

# Seroprevalence of HBV Infection Among Normal Population and Healthcare Workers in Baghdad

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## Abstract

**Objective:** In this study we verified the epidemiology of HBV infection among normal population and healthcare workers (HCWs) in Baghdad by analyzing the prevalence of specific viral markers (anti-HBs, anti-HBc and HBsAg). **Method:** A total of 797 serum specimens (588-normal population, 209-HCWs) were tested using ELISA technique and positive HBsAg specimens were confirmed by VIDAS technique. **Results:** In normal population group, the HBsAg, anti-HBs and anti-HBc Total were 1.02%, 10.54%, 5.44%, respectively. The HBsAg result was significantly lower ( $P < 0.05$ ) than previous studies were done in Iraq. Significant correlation ( $P < 0.05$ ) in prevalence of HBsAg was found between age groups and males had higher positive HBsAg marker than females ( $P < 0.05$ ). The prevalence of anti-HBs was insignificant ( $P > 0.05$ ) between age groups but significant importance ( $P < 0.05$ ) was recorded between both sexes. Highly significant ( $P < 0.01$ ) was recorded between ages groups regarding to anti-HBc Total marker but insignificantly ( $P > 0.05$ ) between both sexes were noticed. In HCWs group the HBsAg, anti-HBs, anti-HBc Total were 0.96%, 26.7%, 1.44% respectively and the prevalence of HBsAg was significantly less ( $P < 0.05$ ) than previous studies. The comparison between normal population and HCWs groups showed significant correlation ( $P < 0.05$ ) related to HBsAg and highly significant correlation ( $P < 0.01$ ) regarding anti-HBs and anti-HBc Total. The HBsAg and anti-HBs among HCWs was significantly increased ( $P < 0.05$ ) with advancing ages, whereas no such variation was observed between both sexes. The HBsAg was high among those with poor health education but it was with significantly importance ( $P < 0.05$ ) among different occupational types as well as among vaccinated, incomplete vaccinated and unvaccinated HCWs groups. Additionally, HCWs who had only 1<sup>st</sup>, only 2<sup>nd</sup> and those who had 3<sup>rd</sup> doses showed significant correlation between number of vaccinated individuals and prevalence of anti-HBs.

**Keywords:** Seroprevalence of HBV, HBsAg, anti-HBs, HCWs, normal population

**Abbreviations:** anti-HBs, antibody anti-HBs; anti-HBc, antibody anti-HBc; HBsAg, antigen HBs

## Introduction

Hepatitis B virus (HBV) is a leading cause of chronic hepatitis, cirrhosis, and hepatocellular carcinoma, accounting at least 2 billion people have been infected with HBV worldwide with an estimated 400 million being chronic carriers of the virus (WHO, 2012). HBV most prevalent in Asia and Sub-Saharan Africa, in the Amazon Basin, and less prevalent in the United States, Northern Europe, Australia and parts of South America; the Middle East, some countries of Eastern Europe and the Mediterranean Basin were considered areas of intermediate endemicity (Franco, et al., 2012). Old figure of seroprevalence of HBV in Iraq indicate that the carrier rate was (3-4.5%) among normal population and 2-3% among healthy blood donors and according to these results; Iraq was categorized under the intermediate endemic countries (Andre, 2000; WHO, 2006). Lower carrier rates (1-2%) was recorded among Iraqi blood donors (Omer, 2004), another study was reported low rate of HBsAg (1.6%) in Iraq, whereas in Baghdad the HBsAg rate was 1.2% (Omer and Al-Salmani, 2007). The present study recorded the seroprevalence of HBsAg, anti-HBs Abs and anti-HBc Total among normal population were 1.02 %, 10.54 % and 5.44 % respectively, and this finding of HBsAg was lower than all that previously reported in Iraq with statistically significant importance ( $P = 0.036$ ).

