ISSN: 0975-833X

Available online at http://www.journalcra.com

International Journal of Current Research
Vol. 12, Issue, 04, pp.11294-11298, April, 2020

DOI: https://doi.org/10.24941/ijcr.38459.04.2020

INTERNATIONAL JOURNAL OF CURRENT RESEARCH

RESEARCH ARTICLE

PREVALENCE AND RISK FACTORS OF HEPATITIS B AMONG BLOOD DONORS IN AL-NAJAF GOVERNORATE

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ARTICLE INFO

Article History:
Received 08th January, 2020
Received in revised form
24th February, 2020
Accepted 18th March, 2020
Published online 30th April, 2020

Key Words:
Rhizobium, Isolation,
Biological nitrogen fixing,
Nodulation, and Biofertilizers.

ABSTRACT

Nutrient deficiency in the soil poses a big challenge to food production globally. The use of artificialnitrogen fertilizer to aid crop yield is a common farming practice, despite its undesirable effects and hazard to the environment and human population. This research work aimed at isolation, identification & characterization of *Rhizobia spp.* from chickpearhizospheric soil samples collected of the southern region of the Faridpur district in Bangladesh. Isolation of *Rhizobium spp.* was culture on Yeast Extract Mannitol Agar (YEMA) medium incubated 3 days at 32^0C. A total of 10 *Rhizobium spp.* isolatewere isolated from rhizospheric soil samples. They are also found to be gram-negative, rod-shaped morphology, fast grower, indole producers and positive for catalase test. All isolates were found with bare absorption of Congo red dye & no growth on YEMA with 2% NaCl. Out of 10 only 3 isolates(FSRS-3, FSRS-7, and FSRS-9)were identified as rhizobia spp. on the basis of the authentication test (nodulation check with *Cicerarietinum*). These threerhizobial isolates may be useful to increase the symbiotic biological nitrogen fixation in legume plant chickpea(*Cicerarietinum*) and can be used as potential biofertilizer owing to their plant growth-promoting characters.

Citation: ***Mahdi Abd Ali Abbood Al-faham, Roohollah Nakhaei Sistani, Musa Nima Mezher AL-Jaifry, Alaa Abdulhussein Rasool and Hashim Ali Abdualmeer Al-sherees,*** **2020.** "Prevalence and risk factors of hepatitis B among blood donors in Al-Najaf governorate", *International Journal of Current Research*. 12. (4). 11294-11298.

INTRODUCTION

Hepatitis B virus (HBV) infection is a global problem. Two billion people were infected by hepatitis B virus and more than 360 million individuals suffer from chronic HBV infection. About 600,000 people die from hepatitis infection or the virus associated liver tumors called Hepatocellular Carcinoma (HCC) (Colin, 2006). In Africa, more than fifty million people are chronically infected with hepatitis B, with about 25% risk of mortality.The rate of hepatitis B carriers in Africa (Sub-Sahara) is about 9%-20% (Ghana Immunization Programme, 2010). The prevalence rate of the disease is about 4.8% to 21% in Ghana (Ghana Immunization Programme, 2010). The causative agent of hepatitis B is an enveloped virus with Single Strand DNA (ssDNA) genome (Taylor, 2013; Tong, 2011). When the virus replicates in the hepatocytes, the liver functions will be impairedand the infection will spread throughout the liver (Adams, 2006; Vinciguerra *et al*., 2013). Liver and hepatitis harm have increased as a consequence of the immune response against the virus in the liver cells (Rehermann, 2005). Blood, blood products and body fluids such as vaginal secretions contain hepatitis B virus, and also the saliva of active carriers contains low concentrations of the virus (CDC, 2010). The average incubation period of HBV is 90 days from the time of exposure to the onset of the symptoms but may vary from six weeks to six months (Mast *et al*., 2005). The HBV transmission through blood and its products is a major health concern, especially in developing countries, where preparing the safe blood is still a primary challenge (MOH-Ghana, 2006; World Health Organization, 2006). The rate of viral hepatitis B infection is proportional to the rate of blood Transfusion Transmitted Hepatitis B Virus (TTHBV). The screening of the blood and its products prevents the transmission of infectious diseases that sometimes may not be cured. The only way to prevent the transmission of such diseases is to use more sensitive methods to examine the blood before transfusion (MOH-Ghana, 2006; World Health Organization, 2006).

