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SEROLOGICAL PREVALENCE OF HEPATITIS-B VIRUS (HBV) IN TEHSIL DARAZINDA SUB DIVISION DERA ISMAIL KHAN, KPK, PAKISTAN

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Abstract

Hepatitis is the most common serious viral liver disease. It is a major threat to public health around the world. The purpose of this study was to determine the prevalence of HBV-positive patients in the general population of Tehsil Darazinda in district D.I. Khan, Pakistan, which is still unknown. A total of 519 blood samples were collected on the basis of signs and symptoms to investigate the infection rate of HBV in the study of population. Among these, 260 were female and 259 were male. All the HBV suspected participants were confirmed through ICT method, which was then confirmed using ELISA. The findings of the HBV DNA screening tests showed that ELISA-based results are more reliable and that positive cases of males account for 58 (22.93%) of the infection cases compared to females, who account for 47 (18%). the study included participants ranging in age from 1 to 75 years of age. The results of an age-wise analysis showed that people in the 16–45 age range are more likely to contract HBV DNA. The survey-based study on the frequency and risk factors linked to the spread of HBV infection found that married individuals had the highest prevalence of HBsAg (64.62%). On the other hand, individuals with a family record of HBV positivity carried the lowest rate of infection (1.43%). The additional factors associated with risk consisted of a history of orthodontic treatment (57.8%), surgical treatment (41.84%), blood transfusion or plasma (42.92%), ear, the nose, piercing, tattooing, or needle use (56.53%), frequent visits to a hairdresser shop (39.45%), and history of injection drug use (38.12%). This study demonstrates the alarming state of HBV circulation and emphasizes the need for improved planning, strategies, and policies to prevent and control HBV.

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INTRODUCTION

Hepatitis is the most common & lethal infectious liver disease worldwide. HBV infection affects approximately 257 million people, resulting in 887,000 deaths (WHO, 2015). Hepatitis B causes a fatal liver infection. Asymptomatic Hepatitis B is the most dangerous among the five different types. Hepatitis A, Hepatitis B, Hepatitis C, Hepatitis D, Hepatitis E. Hepatitis B is a Hepa DNA viridae family. HBV has a 3.2-kilo base pair of double-stranded DNA genome & is enveloped [1]. HBV infects hepatocytes and can cause a variety of liver complications, including cirrhosis, hepatocellular carcinoma (HCC) & other serious liver-diseases [2]. HBV can be transmitted through a variety of channels, including body fluids, saliva, vaginal fluids, menstrual fluids & blood. Other modes of transmission are the usage of contaminated syringes, unscreened blood transfusions & sexual contact with an infected partner or a newborn from an infected mother, unsanitary conditions such as unsterile dental, clinical & therapeutic processes, the utilization of contaminated razors & transplanted body organs [3]. Silent contagious liver infections are prompted by Hepatitis B virus. People are asymptomatic throughout. However, acute illness involves abdominal pain, extreme fatigue, jaundice, vomiting or nausea & dark urine [4-5]. In contrast, Enzyme-Linked Immuno-Sorbent Assay (ELISA), Recombinant Immunoblot Assay (RIBA), Enzyme Immunoassay for the Qualitative Detection (EIA), Immuno-chromatography (ICT) & others in that it is a quick & reliable tool for diagnosing, genotyping & quantifying HCV & HBV genetic material in the blood. Previously, slight studies were conducted in various regions of Pakistan [6-7]. HBV recombinant DNA vaccines have been made available for over 20 years. Three vaccine doses are typically given for hepatitis B. If followed by two more doses, infant hepatitis B vaccination within 24 hours of birth prevents infection & transmission by 90–95%. WHO recommends vaccinating all infants against hepatitis B as soon as feasible after birth [8] treatment Adefovir & Entecavir are medications used to treat HBV infections. Both of these medications appear to be promising new treatments for HBV infection [8]. HBV infected 257 million humans, killing 887,000 [9]. HBsAg is carried by 350–400 million humans. The five major hepatitis viruses—HAV, HBV, HCV, HDV as well as HEV—severely damage the liver, with the prevalence of HBV being highest in the Western Pacific Region (6.2%), followed by the African Region (6.1%). HBV inhibits liver fibrosis, including HCC, normalizes biochemical liver markers & causes liver cirrhosis. Since 1963, over 20 million Pakistanis have hepatitis B [10]. Pakistan's high HBV rate affects poor people & their families. Because 67.5% of Pakistanis live in rural areas with low socio-economic worth, HBV control is crucial. Pakistan's general populace has 3–5% HBV & high-risk populations 10–20%. HBV incidence is 9.3% in Baluchistan, 2.4 % in Punjab, 2.3 % in Sindh, & 1.31 % in Sindh (KP). KPK has the lowest HBV rate at 1.1% [11]. To determine the frequency of HBV among individuals of various age groups in Tehsil Darazinda.

