsAg positives being males. Vaccination plays a major role in controlling infection rates, and early detection and treatment can decrease morbidity and mortality. Both serology and molecular methods can be used to detect HBV infection.

Keywords –hepatitis B virus, meriscreen HBsAg , PCR.

I. INTRODUCTION

When the liver becomes inflamed, it is known as hepatitis. The A, B, C, D, and E hepatotropic viruses are among those that cause it. The two most frequent hepatotropic viruses that may be transmitted by blood are hepatitis B virus (HBV) and hepatitis C virus (HCV). Hepatitis B and C viruses are major contributors to liver-related diseases and deaths. The infection of the liver with hepatitis B may be fatal. Everywhere in the world, people are dealing with this serious health issue. Cirrhosis and hepatocellular cancer are the end results, and it causes a great deal of illness and death.

In 2015, 257 million individuals were expected to be living with chronic hepatitis B infection, as defined as being hepatitis B surface antigen positive, according to the World Health Organization. Cirrhosis and hepatocellular carcinoma (primary liver cancer) were the leading causes of mortality among the estimated 8,87,000 hepatitis B cases in 2015. In the pre-vaccine period, which spanned from the 1980s to the early 2000s, around 5% of the population was chronically infected with hepatitis B virus; however, by 2019, that number had reduced to just under 1%, and 27 million individuals were aware of this fact. One member of the family Hepadnaviridae, HBV is an enclosed DNA virus with partly double-stranded DNA. 3 Hepatitis B virus (HBV) causes a range of diseases, including self-limited hepatitis, acute fulminant hepatitis, chronic hepatitis, and liver cirrhosis and hepatocellular cancer, among other problems.

II. REVIEW OF LITERATURE

Hippocrates, writing in the fifth century B.C., was the first to describe epidemic jaundice. In 1983, German shipyard workers were given a smallpox vaccination that included human lymph, which led to the discovery of the first documented instances of Hepatitis B. In 1940, a bigger incidence of hepatitis was seen in troops who had received the yellow fever vaccination that included human serum, according to British doctor F.O. MacCallum. He first came up with the names "Hepatitis A" and "Hepatitis B" to describe the two types of the illness; the former is spread by infected water and food, while the latter is acquired through direct contact with infected blood. Dr. Baruch Blumberg began collecting blood samples from people all around the globe in the late 1950s so he could study protein polymorphism. In 1963, Dr. Baruch took blood samples from people with hemophilia or leukemia who had undergone many transfusions. Using a panel of 24 sera from healthy persons, these samples were examined for the presence of isoprecipitins. In contrast to the other panel sera, two hemophilia sera showed a distinct precipitin line when tested with an aboriginal Australian sera. The Australian antigen is the name given to this novel protein. Using electron microscopy, D.S. Dane found the whole Hepatitis B virus in blood samples in 1970. This finding was the basis for Dr. Blumberg's 1976 Nobel Prize in medicine.

III. OBJECTIVES OF THE STUDY

- In order to get a feel for the HBsAg seroprevalence in hospitalized populations across various age groups.
- To determine the scope of the disease's spread in the community and the key variables contributing to it.
- The goals of this study are to evaluate the HBsAg marker in patients and to compare it with real-time PCR as a tool to identify active hepatitis B infection in patients suspected of having the virus, and to detect the Hepatitis B surface antigen using a quick card test and HBV DNA using RT-PCR.

IV. MATERIALS AND METHODS

Place of study: KHAJA BANDANAWAZ TEACHING AND GENERAL HOSPITAL, KALABURGI

Study period: 1 Year. August 2021 to July 2022.

Design of Study: A cross sectional hospital based study.

Ethical committee: Prior approval obtained from institutional ethics committee .

Informed consent: Taken from each patient who participated in the study.

Sample size: Calculated by using the formula

$$N=4pq/l^2$$

$$=4 \times 60 \times 40 / 144$$

$$=66$$

Where: p= 60(prevalence)

q=40(100-p)

l=20% of p(Allowable error)