In the health care setting, HBV transmission may occur via several routes, but the most frequent route leading to establishment of HBV infection is through needle stick injury, invasive surgical procedures are another route of HBV transmission (Bhat, et al., 2012). In Iraq few reports exist on the prevalence of HBV among HCWs (Omer et al. 1984; Al-Mashhadani, 1998) and all previous studies demonstrated a high seroprevalence of HBsAg among HCWs ranged from (5 -1%). Our study was recorded the seroprevalence of HBsAg, anti- HBs and anti- HBc Total among HCWs was 0.96 %, 26.79 % and 1.44 % respectively, which was significantly less than previous studies were done in Iraq. Vaccination is considered the most effective measure to reduce the incidence of HBV infection (Boccalini, 2013); Iraq was introducing a program of universal vaccination against HBV in the middle of 1990s. In this study we verified the impact of universal immunization on the epidemiology of HBV infection by analyzing the prevalence of specific viral markers (anti-HBs, anti-HBc and HBsAg) among normal populations and HCWs as a risk group chosen from various hospitals and health center in Baghdad.

## 2. Materials and methods

### 2.1 Study design and setting

This study was carried out at the Viral Hepatitis Reference Laboratory /Central Public Health Laboratories from October 2014 to July 2015, to determine the seroprevalence of HBV infection among normal population and HCWs in Baghdad.

#### 2.1.1 Normal Population Group

This group was with the following criteria; healthy individuals from different age and sex with no signs of HBV infection. They were showed neither clinical symptoms nor abnormalities via biochemical laboratory tests, all individuals were residents in Baghdad. A total of 588 serum specimens from individuals with age of both sexes ranged from one day to 71 years old were collected. This group consisted of 366 (62.24 %) male and 222 (37.75 %) female, with male to female ratio as 1.6-1.

#### 2.1.2 HCWs Group

A total of 209 serum specimens were collected from apparently healthy HCWs selected from different hospitals in Baghdad with age ranged (20 – 60) years old. This group consisted of 112 (53.58 %) male and 97 (46.41 %) female, with male to female ratio 1.15-1. They were submitted for historical questionnaire about a previous vaccination with HBV vaccine and number of doses that had been taken, either with or without vaccine booster dose.

### 2.2 Sample collection

Blood specimens were collected in randomly way, recording name, age, sex, code and date of collection. Specimens were collected from each individual via vein-puncture in 5-ml red-top vacutainer tube (Becton Dickinson, NJ, USA) and allowed the blood clot naturally at room temperature (RT). Serum specimens were separated by centrifugation at 3000 g for 5 min. Then divided into aliquots 250 $\mu$ l (4 tubes for each sample) and stored at -20°C until use. Each part of sera was used once to avoid repeated thawing and freezing. All materials (i.e. reagents and sera) were allowed to stand at RT before use.

### 2.3 Biochemical Assays

Liver function tests, including alanine aminotransferase test (ALT), (normal range, up to 13 u/l) and total serum bilirubin (TSB), (normal range, less than 1mg/dl) levels (Young, 1997) were done for all individuals from both groups at initial examination using commercially available ELISA kits (Randox, UK).

### 2.4 Screening for HBV serological Markers

Serum specimens were screened for HBsAg, anti-HBs and anti-HBc Total using commercial available ELISA Kits (Bioelisa HBsAg, Bioelisa anti-HBs and anti-HBc Biokit, Spain). The positive specimens for HBsAg were confirmed using “Vitec Immune Diagnostic Assay System” (VIDAS) and (Hepanostika HBsAg Uni-Form II confirmatory; Bio Merieux, France) Kit. All criteria provided by the producers for the qualitative evaluation of antibodies and antigen detection, were applied according to specific instruction manual.