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MATERIALS AND METHODS

The diagnostic and research kits are listed in Table 1 below. In brief, samples were collected from all the donors in addition to 20 apparently healthy controls. All the samples were typed for blood groups and tested by ELISA for HBsAg and anti-HBc antibody. Those who were diagnosed as infected by ELIZA as well as 10% of randomly selected samples with negative results were confirmed by ELFA. Then blood and liver factors were tested on patients and 20 healthy controls**.**After examining ELISA and VIDS, all samples were examined with tests for GOT, GPT enzymes, complete blood counts, and lymphocytes, as shown in Figure (1).Samples were collected from 16273 voluntary donors from (20/5/2016) to (12/12/2016). The presence of HBV was tested by enzyme-linked immunosorbent assay (ELISA) for HBsAg and anti-HBc antibody.

METHODS

Diagnosis of HBsAg and anti- HBc by ELISA–III: The detection of HBsAganti-HBc was done by the use of the third generation of ELISA, technique using HBsAg and anti-HBc ELISA from BIO KIT (Spanish) and the assay was performed according to manufacturer ′s instructions.

Confirmatory of HBsAg by VIDAS (Mini VIDAS): The confirmation of HBsAg was done by using VIDAS the assay was performed according to manufacturer ′s instructions. VIDAS HBsAg Ultra (HBs) is an automated qualitative test, the kit HBsAg Ultra from Biomerieux.

RESULTS

Clinical Samples Distribution: A total of 16273 blood were collected in the main blood bank of AL-Najaf governorate. The mean age of the donors is 38.5 ±5.09 years (range: 18–59). All the samples were examined for the presence of HBsAg and anti-HBc antibody. The results are shown in the Table (3). Our results showed that 42 people were HBsAg and anti-HBc positive (0.26%) and 41 out of them (97.6%) was male and the remaining one (2.4%) was female. It was also found that 278 individuals (1.7%) were only positive for anti-HBc antibody and eight individuals (0.05%) were positive for just HBsAg. All the donors were volunteers. The prevalence of HBV exposure in Al-Najaf governorate was 2.01%, which 0.26% of them had an active infection. Eight individuals were only HBsAg positive means that they were at the beginning of HBV infection. Therefore, the incidence rate of HBV is 0.05%. The only anti-HBc antibody positivity indicating previous exposure that might be improved was seen in 278 people. Studies in the Middle East showed that the prevalence of HBsAg ranges from 4% to 5% in Iraq (13). The present study shows the lower rate of this infection in Najaf city.

Gender of donors: The classification of the data based gender in each age range group is shown in Table (4). The only notable fact of these tables is that the infection is less prevalent in women. But this could be a misinterpretation because the number of female participants are too small.

Profession of Patients: The resultsin chart (1) indicate the drivers can be infected by the virus more than the other professions significantly.

Complete Blood Counts (CBC) of patients: The blood factors collectively called CBC were analyzed for the patients and controls Tables (6).

Statistical Analysis: This study was a kind of Cross-sectional study. Statistical analysis was done by using statistical package for social sciences (SPSS) version 17. The ANOVA test was used for the purpose of testing the differences between the means of the different variables, and the T-test was used to evaluate the differences between two groups. The LSD (Least Significant Differences) was used to detected significant differences between parameters, and Z-test was also used. P values less than the 0.05 was considered as statistically significant (14).