V. RESULTS

Interpretation of results-

1. Meriscreen HBsAg kit-



FIGURE 1- Positive test

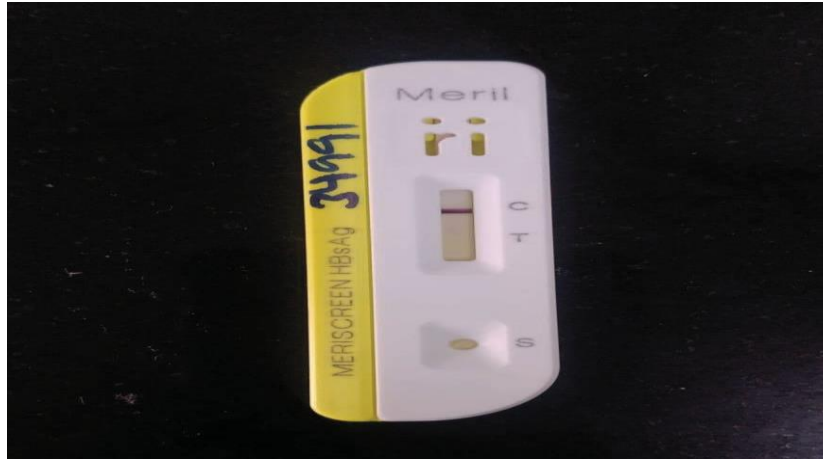


FIGURE 2-Negative test

2. AMPLIFICATION PLOTS OF PCR FOR HBV DNA POSITIVE SAMPLES

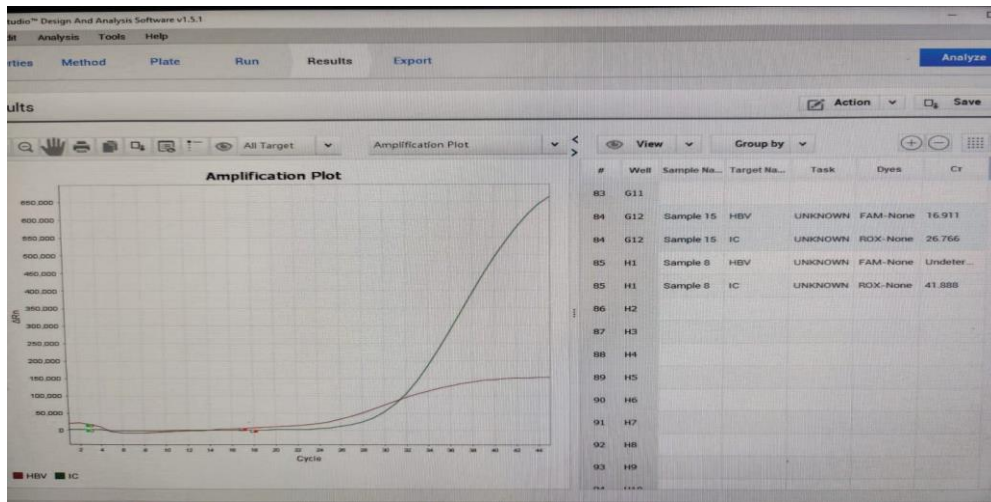


FIGURE 3- Amplification plot 1-showing cycle threshold at 18th cycle for sample no 15. Threshold of standard is 16.5.



FIGURE 4- Amplification plot 2.- cycle threshold seen at 22nd cycle for sample no 11. Standard threshold value is 18.

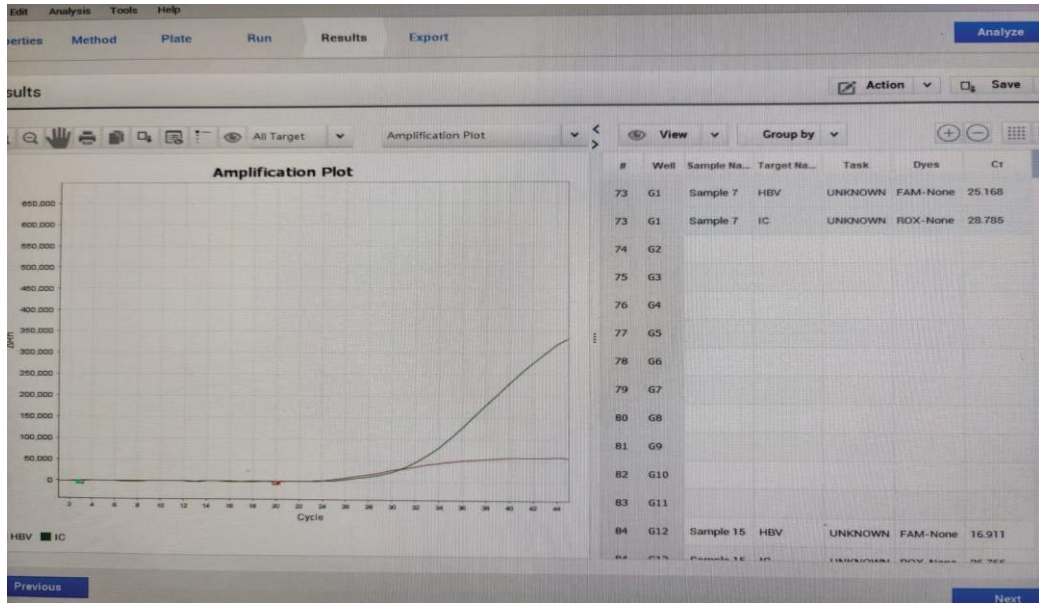


FIGURE 5- Amplification plot 3 -showing threshold value at 20th cycle for sample no 7.

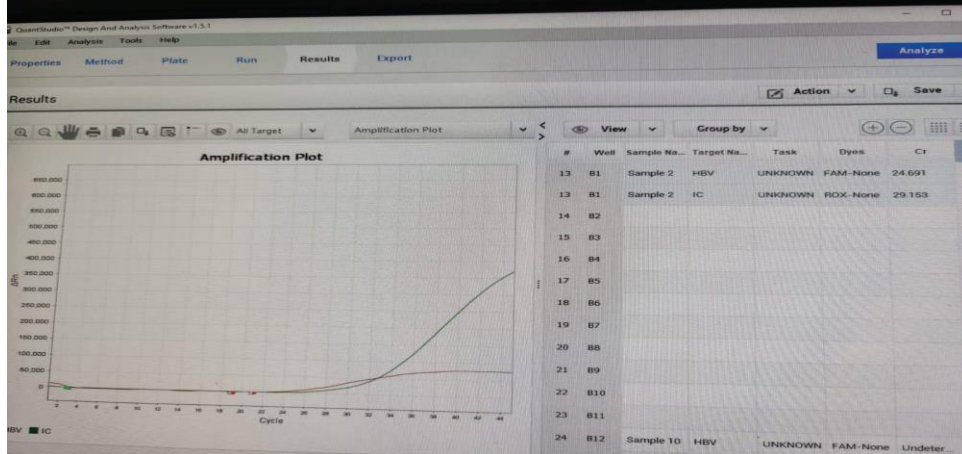


FIGURE 6- Amplification plot 4- showing threshold value at 21st cycle for sample no 2. Threshold value of standard is 19.