### 2.5 Statistical analysis

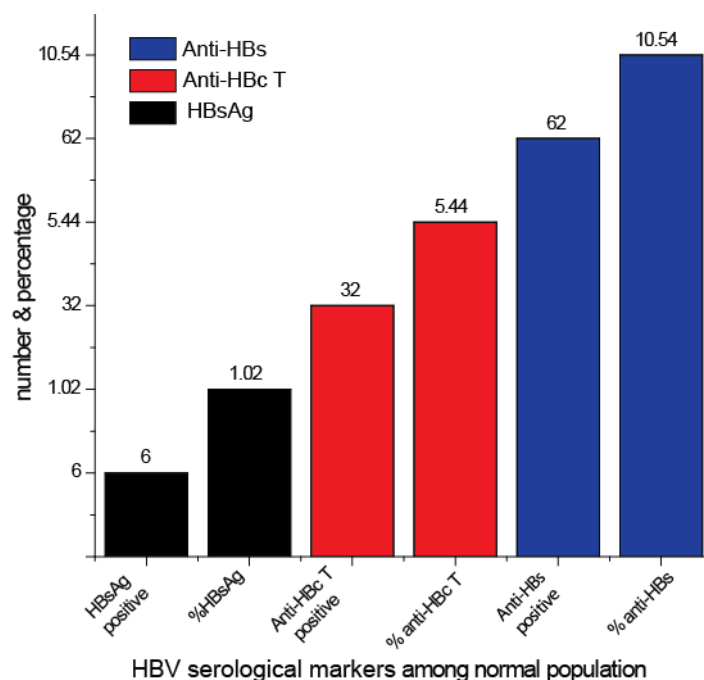
Data were translated into codes using a designed table sheet and then converted to a computerized database. Statistical analyses were done by using SPSS version 15, under windows<sup>XP</sup> and Mini. Tab programmes; the data were given as means  $\pm$  SD of numbers and percentage. And significance differences between the means of certain continuous outcome were assessed by ANOVA table (F-test) or t-test. Chi-square test ( $X^2$ ) was also used to assess significant differences between frequencies.

## 3. Results and discussion

### 3.1 Seroprevalence of HBV markers among normal population

The seroprevalence of HBsAg, anti-HBs and anti-HBc Total was 1.02 %, 10.54 % and 5.44 % respectively (Fig. 1). The results of HBsAg in present study on the one hand, in line with the results that were recorded from 2002 till 2008 by Iraq / CDC in which the prevalence of HBsAg in Baghdad was 1.2% among normal population (Omer and Al- Salmani, 2007).

Indeed, the results of Iraq /CDC were based on the prevalence of HBsAg among blood donors who were mostly urban middle class young males, because of the trend in blood donation in our country, while the present study included different age groups and both sex. On the other hand, the present study results of HBsAg was lower than all that previously reported in Iraq (Al-Azawi and kadori, 1984; Al-Mashhadani, 1998) with significant statistically importance ( $P = 0.036$ ). The low seroprevalence of both anti-HBs and anti-HBc Total in the present study consistent with previous work was done in Iraq (Omer and Al-Salmani 2007), and these findings of both markers indicates low coverage rates of vaccination and low past infections were acquired among normal population (Bonanni, et al., 2003; Jawetz et al., 2007).



**Fig. 1. Seroprevalence of HBsAg, anti-HBs & anti-HBc Total among normal population group**

Then, we compared the seroprevalence of HBsAg in normal population according to the age groups. Table (1) shows, the age groups ranged from one day to 74 years with a mean of age  $22.77 \pm 19.6$ . The highest rate of HBsAg (2.70%) was located in the age group (51 years and above) with statistically significant ( $P < 0.05$ ) followed by the age group (less than one year) it was (2%) with insignificant correlation ( $P > 0.05$ ) and it was decreased in age groups (1-5, 16-20 and 31-40) years old also with insignificant correlation ( $P > 0.05$ ), while this marker did not score at all in other age groups.