2 Materials & Methods

2.1 Study Area

The study was conducted in the Darazinda region of D.I. Khan Tehsil in KPK, Pakistan.

2.2 Patient's data collection

We conducted the examiner throughout the month of January 2024. People suspected of having a liver virus infection provided the data for this study. The research scholar would clarify the goals and justification of the study to the patients in their indigenous language, namely Pashto. The families of the individuals who accepted to take part in the study provided the data points.

2.3 Questionnaire

Before blood collection, participants were required to fill out a questionnaire in the local language of Urdu. The questionnaire covered various aspects such as personal information (name, age, gender), educational and income levels, medical history (including yellowing of the skin, surgeries, dental treatments), hazard factor for spread of diseases (such as needle sticks, intravenous drug usage, tattoos, needle pricks, injections each year, and a history of hepatitis in the family.

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2.4 Sample collection

Blood samples were collected from individuals assumed of having HBV in a gel tube; each sample bore 5 milliliters. For serological and molecular analysis, sera were stored at 20 °C in three separate aliquots following centrifugation (1100-1300 rpm for 15 minutes). Men and women of various ages participated in the most recent testing.

2.5 Serological Studies

Every serum sample underwent testing using ICT and an ELISA kit to detect the incidence of anti-HBsAg anti-bodies [12].

2.5.1 Immunochromatography

The serum samples were first analysed for the existence of anti-HBsAg using immune-chromatography. Siemens Healthineers, Germany provided the ICT equipment for the screening of the samples. Based on the manufacturer's instructions, 10 µl of After adding the patient's serum to a strip well, a buffer clarification was combined using 1 or 2 drops. The existence of two red colour bands indicated a positive result for anti HBV, whereas the presence of HBsAg was shown to be negative when just one red line was present.

2.5.2 Detection of Hepatitis B Antigen (HBeAg)

The samples were screened for the finding of HBeAg using the QuickTiterTM HBeAg enzyme- linked immune-sorbent assay Kit, following procedure provided by the manufacturer. 100 microliters of the sample were introduced to a plate that had been coated with anti-HBeAg antibodies. We then kept the plate at 37 degrees Celsius for 2 hours. Five times, 250µl of 1x wash buffer were used to clean the microwell strips. Based on the manufacturer's instructions, 10-µl of tolerant serum be added to band fine, and a shield clarification consisting of 1 or 2-drops was mix. Each well was treated with 100 µl of FITC-labelled monoclonal anti-HBeAg and incubated at room temperature for one hour. We once again emptied all the wells and then washed them using a 1x concentration of WB. Subsequently, 100-µl volume of H.R.P-conju-gated (anti-FITC) monoclonal anti-body be introduced & allowed to incubate for one-hour at ambient temperature. Clear all of the well & restart the procedure of cleansing. Subsequently, the substrate solution was warmed to the ambient temperature, and 100 µl was uniformly dispensed into each well. The incubation step was conducted at room temperature for approximately 20 minutes. Conducted a comprehensive analysis of the plate's color and quickly stopped the reaction when a rapid color shift was seen by adding a stop solution of 100 µl to all wells. The results were instantly quantified by measuring the absorbance of each individual microwell using a spectrophotometer calibrated at a wavelength of 450 nm.