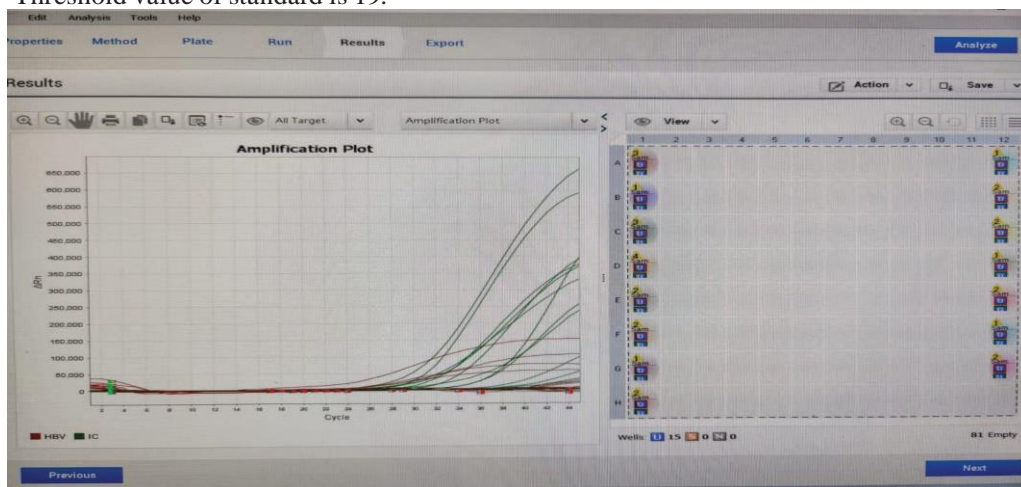


FIGURE 7- Amplification plot 5- . Threshold value of positive samples no's 15,11,7,2 is 18, 22,20,21 respectively.

OBSERVATIONS

TABLE 1 -Age wise distribution of patients.

Age in years	Number of patients	Percentage
<20 yrs	3	4.6
21-30 yrs	17	25.7
31-40 yrs	16	24.3
41-50 yrs	15	22.7
51-60 yrs	9	13.6
>60yrs	6	9.1
Total	66	100
Mean \pm SD	40.36 \pm 12.80	-----

Among the 66 patients, 17 (or 25.7% of the total) fall within the 21–30 age bracket, 16 (or 24.3% of the total) fall within the 31–40 age bracket, and 15 (or 22.7% of the total) fall within the 41–50 age bracket. Patients' ages ranged from nineteen years old at the youngest to sixty-eight years old at the oldest. Patients' average age was 40.36.

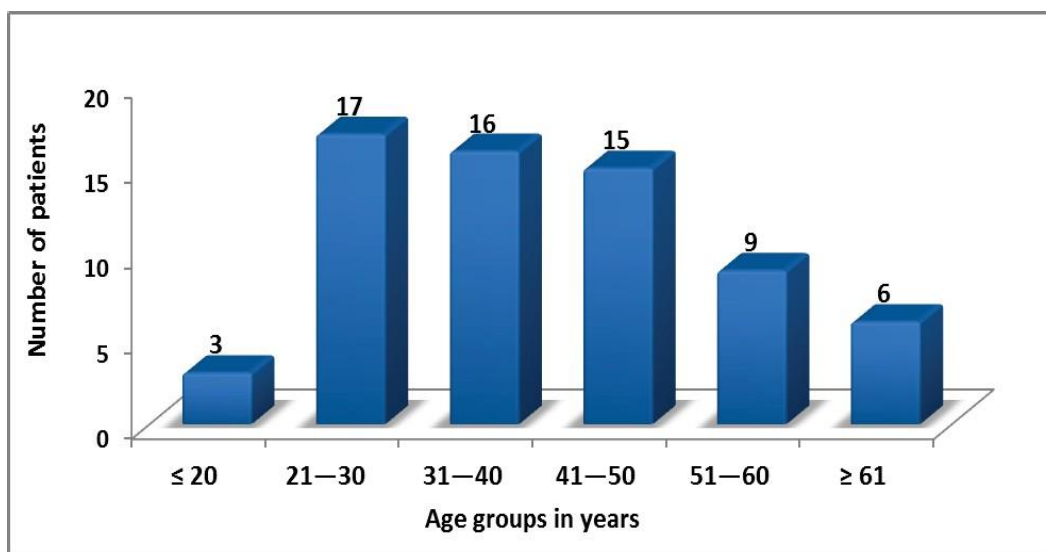


FIGURE 8 :Simple bar diagram represents age wise distribution of patients

Table 2: Gender wise distribution of patients

Gender	Number of patients	Percentage
Males	32	48.5
Females	34	51.5
Total	66	100

A total of 34 patients (51.5%) were female and 32 patients (48.5%) were male, according to the study. There were 1.06 males for every female participant in the research.