DISCUSSION

To do this, 16273 volunteers were enrolled and blood samples were collected and analyzed. It was found that 42 people (0.26%) were infected as indicated by positivity for HBsAg and anti-HBc Antibody. This is lower than the report from Sulaimania governorate (0.67 (14), and it was also lower when compared to the neighboring Arab countries Lebanese blood donors (0.6%) (Ramia *et al*., 2015), and much lower than Abuja (Nigeria) 17.5% (Agbesor *et al*., 2013). It was much lower compared to Omani blood donors where HBsAg was present in 2.8% of the donors (Geraldine *et al*., 2006). Also, it was lower in AL-Anbar governorate, (west of Iraq) that the prevalence of HBsAg among screened total blood donors 16125 was 1.25% (Yassin, 2016). The prevalence of chronic HBV infection is higher in men than women and this may be because of the rarity of volunteer female participants in this study. In the current study, the prevalence of HBV result (2.01%) was similar to that in Sulaimania, Kurdistan Region-Iraq in 2006, which showed that the prevalence of HBV was 2.3% (Mohammed O. Mohammed, 2006). Our results showed the frequency of 0.31 for HBsAg which is lower than the prevalence reported for adults in New Zealand which showed that the prevalence of HBsAg was 0.5% (20). The prevalence of HBsAg in our samples was lower than the result of Shahrekord (a city located in the west of Iran). Results showed HBsAg positivity in 1.78%; It is also lower than Shiraz (South-west Iran) where HBsAg was detected in 1.07% of samples; and Ghazvin (west-central) with HBsAg positivity of 1.08%; and also lower than HBsAg positivity reported from Kashan (center) which was 0.5%; and much lower than Tehran (North-central) with HBsAg positivity 3.4% (Doosti, 2009). Also, it was much lower than the prevalence of HBV among blood donors in Kosovo is 4.2% (Hajrullah Fejza, 2009).

However, my result was lower than that of a study which was done in the western Brazilian Amazon, which included 2656 samples; 3.3% were positive for HBsAg (Sebasteao, 2005). In the current study, there was no significant relation of HBsAg positivity with all ages but only in age (28-37) P-value < 0.05, while in gender showed a relative significance P-value < 0.05; a result which is similar to that of Blakely T. *et al* study (Bandaranayake, 1991). Based on profession the current study revealed a significant relation of HBsAg positivity with the drivers 14 (33.3%) where the X^2 calculation reached to (13.6) which is greater than the value of X^2 (11.07) at a level of significance (P< 0.05) table (6). While for the other jobs it was not significant. This result was compatible with results of a study (Hepatitis B Prevalence and Risk Factors in Blood donors in Ghazvin which revealed that unemployed people,

Table 1. Kits that used by current study

No.	Kits	Manufacture	Origin
1	HBsAg	Bio kit	Spanish
2	Anti-HBc total	Bio kit	Spanish
3	HBsAg Ultra Vidas kit	Bio Merieux	France
4	GOT/AST-P III	Fujifilm	Japan
5	GPT/ALT-P III	Fujifilm	Japan

Table 2. The Equipment used by current study

No.	Equipment and Instruments	Manufacturer	Origin
1	ELISA washer ELx50	Bio Tek	USA
2	ELISA reader ELx 800	Bio Tek	USA
3	ELISA printer Epson LQ 300+II	Bio Tek	USA
4	Incubator	Memmert	Germany
5	Refrigerator	DAIREI	USA
6	Centrifuge	Hettich	Germany
7	Timer with alarm	Rhythm	China
8	Micropipette 10-100	Eppendorf	Germany
9	Hematology Analyzer	Diagon D-Cell 60	Hungary
10	Vidas and Mini vidas	Biomerieux	France
11	FUJIFILM-Dry chemo	FUJIFILM	Japan

Table 3. Numbers of HBV positive according to the markers of HBV

No. of Donors	No. of HBsAg+ anti-HBc positive	No. of only HBsAg positive	No. of only anti-HBc positive	Total
16273	42 (0.26 %)	8 (0.05 %)	278 (1.7 %)	328(2.01%)

Table 4. The classification of samples by age and gender

Age of Groups	No. of donors of males	No. of donors of females	Percent
18-27 yr.	3178 (19.5%)	21 (0.13%)	19.63 %
28-37 yr.	7623 (46.9%)	26 (0.16%)	47.06 %
38-47 yr.	1595 (9.8%)	15 (0.09%)	9.89 %
48-57 yr.	3426 (21.05%)	9 (0.06%)	21.11 %
58 and more	378 (2.3%)	2 (0.01%)	2.31 %
TOTAL	16200(99.55%)	73 (0.45%)	16273(100%)

Table 5. The classification of HBsAg and anti-HBc positive patients based on gender

Gender	No. of Patients	Percent %
*Males	41	97.62 %
Females	1	2.38 %
Total	42	100 %
Test	4.25 Sign Males *P< 0.05	

