**Sero-detection of Hepatitis B virus Surface Ag (HBsAg) among Drugs Upusers in Prisons by using Enzyme Linked Immunosorbent Assay (ELISA) at Khartoum state.**

# Abstract

Hepatitis B virus (HBV), a vaccine-preventable infection, remains one of the leading causes of acute and chronic liver diseases . The resulting cirrhosis and hepatocellular carcinoma were the main contributing factors to HBV burden. Hepatitis B is one of the main infectious diseases among people who use drugs (PWUD). There are several reasons why PWUD are considered more vulnerable to HBV. Needle sharing is one of the major routes of transmission among people who inject drugs (PWID) . Substance use contributes to other certain vulnerabilities, such as homelessness, incarceration and unsafe sexual contacts . In addition, this population has limited access to health services required for timely prevention, diagnosis, and treatmentThe .
Objective: To determine the prevalence of HBsAg among drug users in prison and assess associated risk factors.

Methods and Materials: A case-control study was conducted in Kober and Al Huda prisons, Khartoum State, from May to August 2022.
A total of 150 prisoners with a history of drug use were enrolled. Blood samples were collected and analyzed using ELISA to
detect HBsAg. Statistical analysis was performed to assess associations between infection status and risk factors such as needle sharing.

Results: Among 150 drug users, 30 (20%) were HBsAg positive, while 120 (80%) were negative. The study found a significant
association between needle sharing and HBsAg positivity (p = 0.003). The highest prevalence was among heroin users (8.0%)
and those aged 30-50 years (13.3%). Males had a higher prevalence (15.3%) than females (4.7%), though this difference was not significant (p = 0.168).

Conclusion: HBV prevalence among drug users is considerable. Strengthening harm reduction programs, promoting
vaccination, and implementing regular screening in prisons are crucial to reducing HBV transmission.
Keywords: Drugs Upusers, , ELISA, HBsAg, Khartoum state, Prisoners.

Introduction :

Hepatitis B virus (HBV), a vaccine-preventable infection, remains one of the leading causes of acute and chronic liver diseases [1]. In 2017, there were 13.8 million new cases of chronic HBV globally [2].The resulting cirrhosis and hepatocellular carcinoma were the main contributing factors to HBV burden. In 2017, HBV-related DALY and mortality rate were estimated at 107.2 years and 3.7 per 100,000 in Iran, respectively . Hepatitis B is one of the main infectious diseases among people who use drugs (PWUD). There are several reasons why PWUD are considered more vulnerable to HBV. Needle sharing is one of the major routes of transmission among people who inject drugs (PWID) [3]. Substance use contributes to other certain vulnerabilities [4] such as homelessness [5, 6], incarceration [7], and unsafe sexual contacts . In addition, this population has limited access to health services required for timely prevention, diagnosis, and treatment .The World Health Organization (WHO) considers PWUD as one of the main adult target populations in its strategy for ending HBV . Promoting harm reduction services is introduced as one of the five core interventions for combating viral hepatitis, including HBV; alongside with vaccination, prevention of mother-to-child transmission, safety of medical interventions, and treatments programs .The impact targets for 2030 by the WHO are 90% of reduction in new chronic cases of HBV and 65% reduction in attributed mortality .Substance use has been a public health issue for decades in Iran [8].The Iranian National Mental Health Survey (Iran MHS) estimated the prevalence of any substance use disorder to be 2.8% in the Iranian general population [9]. Although it was previously estimated that more than 20% of PWUD had used drugs through injection in the last 12 months [10], recent studies have shown that the figure has decreased to about 3% [11, 12]. In Iran, drug use and injecting drug use are known as the main risk factors for HIV and HCV [13,14]; therefore, the country has adopted extensive harm reduction measures to control blood-borne infections among PWID for more than a decade.

HBV prevalence among PWUD has been assessed in many studies in Iranian population, and the results were quite different. In addition, HBV prevalence among PWID was assessed in a global systematic review. The provided estimate for HBV infection among Iranian PWID was considerably lower than the corresponding global (9%) and regional Figures (8.1%). However, this was not the case for HIV and HCV estimates . Therefore, in order to provide a more detailed picture of HBV among PWUD in Iran, we conducted a systematic review on all studies providing the prevalence of HBV surface antigen (HBsAg) among both PWID and non-injecting PWUD, according to the socio-demographic characteristics, recruitment settings, high-risk behaviors, and geographical distribution. HBsAg, a surface protein, can be detected in both acute and chronic HBV infection, also indicating that the individual is infectious [15].