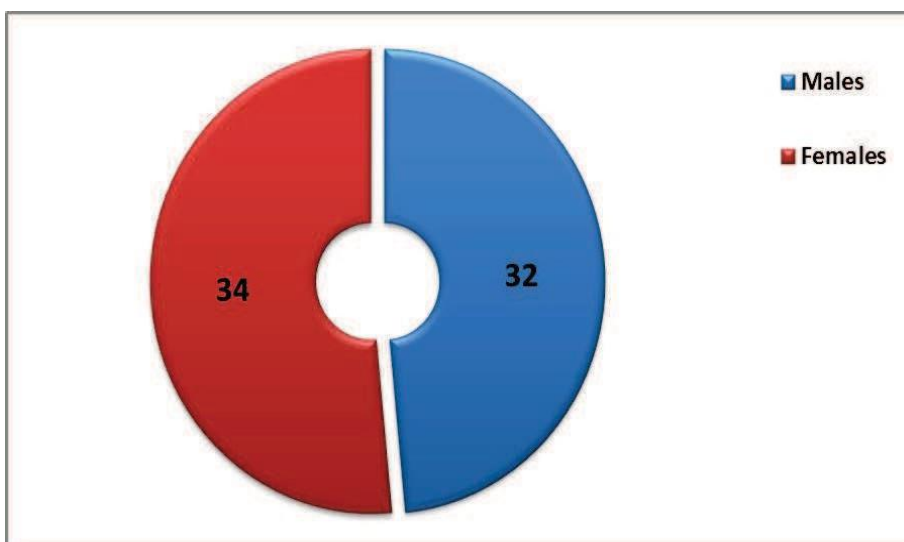


FIGURE 9 :Pie diagram represents gender wise distribution of patient

Table 3: Distribution of patients based on positive past history finding

Past history	Number of patients	Percentage
Blood transfusion	10	15.2
Drug abuse	6	9.1
History of NSI	3	4.5
Body piercing	1	1.5
Female sex worker	1	1.5
Old case of hepatitis	1	1.5
No significant history	44	66.7

Total	66	100
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Among 66 individuals diagnosed with hepatitis B, 44 (66.7%) had no known history of the virus, 10 (15.6%) had received a blood transfusion in the past, 6 (9.1%) had abused drugs, and 3 (4.5%) had experienced non-specific inflammation.

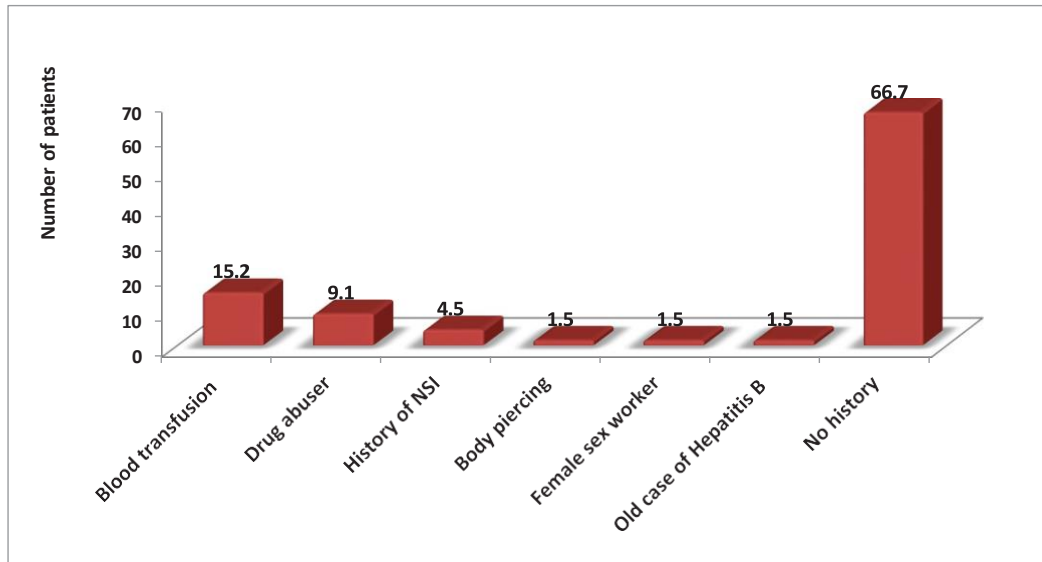


FIGURE 10 - Simple bar diagram represents past history distribution of patients

Table 4 : Distribution of patients according to history of vaccination

Vaccination status	Number of patients	Percentage
Vaccinated	26	39.4
Not vaccinated	27	40.9
Not known	13	19.7
Total	66	100

Of the 66 individuals with hepatitis B who participated in the trial, 26 (or 39.4%) had received the vaccine, 27 (or 40.9%) had not, and 13 (or 19.7%) had no idea that it was an option.