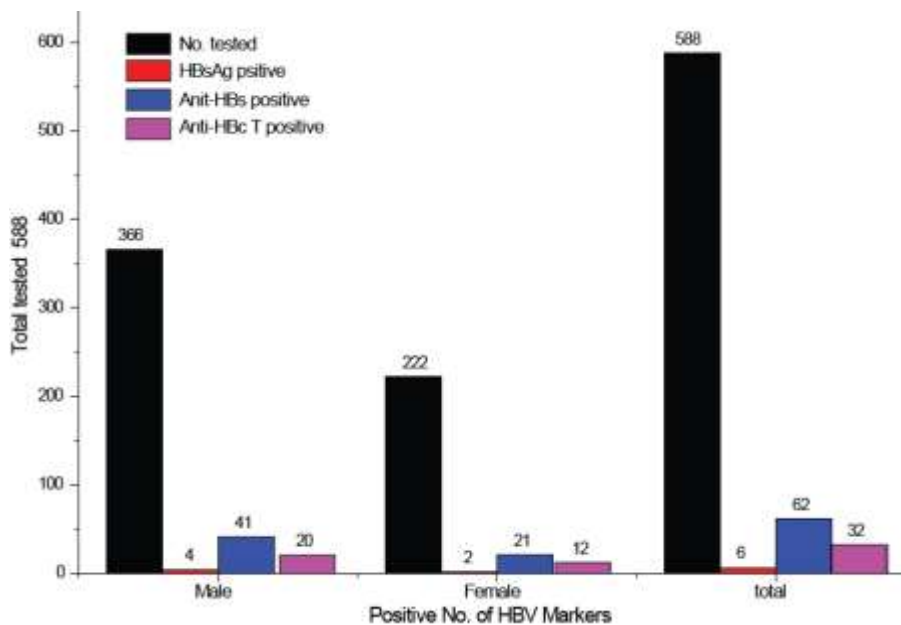
**Table (1): Seroprevalence of HBsAg according to the age among normal population**

No.	Age group (year)	Mean $\pm$ SD	No. tested	HBsAg positive No	%
1	< 1	5.5 $\pm$ 3.93	50	1	2*
2	1- 5	3.2 $\pm$ 1.27	56	1	1.78*
3	6- 10	7.9 $\pm$ 1.47	51	0	0
4	11- 15	13.02 $\pm$ 1.5	50	0	0
5	16- 20	18.2 $\pm$ 1.45	73	1	1.36*
6	21- 30	25.5 $\pm$ 3.11	83	0	0
7	31- 40	35 $\pm$ 2.8	80	1	1.25*
8	41- 50	45 $\pm$ 3.7	71	0	0
9	$\geq$ 51	57 $\pm$ 4.53	74	2	2.70**
Total			588	6	1.02

\* Significant difference when ( $P < 0.05$ ), \*\* non- significant difference ( $P > 0.05$ )

However, the prevalence of HBsAg marker among infants and children may be related to perinatal transmission from mother to her baby (Ye et al., 2006; Schillie, et al., 2015). Notably, those babies might have not enrolled in the National Immunization Program. While the prevalence of HBsAg among adult age groups may return to blood, blood products transfusion or other horizontal risk factors such as sexual transmission similar to many developing countries (Chowdhury, 2004; Franco et al., 2012 ).

Figure 2 shows, the seroprevalence of HBV serological markers in normal population according to the sex. Out of 366 males were tested, the HBsAg, anti-HBc Total and anti-HBs rates was 1.09 %, 5.46 %, and 11.20 % respectively, whereas out of 222 females were tested, it was 0.90 %, 5.40 % 9.45 % respectively. This distribution showed the males had highly positive HBsAg marker than females with statistically significant importance ( $P < 0.05$ ).