Drug usage and addiction is one of the major health problems with serious negative outcomes A According to the data from World Health Organization (WHO); one of each 20 adults of 250 million people between ages 15-64 worldwide have at least one drug usage history and approximately 12 million people use drugs intravenously [16]. Drug usage in Turkey appears to be a serious problem which should be addressed with its medical outcomes [17]. According to the 2018 National Drug Report of Turkey, the drugs which are detected nationwide can be aligned in decreasing order according to the amount and number of people arrested with the drug as marijuana, synthetic cannabinoids, heroin, methamphetamine and ecstasy [18].Drug usage is considered an important risk factor for the contamination of some infectious diseases and it increases the global burden of these infections. Especially the prevalence of blood-borne viral infectious diseases is higher among intravenous drug users compared to the general population [19,20].WHO estimates a prevalence of 9% for Hepatitis B Virus (HBV) infections among intravenous drug users in 2014 .In addition to the high prevalence among intravenous drug users, there are some studies showing increased prevalence of HCV and HBV infections among non-intravenous drug users [21-24 ].In the countries where illegal drug usage is common, it’s obligatory to take legal precautions because of the increasing rate of both individual and social problems of drug addicts. In this context, drug addicts who use, sell, produce drugs; act criminal while under the effect of drugs or while supplying drugs are scheduled in probation and connectedly, obligatory treatment. The main idea of this application is that community based treatment sanctions would be more effective than imprisonment for drug addiction and the ones who are punished would adapt easier to the society with this kind of programs of protection [25].

HBV is transmitted parenterally by contaminated blood or other body fluids through blood vessels, skin or mucous membranes. The virus can be detected in all human body fluids, the virus concentration in the fluids is highest in the blood or serous exudates, and it is relatively low in saliva, semen, vaginal fluids [26]. Thus, there is almost no risk of transmission in the course of daily life, and the infection through fecal-oral transmission does not occur.

 **Materials and Methods**

The study was case control study conducted in Kober Prison, Al Huda prison ,Khartoum state, Prisoner's with a history of drug upuse.

Specimen Collection and Transportation:

 Sample Collection:

Either fresh serum or plasma samples can be used for this assay. Blood collected by venipuncture should be allowed to clot naturally and completely-the serum /plasma must be separated from the clot as early as possible as to avoid hemolysis of the RBC.

 Transportation and Storage:

Store samples at 2-8c.Samples not required for assaying within 3 days should be stored frozen (-20 C or lower).Multiple freeze-thaw cycles should be avoided. For shipment ,samples should be packaged and labeled in accordance with the existing local and international regulations for transport of clinical samples and ethological agents.

**Detection of Hepatitis B Virus by Enzyme Linked Immunosorbent Assay (ELISA).**

HBV infection was detected by assay of the hepatitis B surface antigen (HBsAg) using the HBsAg EIA Test Kit. It is a third generation immunoassay for the qualitative detection of the presence of Hepatitis B surface antigen in serum or plasma specimen. The test utilizes monoclonal antibodies according to manufacturer’s instruction to selectively detect various subtypes of HBsAg in serum (ACON Laboratories Incorporation, San Diego, U.S.A.)

The HBsAg EIA Test Kit is a solid phase qualitative enzyme immunoassay based on a sandwich principle for the detection of HBsAg in human serum or plasma. The microwell plate was coated with monoclonal antibodies specific to various subtypes of HBsAg. During testing, the specimen and the enzyme-conjugated

HBsAg antibodies were added to the antibody coated antibody microwell plate and

simultaneously bind to the conjugate to form immobilized antibody-HBsAg-conjugate complexes. If the specimen does not contain HBsAg, the complexes will not be formed. After initial incubation, the microwell plate was washed to remove the unbound materials.

Substrate A and Substrate B were added and then incubated to produce blue color,

indicating the amount of HBsAg present in the specimen. Sulfuric acid solution was added to the microwell plate to stop the reaction which produces a color change from blue to yellow.

The color intensity, which corresponds to the amount of HBsAg present in the specimen, was measured with a microplate reader at 450nm.

The test was carried out and interpreted according to the manufacturer’s instruction.

Data analysis: Statistical analysis was done by using the Statistical Package for Social Science program (version 20)

Ethical clearance was taken from the Research Ethical Committee at ministry of health –khartoum state , al-huda prison administration and formal consent from the prisoners under study .