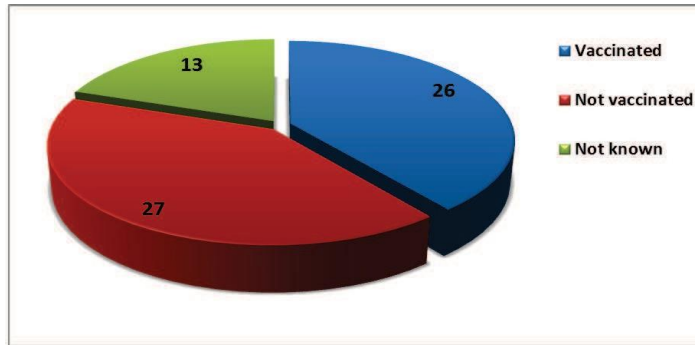


FIGURE 11-Pie diagram represents history of vaccination

Table 5: Incidence of Hepatitis B positive

RESULT	Number of patients	Percentage
Hepatitis B positive	4	6.1%
Hepatitis B negative	62	93.9%
Total	66	100%

Of the 66 patients included in the research, 62 (or 93.9% of the total) tested negative for HBsAg, whereas 4 (or 6.1% of the total) tested positive. There was a 6.1% prevalence of HBsAg in the research.

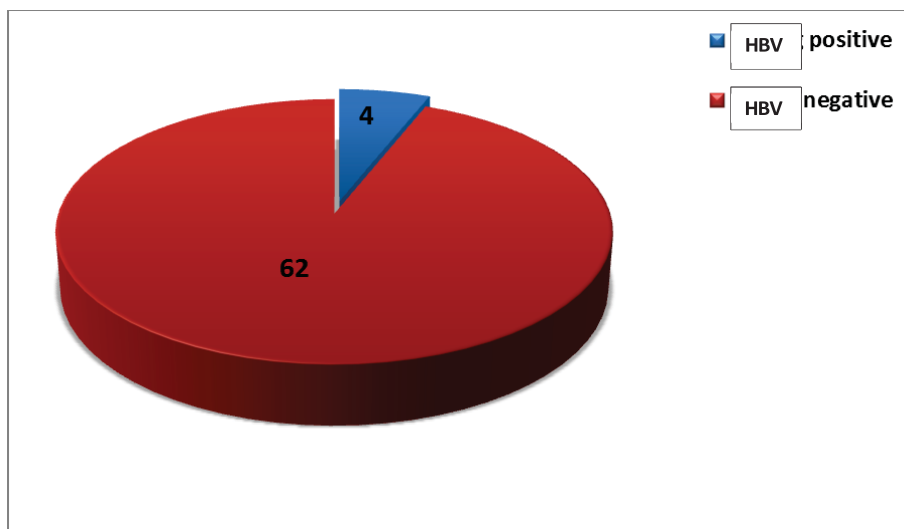


FIGURE 12-Pie diagram represents Incidence of Hepatitis B positive

Table 6: Age wise comparison of Hepatitis B positive patients

Age in years	Total	Hepatitis B positive patients		Hepatitis B negative patients	
		Number	Percentage	Number	Percentage
<20years	3	0	0.0	3	100.0
21-30 years	17	1	4.9	16	94.1
31-40 years	16	2	12.5	14	87.5
41-50 years	15	0	0.0	15	100.0
51-60 years	9	1	11.1	8	88.9
>61 years	6	0	0.0	6	100.0
Total	66	4	6.1	62	93.9
Fishers exact test		P=0.073, NS			

Of the four patients who tested positive for hepatitis B, two (or 50.0% of the total) were within the 31–40 age bracket, one (or 25.0% of the total) was in the 21–30 age bracket, and one (or 25.0% of the total) was in the 51–60 age bracket. Regarding age categories, there was no statistically significant difference in the distribution of patients who tested positive for Hepatitis B ($P>0.05$).