2.7 Data Analysis

The Chi-Square test was used to analyses risk factors in the research. The data analysis was performed employing SPSS, a statistical software version 22. The significance level was found to be less than 0.05.

3 Results& Discussions

3.1 Demographics of HBV

The data was analyzed using demographic factors. In order to assess the prevalence of HBV infection in the population of Tehsil Darazanda, samples were collected from persons who were thought to have HBV infection based on their presenting signs and symptoms. The predominant indications and manifestations of HBV encompass pyrexia, exhaustion, anorexia, diarrhea, stomach unease, darkened urine, pale-colored fecal excretions, arthralgia, and icterus (yellowing of the skin or sclerae). The current study investigated 519 individuals suspected of HBV infections. The participants were divided based on gender, with 259 (49.9%) respondents identifying as male and 260 (50.1%) as female. We divided individuals suspected of having HBV based on their age. We classified the study participants into five different age groups, ranging from 1 to 75 years old. Age group I comprised 16 (3.1%) suspects, followed by group II with 197(38%), group III with 210(40.5%), group IV with 70(13.5%) and group V with 26(5%) individuals.

Among the 519 individuals suspected of having HBV, ICT confirmed 117 (22.5%) as HBV-positive. As a result, 402 people (or 77.5% of the total) tested negative for HBV. The ELISA test revealed 105 positive results out of the 147 samples that tested positive for ICT. With 519 possible cases, this means

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that 20.2% tested positive. Finally, a questionnaire-based survey was done to determine possible hazardous variables associated with HBV infection. The history of injectable drug addiction 281 (54.14%), history of frequently barbershop shop visits 255 (49.13%), married person 245 (47.20%), ear, nose, piercing, or tattoos 225 (43.35%), history of surgery 213 (41%), history of dental producer 198 (38.15%), history of blood or plasma transfusion 167 (32.17%) and 18 (3.46%) members of the family have tested positive for HBV. (Table No.4.1).

Table No; 3.1 Demographics of HBV

S.#	Demographics Variable	No 519 (100%)
01	Gender	
	Male	259 (49.9%)
	Female	260 (50.1%)
02	Age range in years	≤15 - >55 years
03	Age range	
	I (≤ 15 years)	16 (3.1%)
	II (16-30)	197(38%)
	III (31-45 years)	210(40.5%)
	IV (46-55 years)	70(13.5%)
	V(≥ 56 years)	26(5%)
04	ICT test	
	• Positive	117 (22.5%)
	• Negative	402 (77.5%)
05	ELISA test	
	• Positive	105 (20.2%)
	• Negative	413 (79.8%)
06	Risk factors	
	• History of family member with Positive)	18 (3.46%)
	• DW (Dental work)	198 (38.15%)
	• HS (history of surgery)	213 (41%)
	• HTP (history of transfusion or plasma)	167 (32.17%)
	• HPE N, S (history piercing ear, nose, or siring)	225 (43.35%)
	• Are you married	245 (47.20%)
	• History of injection Drug use, outdoor patient	281 (54.14%)
	• History of frequent visiting a barber shop	255(49.13%)

3.2 Gender Wise HBV Prevalence.

In order to find out the prevalence of HBV disease in our study population, a gender-based evaluation was carried out on a sample of participants. The HBV testing pool consisted of 519 individuals. There were a total of 259 male and 260 female participants. Among them, 62 men and 55 women tested positive. In this respect, men were 23.93% positive for HBV, whereas females were 19% positive (Table No 4.2). These findings suggest that HBV is more frequent in Males than Female in District Darazinda(Figs. 4.1and 4.2).

Table No; 3.2 Gender Wise HBV Prevalence.