Results

This study was conducted at two places: 99(66.0%) of them at kober prison for male and 51(34.0%) at Al Huda prisons for female. The studied participants (n= 150) were men and women, the men were (99), and the women were (51). The mean standard of the age of the subjects was 39.4. And the majority of them had less school education. More than 44% of the participant had a history of needles sharing and this had a significant association with HBsAg positivity. Of the 150 IVDU, 30(20%) were positive for HBsAg, 23(15.3%) from male with the p.value was 0.168 and this was not significant for HBsAg, and about 7 (4.7%) from female. Less than <30 years of prisoners, the total was about 36 (24.0%) with the positive results about 6(4.0%) and with p.value about 0.447 , in prisoners with age from 30-50 years the total was about 85(56.7%) with the positive results about 20(13.3%). Prisoners more than >50 years was about 29(19.3) with the positive results about 4(2.7%), all total of results was about 150(100%).The Frequency of most type of Drugs used by prisoners it` was wine about 55(36.7%) ,Heroin was 44(29.3%), MDME 19(12.7%), Cocaine 18(12.0%), Opium 8(5.3%), Heroin&MDME 2 (1.3%), Wine& Heroin 2 (1.3%), Heroin&Opium 1 (.7%) and Wine&MDME 1(.7%). All total was about 150(100%), about 66(44.0%) of prisoners who Shared the same needle to used drugs they had a positive results for Hepatitis B surface antigen marker about 23( 15.3%) with p.value about (0.003%) and this was significant. And about 84 (56.0%) of prisoners did not shared needles the positive results of Hepatitis B surface antigen marker was about 7(4.7%). The total of positive result was higher in male than female due to prevalence of drugs in male, and the male used drugs more than female. In prisoners who using heroin drug the positive results was about 12 (8.0%) this is higher result and its due to prevalence of Heroin drug and it’s used through sharing the same needles and syringes. While prisoners who used cocaine drug the positive results of HBsAg was about 3(2.0%) with the p.value about (0.156). In prisoners used wine the positive results was about 8(5.3%), In prisoners using MDME drug the positive results was about 4(2.7%) while in prisoners using opium the positive results was about 1 (0.7%). In prisoners using Heroin&MDME the positive results was 0(0.0%) and its lowest result. In prisoners using Heroin&opium the positive was about 0(0.0%) also its lowest result, while In prisoners who used Wine&Heroin the positive results was about 2(1.3%). Last result showed In prisoners who used Wine&MDME the positive results its low and it was about 0(0.0%).

The total of Positive results in all types of Drugs was about 30(20.0%) positive results and the Negative results were about 120(80.0%). The total of all results (Positive&Negative) was about 150(100%) results.

1. **Table 1 : Distribution of area of collection**

|  |  |  |
| --- | --- | --- |
| **Area of collection** | **Frequency** | **Percent** |
| Kober prison | 99 | 66.0 |
| Al huda prison | 51 | 34.0 |
| Total | 150 | 100.0 |

1. Table 2 : Distribution of gender

|  |  |  |
| --- | --- | --- |
| **Gender** | **Frequency** | **Percent** |
| Male | 99 | 66.0 |
| Female | 51 | 34.0 |
| Total | 150 | 100.0 |

1. Table 3 : Distribution of age

|  |  |  |
| --- | --- | --- |
| **Age**  | **Frequency** | **Percent** |
| < 30 years | 36 | 24.0 |
| 30 - 50 years | 85 | 56.7 |
| > 50 years | 29 | 19.3 |
| Total | 150 | 100.0 |

1. Table 4 : Distribution of types of Drugs

|  |  |  |
| --- | --- | --- |
| **Type of alcohol** | **Frequency** | **Percent** |
| Cocaine | 18 | 12.0 |
| Heroin | 44 | 29.3 |
| MDME | 19 | 12.7 |
| Opium | 8 | 5.3 |
| Wine | 55 | 36.7 |
| Heroin&MDME | 2 | 1.3 |
| Heroin & opium | 1 | .7 |
| Wine & heroin | 2 | 1.3 |
| Wine & MDME | 1 | .7 |
| Total | 150 | 100.0 |

1. Table 5 : Distribution of needle sharing

|  |  |  |
| --- | --- | --- |
| **Needle sharing** | **Frequency** | **Percent** |
| Yes | 66 | 44.0 |
| No | 84 | 56.0 |
| Total | 150 | 100.0 |

1. Table 6 : Distribution of results of Hepatitis B Surface Ag

|  |  |  |
| --- | --- | --- |
| **Results** | **Frequency** | **Percent** |
| Positive | 30 | 20.0 |
| Negative | 120 | 80.0 |
| Total | 150 | 100.0 |

1. Table 7 : Association between Hepatitis B Surface Ag result and needle **sharing**

|  |  |  |  |
| --- | --- | --- | --- |
| **Needle sharing** | **Results** | **Total** | **P. value**  |
| **Positive** | **Negative** |
| Yes | 23 (15.3%) | 43 (28.7%) | 66 (44.0%) | 0.003\* |
| No | 7 (4.7%) | 77 (51.3%) | 84 (56.0%) |
| Total | 30 (20.0%) | 120 (80.0%) | 150 (100.0%) |