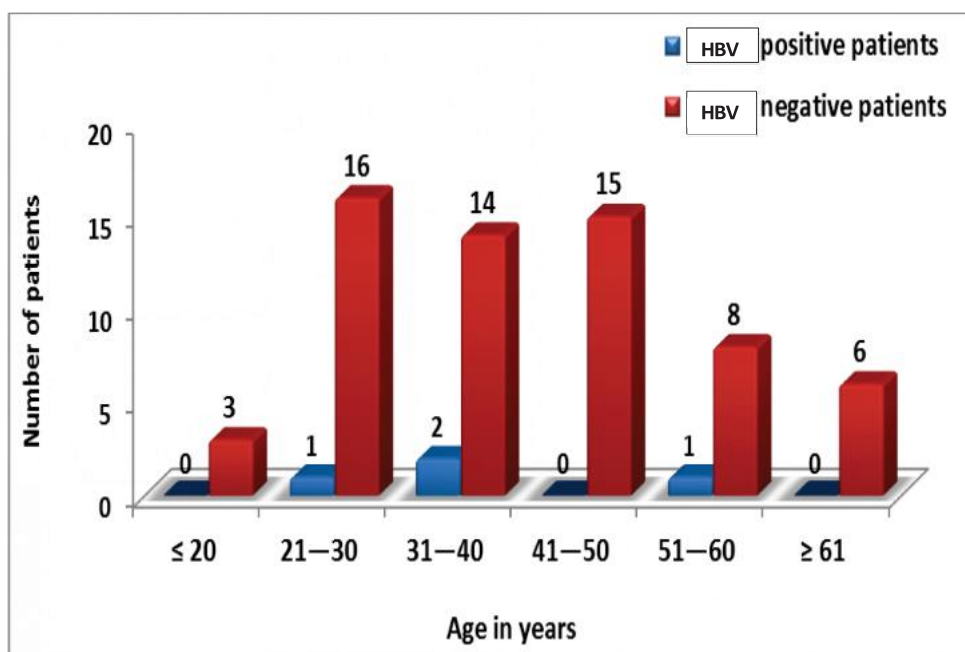


FIGURE 13: Multiple bar diagram represents age wise comparison of Hepatitis B positive patients

Table 7- Gender wise comparison of Hepatitis B positive patients

Gender	Total	Hepatitis B positive patients		Hepatitis B negative patients	
		Number	Percentage	Number	Percentage
Males	32	3	9.4	29	90.6

Females	34	1	2.9	33	97.1
Total	66	4	6.1	62	93.9
Fishers exact test	P= 0.205, NS				

Out of 4 HBsAg positive patients; 3 (75.0%) of Hepatitis B positive patients were males and 1 (25.0%) Hepatitis B positive patient was female. Percentage of Hepatitis B positive patients of males were predominant as compare to females, but not statistical significant ($P>0.05$).

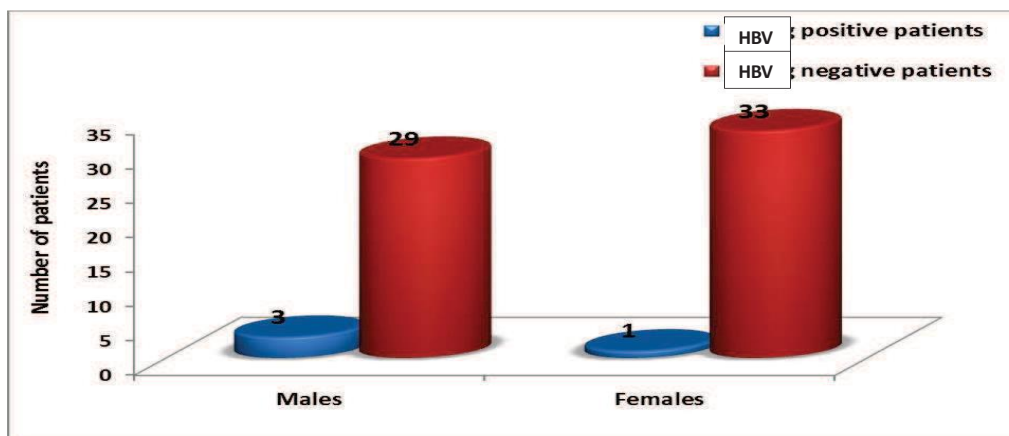


FIGURE 14-Multiple bar diagram represents gender wise comparison of Hepatitis B positive patients

Table 8: Correlate and discordant of patients with PCR

SL NO.	RESULT	HBsAg	PCR	NO. OF CASES	PERCENTAGE
1	POSITIVE	POSITIVE	CORRELATE	2	50.0
2	NEGATIVE	NEGATIVE	DISCORDANT	2	50.0
	TOTAL	-----		4	100

In the study; Out of 4 positive patients 2 (50.0%) of patients were correlate with PCR and 2 (50.0%) were discordant with PCR.

Table 9: Statistical Analysis of RT-PCR test.

1	Sensitivity	66.7%
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2	Specificity	100.0%
3	Positive predictive value	100.0%
4	Negative predictive value	50.0%
5	Diagnostic accuracy test	75.0%

DISCUSSION

A major issue in public health across the world, hepatitis B is a major concern. Blood and blood products are the primary vectors for the transmission of HBV infections. There are two billion HBV infections and 350 million persons who carry the virus chronically over the world. The locations are categorized as having a high prevalence (>8%), an intermediate prevalence (2-7%) or a low prevalence (<2%) of HBV infections. Reducing the hepatitis B carrier status, the World Health Organization (WHO) advocated for universal hepatitis B vaccination. Cirrhosis of the liver and hepatocellular cancer may be triggered by this. The scope and transmission of diseases in a community may be better understood with the use of seroprevalence studies.

This research comprised sixty-six individuals with probable hepatitis B who visited the outpatient and inpatient departments of Khaja Bandanawaz Teaching and General Hospital in Kalaburagi. Below, we explain the results that were acquired after assembling the data and comparing them with comparable research.

Prevalence of Hepatitis B:

A prevalence rate of 6.1% for hepatitis B was found in this investigation.

Given that 3% to 8% of the world's population has hepatitis B, this estimate is nearly reasonable. 71

This incidence of prevalence is similar to the 4.6% seen in the 2009 research by Sameen Afzal Junejo et al. In 2008, researchers Noorali et al. and Hakim et al. determined that 4.5% of the population had hepatitis B.

Wang C et al.(2009) and Yan YX et al. (2010) found a prevalence of 11.9% and 12.5%, respectively, which is much higher than what we observed in our research.