	Gander					
	Male			Female		
HBV Prevalence	No	Positive	Negative	No	Positive	Negative
	259	62	197	260	55	205

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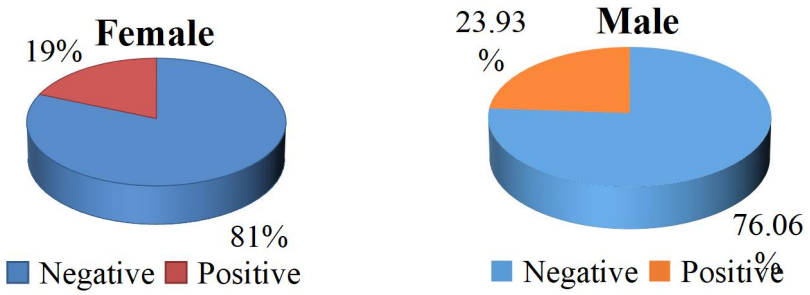
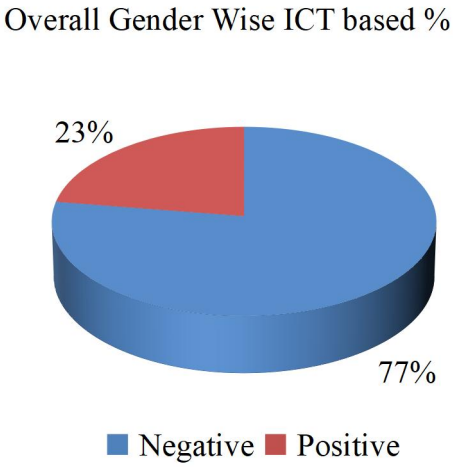


Figure; 3.1 Sero-prevalence of HBV gander in male female



Figure; 3.2 Sero-prevalence of HBV Overall Gender Wise ICT based % age

3.3; Prevalence of ICT in different age groups of HBV

The study included participants ranging in age from 1 to 75 years. These participants were separated into five age groups: group I & II comprised (1-15, 16-30 years), group III & IV were comprising (31-45, 46-60 years), and group (V) for 61–75 years. Among the initial group of approximately 16 individuals, an overall total of 9 participants had positive results for ICT, resulting in an infection rate of 56.25%. Among a total of 197 participants, 52 positive cases were found using the ICT-based second-age diagnostic. Resulting in a positivity rate of 26.39%. Among the 210 individuals in the third age group, 31 tested positive, resulting in an infection rate of 14.6%. Out of the whole sample size of 70 individuals in the fourth age group, 19 individuals tested positive, resulting in an infection rate of 27.14%. Among the 26 participants in the last V age group, 6 people tested positive, resulting in a positivity percentage of 23.07%. Based on the study conducted, it was observed that the II and III age groups had a greater prevalence rate compared to the other categories. The combined examination of ICT outcomes across all age cohorts resulted in a consecutive positive rate of 22.54% for both men and females. The prevalence rate of HBV infection is statistically considerable ($P<0.05$) (Table. 4.3, Figure 4.2, & 4.1.3).