**Fig. 2. The distribution of HBV serological markers among normal population group according to sex**

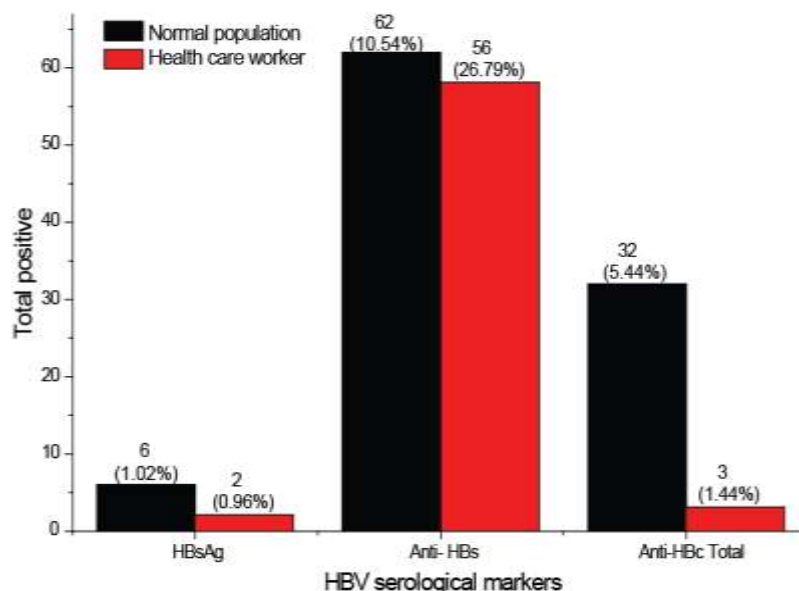
The differences between both sexes could be explained on the basis that males may have a greater chance to come in contact with risk of horizontal transmission factors of HBV infection than females, or alcohol intake being common in males that may enhance the liver damage caused by HBV infection (Ugwu, et al., 2008). This observation is in consistent with earlier studies were done in Iraq (Sabri, 2003). Regarding the seroprevalence of anti-HBc Total, insignificant correlation was recorded in this study between male and female ( $P > 0.05$ ). Furthermore, those individuals who were positive to the anti-HBs and anti-HBc Total together, that indicates they acquired past infection with immunity. On the contrary, the remaining individuals in both groups who had anti-HBs alone, that indicate those individuals were enrolled in the National Immunization Program (WHO, 2012). Such of this result, however, point out fallen seroprevalence of HBV infection among normal population in Baghdad. Since the development of a vaccine to prevent acute HBV infection in the early 1980s, the incidence of acute HBV infection in the general population of the United States has sharply fallen (Lewis et al., 2015). The CDC estimates that the incidence of new HBV infections had fallen 5-fold between 1980 and 2010 (USA, CDC, 2014).

### 3.2 Seroprevalence of HBV markers among HCWs

Figure 3 reveals the HCWs that were screened for the detection of HBsAg, anti-HBs and anti-HBc Total, with results of 0.96 %, 26.79 % and 1.44 % respectively. This result of HBsAg compatible with many reports around the world (Schiff, 2004; Djeriri et al. 2008), but it is incompatible with all previously published in Iraq (Al-Alwan et al., 1986; Al-Mashhadani, 1998). The highly differences and improving in the results in present study compared with previous results may be due to the introduction of vaccination program among HCWs, as well as, may be related to the increase awareness and health educations.

In comparison between normal population and HCWs groups, Figure 3 shows the high prevalence rate of HBsAg (1.02%) among normal population compared with rate of (0.96%) among HCWs with statistically significant ( $P < 0.05$ ), this fallen in HBsAg results among HCWs may be due to the “National Hepatitis Control Program” especially vaccination, increasing awareness and health education, increasing use of barrier

precautions and personal protective devices as well as improvement of general hygiene condition in the hospital setting by availability of disposable syringes and other medical equipments as possible, in addition to using adequately sterilization and disinfected instruments.