About 3% to 4.2% of the population in India has hepatitis B. According to the majority of research, the total rate of HBsAg positive falls anywhere between two percent and eight percent.

Batham A et al. demonstrated that, according to a meta-analysis of population weights, the estimated positive prevalence is 3.1% in non-tribal groups and 11.85% in tribal communities.

Environmental, societal, and genetic factors may explain why this research found a greater incidence.

Age wise distribution of patients:

With a mean age of 40.36 years, the age groups included in the current research ranged from 18 to 68 years.

Table no. 4 shows that the age group between 31 and 40 years had the greatest frequency, at 24.3%. Khan F et al. found a frequency of 23.83% in the 31–40 age range, which is in line with our results.

This research contradicts a comparable one conducted in northern India by Rabyang et al., who found a rate of 6.10 percent for the 31–40 age bracket

In the age groups of 21–30 and 51–60, the prevalence rate was 25%, which is in line with previous studies

(Khakharvipul M et al., 58 Sangramsinghpatel et al.).

Contrasting with the current research, a comparable one conducted in northern India by Rabyang et al. found a rate of 5.34% for the age groups of 21–30 and 51–60 years. In this particular investigation, we did not find any statistically significant differences across the age groups.

Gender wise distribution of patients :

Of the 66 patients included in the current research, 52.5% were female and 48.5% were male. Rabyang et al. and Abdul Malik et al. came to similar conclusions.

Males (9.4% of the total) fared better than females (2.9%). But, like with Rabyang et al., it is not statistically significant.

It was noted by both Patil SS et al.⁶⁰ and Nafees et al. that the frequency was 10.3% among men and 3.7% among females.

Researchers in Sudan and Iran found that the frequency was 84% in men and 17% in females.

Despite the fact that several research, including the current one, have shown a female preponderance (73.8% vs. 26.2%), a Ghanaian study by V.E. Senoo-Dogbey and D.A. Wuaku discovered the opposite.

A different cross-sectional research conducted by B.Mangkara et al. found that the prevalence of females was greater (67.2%) than men (33.8%).

Distribution of patients based on significant Past history.

Of the 66 individuals diagnosed with hepatitis B, 44 (66.7%) had no prior history of the virus. In contrast, 10 patients (15.6%) have previously had blood transfusions.

A total of 2.4% of the patients in the Rabyang et al. research had a transfusion history. These results are inconsistent and do not apply to the current investigation.

Measures such as (1) donor education, (2) stringent donor selection criteria, (3) enhanced serological screening processes, and (4) improved blood collection and transfusion techniques may have contributed to the drop in seropositivity.

There was a history of intravenous drug misuse in 6 individuals (9.1%) in this research. This result was in line with what Sandesh K, Varghese T, Harikumar R, et al. ⁶² discovered in their research of risk categories in north Kerala, which was about 8%.

Jamie et al. discovered that 19.8% of participants had a history of drug misuse.

The results were fairly similar to those of Ali et al. (6%), with 3 patients (4.5%) having a history of NSI.

Contrary to the current investigation, Rabyang et al. demonstrated a high percentage of 39.7 percent of patients with NSI.

Distribution of patients on past history of Vaccination -

This research's results are in line with those of A.S. Saddik, A.A. Alzailayee, and M.B. Foda, where 41% of 66 hepatitis B patients received a vaccine. Vaccination rates in the current study were 26 (39.4%).

Of the participants surveyed by V.E. Senoo-Dogbey and D.A. Wauku, 53.5% received the vaccine. This result is marginally more than what the current research found.

Among the patients surveyed for this research, 40.9% had not had vaccinations and 19.7% were unaware of whether or not they had been vaccinated.

Vaccinated persons faced practically zero-level common risk factors of HBV infection compared to unvaccinated individuals, according to a study by Yiwei guo et al., which might indirectly suggest that vaccination could prevent people from HBV infection. This result agrees with what we found in our current investigation.

Serologic versus RT-PCR Technique-

Only two of the sixty-six samples tested positive for HBsAg by RDT, whereas four indicated the presence of HBV DNA by RT-PCR. Two instances (50%) match the results of RT-PCR, whereas two cases (50%) do not.

Occult hepatitis, in which there is no HBsAg but HBV DNA in the blood, may explain the contradictory PCR findings shown in this investigation.

Maria Belopol'skaya et al. also found anything similar in their investigation.

Results from serological testing and real-time polymerase chain reaction (RT-PCR) for hepatitis B virus infection were shown to be 100% concordant in the investigations conducted by A.S. Sadak, A.A. Alzailayee, and M.B. Fedat ⁶⁷ and Hudu.S.A et al.

A recent research conducted in June 2022 by Iker Falces-Romero et al. found 99% concordance with PCR and a mere 1% result that did not match.

In 2010, Al Shaban Z.O. et al. performed another investigation in which 6.25% of the samples tested positive by serology were compared to 14.58% by RT-PCR.

Statistical Analysis:

SENSITIVITY-

Consistent with the results of D. Bulent et al. (74.3%), the current investigation found that RT-PCR had a sensitivity of 66.7%.

A separate research by C. Daniel et al. found that a multiplex RT-PCR test could identify hepatitis B, hepatitis C, and human immunodeficiency virus type 1 with a sensitivity of 95%. This number is much higher than the 66.7% found in the current research.