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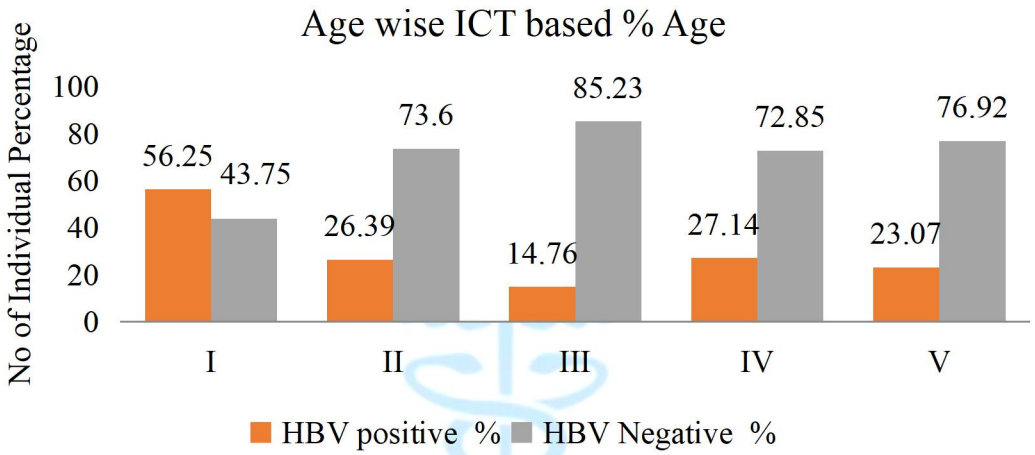
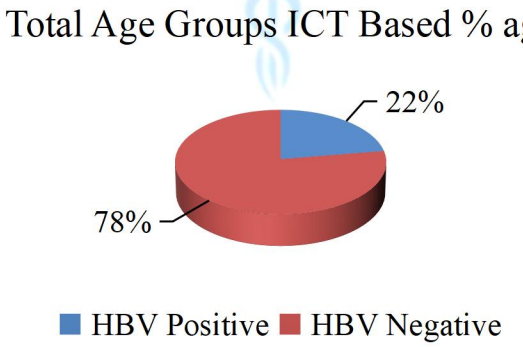
Age group	Total No	HBV Positive cases (%)	HBV Negative cases (%)	P value
I	16	9 (56.25%)	7 (43.75%)	.000
II	197	52 (26.39%)	145 (73.60%)	
III	210	31 (14.76%)	179 (85.23%)	
IV	70	19 (27.14%)	51 (72.85%)	
V	26	6 (23.07%)	20 (76.92%)	
Total	519	117 (22.54%)	402 (77.45%)	

Table No; 3.3 Prevalence of ICT in different age groups of HBV



Figure; 3.3 Prevalence of HBV with confirm to ICT in various different age groups.



Figure; 3.4 Prevalence of HBV with confirm to ICT in various different age groups.

3.4; HBV Prevalence on the bases of ICT Risk Factor

There are several risk factors for HBV infection that contribute to the prevalence of HBV in the community. We used a predesigned questionnaire approach to investigate the transmission of HBV infection and determine a range of risk variables. We have identified a number of risk variables that significantly correlate with HBV infection. The potential risk factors associated with the transmission of HBV include marital status (specifically, being married), a prior history of (DP) dental procedures, participating in ear, nose, piercing or tattooing, utilization of non-disposable-syringes, a record of blood or plasma transfusions, surgical interventions, common visits to barbershops, & dependence to inject able drugs. The results obtained from the questionnaire proved to be statistically significant in

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relation to the risk variables linked with serum hepatitis illness (p > 0.05). The incidence of HBsAg be greatest among patients with a patient with a history of inject able drug addiction (281 (54.14%), patients (64.6%), while it was lowly among patients through a family history of HBV-positive individuals (3.46%). Additional risk factors that have been identified include a history of frequently barbershop shop visits by 255 (49.13%) participants in activities such as married persons (245 (47.20%), ear, nose, piercing, or tattoos (225 (43.35%), history of surgery (213 (41%), history of dental producers (38.15%), and history of blood or plasma transfusion (167 (32.17%). The statistical analysis reveals a considerable prevalence rate of HBV infection (p<0.05) (Table No 4.4, Figure 4.5).

Table No; 3.4 The rate of HBV based on ICT indicators of risk

I.C.T based hazard factor	N. of Total	No	Yes	% age	P value
Family member with Positive	117	111	6	1.4	
History of dental work	117	53	64	57.8	
Surgery history	117	68	49	41.8	
Transfusion plasma or blood of history	117	60	57	42.9	
Ear, nose, piercing, tattooing, syringe of history	117	73	44	56.5	0.0001
Married	117	27	90	64.6	
Barbers Shop history	117	60	57	39.45	
Injection Drugs use of History	117	63	54	38.1	