**Fig. 3. Seroprevalence of HBV markers among HCWs and normal population**

It should be noted, although HBV infection was higher among HCWs than that of the normal population before development of the HBV vaccine, this situation has been reversed, with many HCWs having previously received the vaccine (Mahoney, et al., 1997; Bhat et al., 2012). Also the anti-HBc Total rate was a greater proportion among normal population group (5.44%) compared with HCWs group (1.44%) which displayed highly significant importance ( $P < 0.01$ ), indicated high prevalence of HBV infection among normal population than HCWs group (Boccalini et al., 2013; Lewis et al., 2015). This finding may due to improvement of vaccination program among HCWs group than normal population group. In regard to anti-HBs, the higher rate (26.79%) was observed among HCWs group compared with this was recorded among normal population group (10.54%) with statistically significant correlation ( $P < 0.05$ ), the high variation may due to high differences in vaccination coverage between two populations.

**Table (2): seroprevalence of HBsAg and anti-HBs among HCWs according to their occupation**

Occupation	No. tested	No. HBsAg positive	%HBsAg positive	No. anti-HBs positive	% anti-HBs positive
Physician	39	0	-----	22	56.4*
Lab. Staff	62	0	-----	11	17.7*
Paramedical staff	36	0	-----	9	25.0*
Nurses	34	0	-----	9	26.4*
Workers**	38	2	0.006*	5	13.1*
Total	209	2	-----	56	26.7

\* statistically significant ( $P < 0.05$ ), \*\* Supporting nonprofessional staffs, which clean the wards and instruments and food handlers

Table (2) illustrates the seroprevalence of HBsAg marker among HCWs according to their occupation. The HBsAg marker was recorded only among “workers” group with statistically significant importance ( $P < 0.05$ ), whereas no evidence of HBsAg marker was found in the other groups.

This result is incompatible with previous studies were done in Baghdad, (Al-Mashhadani, 1998). The differences between the present study result and previous studies results may be due to improvement in hygiene situation among various HCWs groups due to the introduction of control and vaccination program, increased awareness and health education, but it may be reflect a poorly improvement among workers group; because majority of them were non-professional staff, older age groups, poorly health educated and from low socio-economic status, and most of them were working in high intensity areas (clean the wards and instruments) with direct contact with wastes and patient's blood and other fluids.

Table (2) also illustrates the distribution of anti-HBs serological marker among HCWs according to their occupation. The total prevalence among all groups was (26.7%) and the statistical analysis showed significant correlation ( $P < 0.05$ ) among all groups compared with total population percentage. The highest rate (56.4%) was found among physician group followed by nurses, paramedical staff and laboratory staff groups with rate of 26.4 %, 25.0 % and 17.7 % respectively. Whereas the lowest rate (13.1%) were located in workers group, this result in a concordance with previous study in Iraq (Al-Mashhadani, 1998) and it is may be related to low healthy education among workers group compared with physicians group.

The seroprevalence of HBsAg marker among HCWs according to the age is shown in table (3), the highest rate (4.76%) was found in the age group 51- 60 years old followed by age group 41-50 year old with rate of (2.22%) with significant importance ( $P < 0.05$ ) between above age groups compared with total population percentage, whereas no evidence of HBsAg marker was found in the younger age groups (20-30, 31-40) years old. These results are not consistent with previous study in Baghdad (Al-Mashhadani, 1998). But these results coincide with other study; the increased prevalence of HBsAg marker with advancing age among HCWs may be due to non enrollment those individuals in vaccination program in their life. Or those individuals with anti-HBs positive, immunization decreased with increasing age (Ciorlia and Zanetta, 2005; CDC, 2008).