Another research that contradicts the current one, by S.Priyanka et al., found a diagnostic sensitivity of 97.4 percent.

V. Alicia et al. found a sensitivity of 90% in their investigation.SPECIFICITY-

Consistent with previous research by V. Alicia et al., D. Buulent et al., and C. Daniel et al., the current investigation found that RT-PCR had a 100% specificity.

There was complete specificity in the research by P. Shantanu, J. Amita, and J. Bhawana. In contrast to the current study, the lone research by S.Priyanka et al. demonstrated a much lower specificity of 99.4 percent..

POSITIVE PREDICTIVE VALUE-

A perfect 100% positive prediction value was achieved in this investigation.

The current research is almost identical to S.Priyanka et al., which demonstrated a lower positive predictive value of 97.4%..

NEGATIVE PREDICTIVE VALUE-

In contrast to the 99.4 percent found in the research by S. Priyanka et al., the current investigation reveals a negative predictive value of 50%.

In terms of negative predictive value, no other studies corroborated the results of the current investigation.

DIAGNOSTIC ACCURACY –

The current research found that the test had a diagnosis accuracy of 75%. We were unable to locate any studies that corroborated or contradicted these results.

Table 10: COMPARISION OF STATISTICAL ANALYSIS OF DIFFERENT STUDIES

	PRESENT STUDY	D.Bulient et al	C.Daniel et al	S. Priyanka et al	V.Alicia et al

SENSITIVITY	66.7%	74.3%	95%	97.45	90%
SPECIFICITY	100%	100%	100%	99.4%	100%
POSITIVE PREDICTIVE VALUE	100%	-	-	97.4%	-
NEGATIVE PREDICTIVE VALUE	50%	-	-	99.4%	-
DIAGNOSTIC ACCURACY	75%	-	-	-	-

VI. SUMMARY AND CONCLUSION

CONCLUSION

The incidence of hepatitis B in tertiary care hospital patients was assessed in this research. When comparing sexes, men had a much higher frequency of hepatitis B (6.1%).

In the age range of 31–40 years, the greatest frequency was seen at 24.3%. In this investigation, we found no statistically significant variation among age groups. Disease transmission patterns and size are estimated using prevalence studies.

A very little medical history was present in 66.7% of individuals. There was a 15.6% prevalence of transfusion history, a 9.1% prevalence of intravenous drug misuse, and a 4.5% prevalence of needle stick injuries.

26 patients (or 39.4% of the total) had received the Hepatitis B vaccine, whereas 27 patients (or 40.9% of the total) had not. Only 13 out of 63 patients (19.7%) knew whether they had been vaccinated.

The HBsAg rapid card test is quick, painless, and inexpensive.

Since real-time PCR is highly specific for HBV DNA, it is considered the gold standard. Treatment plans benefit greatly from early identification and diagnosis. In addition to reducing the transmission of hepatitis B, it aids in blood transfusion screening.

Only two of the sixty-six samples tested positive for HBsAg by RDT, whereas four indicated the presence of HBV DNA by RT-PCR. Two instances (50%) match the results of RT-PCR, whereas two cases (50%) do not.

Positive predictive value (PPV) is 100%, specificity is 100%, and sensitivity is 66.7% for RT-PCR in this research. The diagnostic accuracy is 75% and the negative predictive value is 50%.

In order to better create, modify, and avoid tactics, more research on genotype distribution is necessary. Prompt identification helps to halt the development of the illness and its consequences.

SUMMARY

- Participants comprised sixty-six individuals who had a history of possible hepatitis B virus infection.
- Patients' blood and serum were tested for HBsAg using a meriscreen fast card test and for HBV

DNA concurrently using RT-PCR.

- The majority of the patients, 25.7%, were between the ages of 21 and 30.
- Of the patients, 32 were men and 34 were females, making it 51.5% female.
- A very little medical history was present in 66.7% of individuals. Out of all the participants, 10, or 15.6%, had a transfusion history.
- Twenty-seven individuals, or 40.9%, did not get a Hepatitis B vaccine, whereas twenty-six, or 39.4%, did. Thirteen patients (19.7%) were unaware of whether they had been vaccinated.
- Two instances (or 3.05 percent) were found to be positive on serology by meriscreen card test, according to analysis by HBsAg.
- We confirmed 4 cases (6.1%) with HBV DNA using PCR analysis.
- Out of the four patients that tested positive, two (or 50%) fall within the 31–40 age bracket, one (or 25%) falls within the 21–30 age bracket, and one (or 25%) falls within the 51–60 age bracket.
- When looking at the distribution of Hepatitis B positive patients by age group, there was no statistically significant difference ($P>0.05$).
- Although the male prevalence of hepatitis B positive patients was higher than the female prevalence, the difference was not statistically significant ($P>0.05$).
- Of the four patients who tested positive, two (or 50%) showed PCR concordance and two (or 50%) showed PCR discordance.
- Possible occult hepatitis, defined as the lack of HBsAg but the presence of HBV DNA in blood, might explain the contradictory PCR findings seen in this investigation.
- The sensitivity of PCR for HBV DNA is 66.7%, specificity is 100%, positive predictive value is 100%, and accuracy is 75%.

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