1. Table 8 : Association between Hepatitis B Surface Ag result and gender

|  |  |  |  |
| --- | --- | --- | --- |
| **Gender** | **Results** | **Total** | **P. value**  |
| **Positive** | **Negative** |
| Male | 23 (15.3%) | 76 (50.7%) | 99 (66.0%) | 0.168 |
| Female | 7 (4.7%) | 44 (29.3%) | 51 (34.0%) |
| Total | 30 (20.0%) | 120 (80.0%) | 1. (100.0%)
 |

1. Table 9 : Association between Hepatitis B Surface Ag result and age:

|  |  |  |  |
| --- | --- | --- | --- |
| **Age**  | **Results** | **Total** | **P. value**  |
| **Positive** | **Negative** |
| < 30 years | 6 (4.0%) | 30 (20.0%) | 36 (24.0%) | 0.447 |
| 30 - 50 years | 20 (13.3%) | 65 (43.3%) | 85 (56.7%) |
| > 50 years | 4 (2.7%) | 25 (16.7%) | 29 (19.3%) |
| Total | 30 (20.0%) | 120 (80.0%) | 150 (100.0%) |

1. Table 10: Association between Hepatitis B Surface Ag result and type of alcohol

|  |  |  |  |
| --- | --- | --- | --- |
| **Type of alcohol** | **Results** | **Total** | **P. value** |
| **Positive** | **Negative** |
| Cocaine | 3 (2.0%) | 15 (10.0%) | 18 (12.0%) | 0.156 |
| Heroin | 12 (8.0%) | 32 (21.3%) | 44 (29.3%) |
| MDME | 4 (2.7%) | 15 (10.0%) | 19 (12.7%) |
| Opium | 1 (0.7%) | 7 (4.7%) | 8 (5.3%) |
| Wine | 8 (5.3%) | 47 (31.3%) | 55 (36.7%) |
| Heroin&MDME | 0 (0.0%) | 2 (1.3%) | 2 (1.3%) |
| Heroin & opium | 0 (0.0%) | 1 (0.7%) | 1 (0.7%) |
| Wine & heroin | 2 (1.3%) | 0 (0.0%) | 2 (1.3%) |
| Wine & MDME | 0 (0.0%) | 1 (0.7%) | 1 (0.7%) |
| Total  | 30 (20.0%) | 120 (80.0%) | 150 (100.0%) |

 **Discussion:**

A total of 150 Drugs up user’s prisoners participated in this study. The study was conducted in two places 99 (66.0%) at kober prison for male, 51 (34.0%) at Al Huda prison for female, however, 99(66.0%) of prisoners were male and 51(34.0%) were female. with male to female ratio 3:1 compared with similar study reported k stark *et al* in J Epidemiol in December 1997. They found that the increased risk of HBV among IDU who had shared syringes in prison warrants specific preventive action (26).Also similar to study conducted by **Shokouh Ghafari *et al****.* In Iran in 2019, the results of prevalence of HBsAg among drug using prisoners were (20.7%).

Dissimilar to study conducted by Mathurin Pierre Kowo *et al* in the Douala New Bell Prison, Cameroon in 2021. Results of the 940 prisoners selected, 94% were male, The mean age of the study population was 33.81 ± 10.35 years. HBV prevalence was 12.9% (95% CI: 10.7-15%). The use of non-injectable illicit drugs (OR: 3.5; 95% CI: 1.9-6.2; P<0.001), sharing of needle or razors (aOR: 24.1; 95% CI: 12.9-45.0; P<0.001), sharing of tooth brushes(aOR: 2.7; 95% CI: 0.9-7.4) (P=0.053), having tattoos or piercings (aOR: 1.9; 95% CI: 1.1-3.1; P=0.01) were significantly associated with HBs Ag seropositivity.

Also dissimilar to study conducted by Jung-Ah Min *et al* In J Med Virol in April 2013. The study were conducted to detect Prevalence and associated clinical characteristics of hepatitis B, C, and HIV infections among injecting drug users in Korea. They found that the seroprevalence of anti-HCV and HBV surface antigen (HBsAg) was 48.4% (n = 154) and 6.6% (n = 21), respectively. HBV/HCV co-infection was found in 4.1% (n = 13).

 **Conclusions:**

The study concluded that there were 30 (20%) cases were positive to Hepatitis B surface Antigen ( HBsAg), and 120 (80%) cases were Negative to Hepatitis B surface Antigen (HBsAg).

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