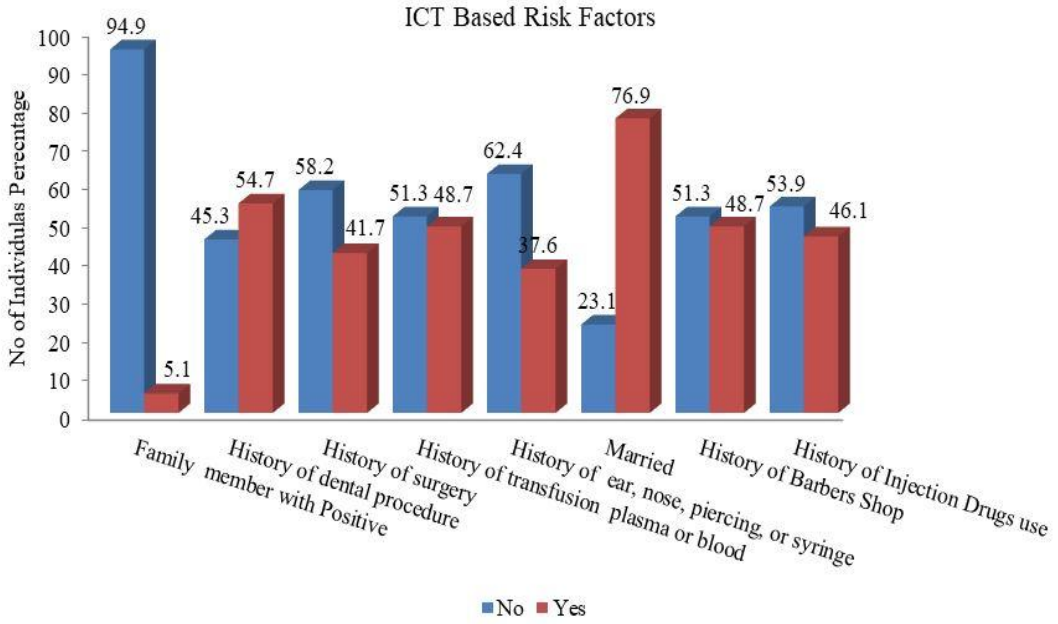

Figure: 3.5 On the bases of ICT prevalence of HBV hazard factor percentage.

3.5; Frequency of HBV was examined gender-wise using Immunoassay.

All 117 suspected I.C.T samples were confirmed through enzyme linked immunosorbent assays (ELISA). According to the ELISA-based screening, out of the 62 men who tested positive for ICT, 58 were positive, while out of the 55 females who tested positive, 47 were positive. The prevalence of HBV infection in men is 22.93%, while in females it is 18.07% are given in (Table No 4.5. Figure 4.6).

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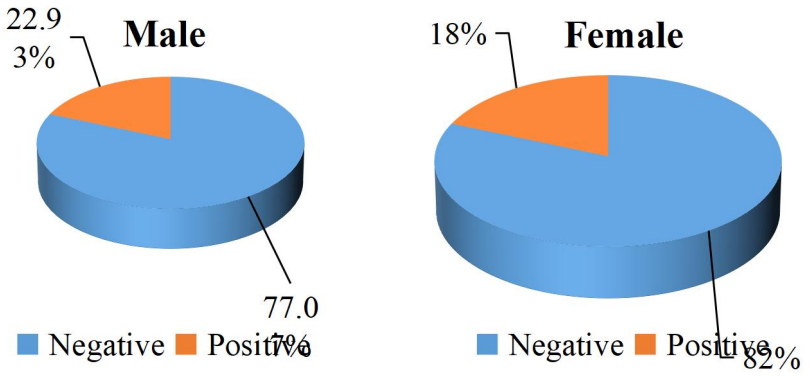
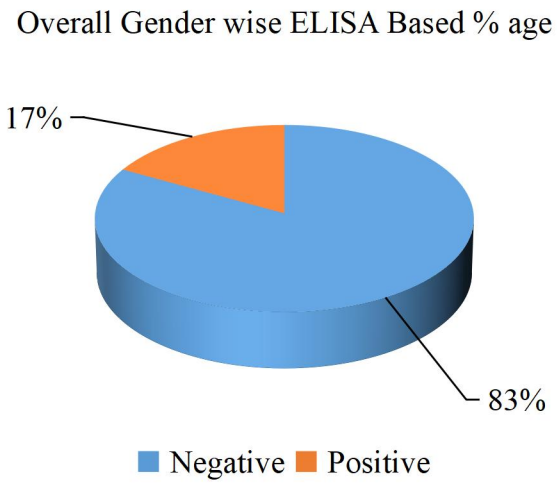
Table No; 3.5 Frequency of HBV was examined gender-wise using Immunoassay.