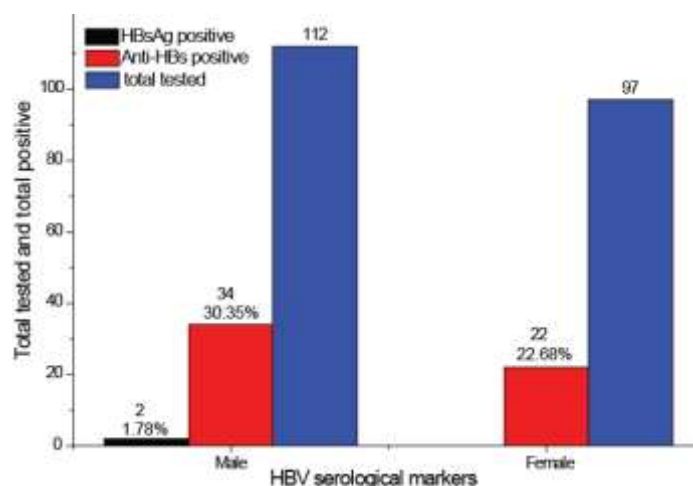
**Table (3): Sero-prevalence of HBsAg and anti-HBs markers among HCWs according to their age**

Age groups (year)	No. tested	No. HBsAg positive	% HBsAg positive	No. anti-HBs positive	%anti-HBs positive
21-30	75	0	0	7	9.33*
31-40	68	0	0	18	6.47*
41-50	45	1	2.22	16	5.55*
51-60	21	1	4.76	15	71.42*
Total	209	2	0.95	56	6.79*

\*Significant correlation ( $P < 0.05$ )

The seroprevalence of anti-HBs marker among HCWs according to the age is shown in (Table 3). The highest rate (71.42 %) was recorded among individuals in age group 51-60 years, followed by the age group 41-50 years with rate of (35.55 %), and the rate of (26.47 %) was noticed in the age group 31-40 years. Whereas the lowest rate (9.33%) was found among individuals in age group 21-30 years old. A significant importance ( $P < 0.05$ ) was registered among all age groups. However, these results indicated that the frequency of prevalent the anti-HBs positive in HCWs was increased with the advancing age and may reflect a frequent exposure to minute amounts of contaminated blood, blood products and other body fluids with development of antibodies and clearance of the antigen with advanced age, so this result is agreed with a previous study (Al-Mashhadani, 1998). Many studies was reported that the advancing age and increased working experience with higher rate of vaccination were significant factors which provide high rate of anti-HBs antibodies (Khan et al., 2007).

The seroprevalence of HBV markers among HCWs according to their sex (Fig. 4), showed high rate of HBsAg maker (1.78 %) among males, whereas no evidence of this marker among females with insignificant differences ( $P > 0.05$ ). This result agrees with previous result in Iraq (Al-Alwan et al. 1986; Al-Mashhadani, 1998). However, epidemiologic studies of gender or ethnic populations have shown inconsistent gender differences among chronic hepatitis B carriers (Chironna, et al., 2003). Additionally, male HCWs are more involved in private medical and laboratory practice in Iraq; therefore, they have additional risk of exposure to HBV infection.

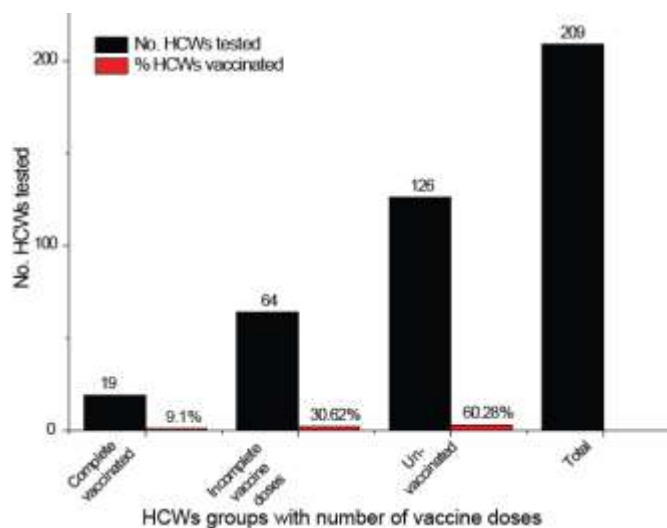


**Fig.4. Seroprevalence of HBsAg and anti-HBs among HCWs according to the sex**

Figure 4, also shows high rate of anti-HBs (30.35 %) among males HCWs, than female (22.68 %) with insignificant importance ( $P > 0.05$ ). These findings agree with previous results was recorded in Iraq (Al-Mashhadani 1998), as well as in line with many studies around the world (Al-Sohaibani, et al., 1995).