*Statistically significant P value < 0.05

Table 6. The classification of HBV patients and healthy control according to CBC

RESULT		N.of Control	Percent %	N. of Patients	Percent%	T test P< 0.05
WBC	Normal	11	55	19	45.2	3.2
	Low	1	5	0	0	2.3
	High	8	40	23	54.2	4.3
HB	Normal	10	50	39	92.9	6.6
	Low	0	0	0	0	NON
	High	10	50	3	7.1	8.1
	Normal	6	30	31	73.8	7.8
RBC	Low	0	0	0	0	NON
	High	14	70	11	26.2	7.6
PLT	Normal	17	85	32	76.2	3.3
	Low	0	0	2	4.8	2.5
	High	3	15	8	19	2.2
MCHC	Normal	2	10	4	9.5	NON
	Low	18	90	37	88.1	NON
	High	0	0	1	2.4	NON
TOATL		20	100	42	100	

*Statistically significant P value < 0.05.

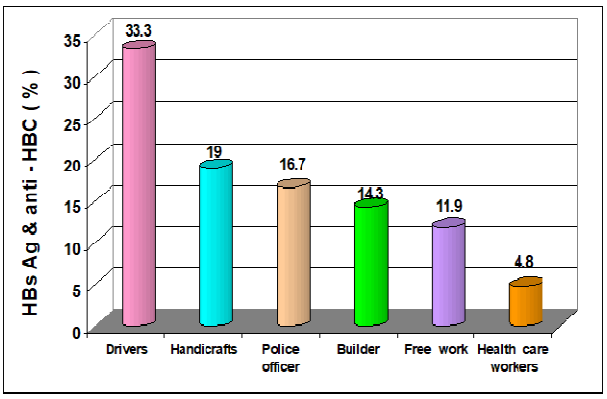

Chart 1. Distribution of HBsAg and anti-HBc positive patients based on profession

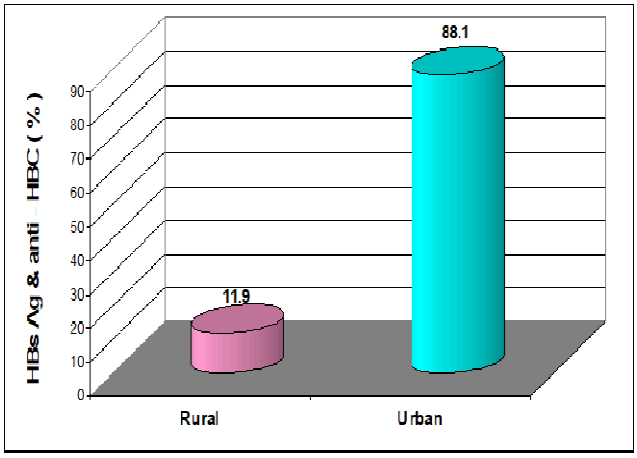

Chart 2. Distribution of HBsAg and anti-HBc positive patients according to residency

drivers, and barbers in Iran are at high risk (Doosti, 2009), the barbers are also a high-risk group for HBV infection in Turkey (Candan, 2002), and drivers are a high-risk group as shown in another study in Iran (Doosti *et al*., 2009).

In the current study, the driving was proposed as a risk factor for HBV infection. This may be because the drivers may deal with sharp tools during repairing cars or may have a low level of education and therefore have risky behaviors or may have extramarital sexual contact.

Conclusion

- The prevalence of HBV among blood donors in Najaf is 2.01%.
- 2-The ratio of infected male to infected female blood donors was approximately 97.62%: 2.38%.
- The prevalence of HBV among blood donors in the urban area (88.1%) is higher than the rural(11.9)area.
- 4-The driving profession has the higher risk for HBV infection among other professions.
- 5-The age group with the highest frequency of viral hepatitis B in Najaf governorate is 28-37 years old.
- 6- The study showed significant differences between patient's hepatitis B and control in SGOT and SGPT.
- 7- The study showed a linear relationship between SGOT and SGPT.
- 8-The current study showed a P-probability of less than 0.05 in lymphocytes among patients with hepatitis B and control.

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