Prevalence of HBV through ELISA	Gander					
	Male			Female		
	N	+ve	-ve	N	+ve	-ve
	259	58	4	260	47	8



Figure; 3.6 Prevalence of HBV Gender Wise tested through ELISA.



Figure; 3.7 Overall gender wise through ELISA percentage.

3.6 ELISA based Frequency of H.B.V in various age groups investigation.

The immunoassay-based examination showed that out of 16 participants in the 1st age group, 9 confirmed positive for ELISA, indicating an illness rate of 56.25%. The I.C.T-based study of the 2nd age identified 47 confirmed cases out of 197 individuals, which is a positivity rate of 23.85% of the total. Among the 210 individuals in the third age group, 27 were positive, resulting in an infection incidence of 12.85%. In the fourth age group, which comprised 70 individuals, a total of 17 individuals were verified positive, resulting in an infection rate of 24.28%. Among the 26 participants in the last five age group, 5 individuals were tested positive, leading to a positivity rate of 19.23%. These analyses revealed that age groups II and III reported significantly higher incidence rates than the other age groups. A comprehensive analysis of ELISA results across different age groups consistently revealed a positivity rate of 20.23% for both males and females. A statistically significant prevalence rate ($P<0.05$) was observed in (Table 4.6, Figure 4.8, and Figure 4.9).

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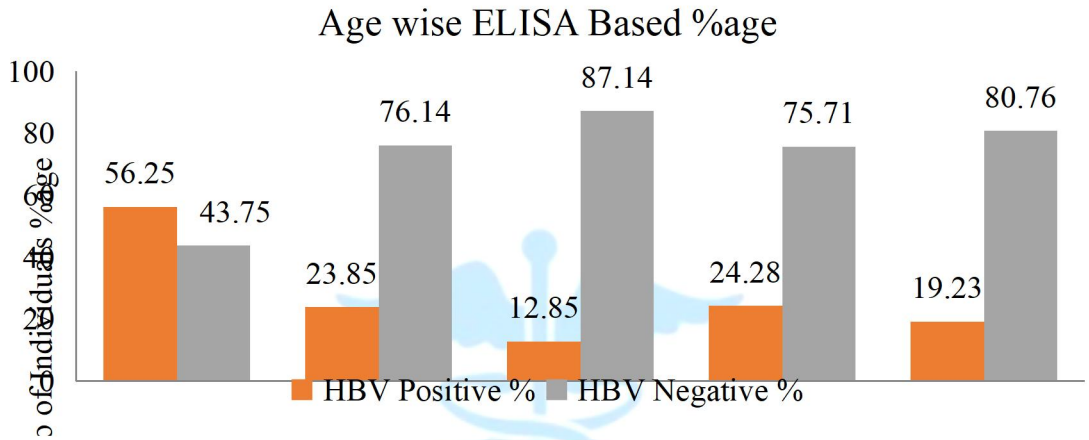
Table No; 3.6 ELISA based Prevalence of HBV in different age groups tested.

Age groups	N	Positive HBV N (%)	Negative HBV N (%)	P value
I	16	9 (56.25%)	7 (43.75%)	.003
II	197	47 (23.85%)	150 (76.14%)	
III	210	27 (12.85%)	183 (87.14%)	
IV	70	17 (24.28%)	53 (75.71%)	
V	26	5 (19.23%)	21 (80.76%)	
Total	519	105 (20.23%)	414(79.76%)	



Figure; 3.8 ELISA based frequency of HBV in various age groups tested.