### 3.2.1 The HBV vaccine status among HCWs workers

A total of 56 (26.79 %) individuals were found to be anti-HBs positive (Fig. 5), such low rate may be due to low number of HCWs who had been vaccinated, there was 9.1 % vaccinated HCWs who received complete series of 3 vaccine doses, 30.62% had incomplete vaccine doses, and 60.28 % were unvaccinated individuals, with significant differences ( $P < 0.05$ ) among these three groups. Despite the availability of vaccination for more than two decades, vaccination of 100 % coverage has not yet been achieved.



**Fig. 5. Vaccine status among HCWs**

The most frequently quoted reason among the unvaccinated HCWs in this study was the ignorance (lack of interest and lack of knowledge) about the importance of HBV prevention, as well as the another common reason that phobic from vaccine itself, because most of HCWs thought that plasma-derived vaccine may cause infection via reactivation, whereas vaccine that used in Iraqi hospitals and health centers is genetically engineered vaccine (Recombinant DNA yeast-derived or mammalian cell-derived). More education or announcement is needed for HCWs group about HBV vaccination.

Table (4) shows vaccine administration among HCWs group related to vaccine doses and seroprevalence rate of anti-HBs antibody among vaccinated HCWs. The highest group of them who had only 1<sup>st</sup> vaccine dose was (53.0 %) with seroprevalence rate of anti-HBs was only (40.9 %) among this group with significant differences ( $P < 0.05$ ).

**Table (4): The effectiveness of vaccine among HCWs related to vaccine doses**

Vaccine doses	Vaccine administration	Vaccine administration	Anti-HBs positive	Anti-HBs positive
	No.	%	No.	%
1 <sup>st</sup>	44	53.0	18	40.9*
2 <sup>nd</sup>	20	24.1	19	95*
3 <sup>rd</sup>	19	22.9	18	94.7*
Total vaccinated	83	100	55	66.26*

\*Significant differences (P < 0.05)

The second group who had received 2<sup>nd</sup> vaccine doses performed (24.1%) and with significant (P < 0.05) seroprevalence rate of anti-HBs antibody (95%), whereas the lowest rate (22.9%) of administration was located among those who had complete series of three doses with seroprevalence rate of anti- HBs (94.7%) with significant importance (P < 0.05). These results indicate the absence of follow up for complete vaccination program. However, it also indicate high effectiveness of vaccine related to the doses, since 3 series vaccine doses resulted in protection 98-100 % of infants and 90-95 % of teens and adults (USA / CDC, 2008; Bhat, et al, 2012).

### Conclusions

This study demonstrated that seroprevalence of HBsAg among normal population was lower than previous figures in Baghdad. It was higher among males than females. The adults had high seroprevalence of HBsAg and anti-HBc Total compared with infants and children. In contrast anti-HBs were high among infants and children. No significant importance in seroprevalence of anti-HBc Total between both sexes, while the seroprevalence of anti-HBs was high among males than females. As well as it demonstrated the seroprevalence of HBsAg among HCWs was lower than previous figures in Baghdad, and it was more prevalent among males than females. The prevalence of anti-HBs among HCWs increased with advanced age, it is higher in males than females. Low rate of complete while high rate of incomplete series vaccine doses among HCWs and high rate of vaccinated HCWs was located among those with high healthy education more than those from low levels. Seroprevalence of HBsAg and anti-HBc Total was lower among HCWs than normal population, while anti-HBs was more prevalent in HCWs compared to normal population group.

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