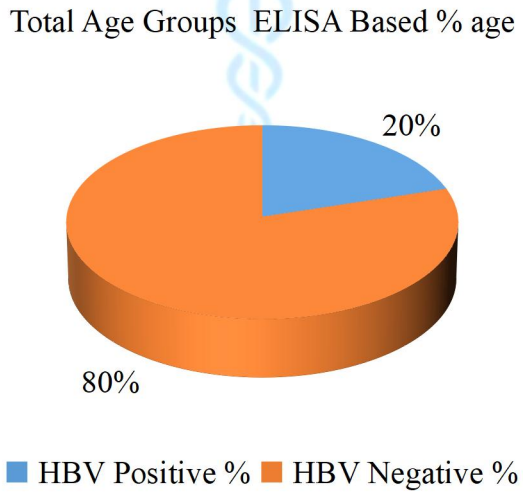


Fig 3.9 ELISA based Prevalence of HBV in different age groups tested

4 Discussion

Hepatitis is a significant global health issue [13]. The Hepatitis B virus is a significant contributor to illness and death on a worldwide scale. Over 1 million mortalities, each year, these virus diseases affect almost a third of the world's population. HBV are probable etiological agents of liver cirrhosis, cancer, end-stage liver disease (ESLD), and other hepatic disorders. The number of cases of HBV contrasts throughout various regions of the world [14]. In Asia and Africa, the incidence is around 15%, whereas in the United States and Britain, it ranges from 0.1% to

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0.2%. In Greece and southern Italy, the rate is 3% [15]. The prevalence of the hepatitis B virus (HBV) in Pakistan is around 10% [16]. According to recent research, the prevalence of HBsAg among healthy blood donors is estimated to be 0.82% [17]. The prevalence rates of hepatitis B virus (HBV) are influenced by several factors, including economic status, health education, horizontal movement, and a history of visiting medical facilities or taking plasma, blood, or bodily fluid transfusions conducted a study [18].

Our research aims to evaluate the incidence of HBV, initial epidemiology, and the incidence of HBV-associated risk factors in the general demographic of Darazinda, a district in western Pakistan. According to the signs and symptoms, we have investigated 519 individuals were preferred for HBV screening. The subjects suffered HBsAg screening using ICT method, which was then confirmed using ELISA and PCR. Initially, the selected participant's blood samples were screened using an immune-chromatographic test and their sex was taken into consideration for investigation. According to the ICT, the prevalence rate of HBV is19% in females and 23.93% in males. The ELISA analysis revealed that the prevalence of HBV positivity in males was 22.93%, while in females it was 18%.

This H.B.V reported is in accordance with the consequences by [19].One potential argument for an amplified occurrence of HBV in males compared to females could be linked to the elevated susceptibility of males to risk factors, particularly due to their greater involvement in society in rural regions of Pakistan [19]. According to the present study, the age groups of 16 to 30 (years) presented the highest incidences of disease, which is similar to the findings of many researchers in different areas of the country [20-21]. In the same way, it was found that individuals between the ages of 31 and 45 were equally susceptible to HBV infection, and the results of different research [22] confirm the findings presented by the author. It was least frequent in the age range (1–15 years), which is according to [23], as well as the age range (61–75 years). Hepatitis B infection is least prevalent in individuals aged 60 and older, according to the findings of many other studies [24]. The age groups 16–30 and 31–45 years age may have higher incidence because they are more susceptible to risk factors, according to one opinion.

The survey-based study on the risk factors linked to the spread of HBV infection found that married individuals had the highest prevalence of HBsAg (64.62%), which suggests that sexual activity plays a big role [25-26]. On the other hand, individuals with a family record of HBV positivity carried the lowest rate of infection (1.43%). The additional factors associated with risk consisted of a history of orthodontic treatment (57.8%), surgical treatment (41.84%), blood transfusion or plasma (42.92%), ear, the nose, piercing, tattooing, or needle use (56.53%), frequent visits to a hairdresser shop (39.45%), and history of injection drug use (38.12%) [27-28]. These indicate a similar pattern to the one previously investigated by [29-30].

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