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Original article

Seroprevalence and Associated Risk Factors of Hepatitis E Virus Infection Among Blood Donors in Tripoli, Libya

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ABSTRACT

Hepatitis E (HE) is a transfusion-transmitted viral disease caused by the Hepatitis E virus (HEV). There are currently no national seroprevalence reports for Libyan blood donors. This study therefore aimed to evaluate HEV risk to blood safety by assessing the prevalence of anti-HEV IgG and anti-HEV IgM among Libyan healthy blood donors. A total of 267 serum samples were collected from blood donors. Using a questionnaire form, the risk factors for HEV infection of all blood donors were gathered. The sera of the blood donors were screened for anti-HEV IgM and anti-HEV IgG using ELISA kits (DIA. PRO Srl, Milan, Italy). The statistical significance of the association between dependent and independent variables was evaluated using the chi-square test. A P-value < 0.05 was considered an indicator of statistical significance. The overall seroprevalence of HEV infection was 5.24% (14/267). Of the 267 blood donors, 12 (4.49%) tested positive for anti-HEV IgG antibodies. In contrast, 2 (0.74%) tested positive for anti-HEV antibodies (IgM and IgG). Our seroprevalence of anti-HEV IgG was significantly associated with increased age, marital status, the technique of washing hands before eating, and smoking habit, and it was significantly low in blood donors who eat in cafes and restaurants. The overall seroprevalence of HEV infection was low in Libya. HEV is not currently prevalent among blood donors in Tripoli, Libya and poses little threat to the national blood supply. Therefore, current blood donor screening protocols are sufficient.

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INTRODUCTION

Hepatitis E (HE) is an emerging viral disease caused by the Hepatitis E virus (HEV) [1]. The journey to the discovery of HEV started with the realization that an epidemic of jaundice that occurred in New Delhi, India, between 1955 and 1956 was due to an enterically transmitted non-A non-B hepatitis. Also, in 1980, Khuroo assumed that the cause of the epidemic of non-A, non-B viral hepatitis that happened in Kashmir, India, in 1978 was likely enterically transmitted [2].

In 1983, Balayan and his research team were able to confirm the discovery of a new hepatitis virus by detecting viral particles in a stool sample using electron microscopy [3].
HEV is a positive-sense single-stranded RNA virus. It is small and present in stool and bile as a non-enveloped virus of 32–34 nm, while in circulating blood, the virus can be found as an enveloped particle with a diameter of 40 nm [4,5]. In the family *Hepeviridae*, HEV is the only member of the genus *Orthohepevirus* [6]. There are eight HEV genotypes, of which only four are confirmed to cause diseases in humans: HEV-1 through HEV-4. HEV-1 and HEV-2 are found in underdeveloped nations, while HEV-3 and HEV-4 are prevalent in developed nations [7]. HEV-1 and HEV-2 are associated with infection in humans, and epidemics frequently occur in nations with inadequate sanitation [8]. HEV-3 and HEV-4 are transmitted by consuming of undercooked pig meat or its derivatives. The HEV genotypes originating from rabbits, pigs, camels, and rats have zoonotic potential [9].
HEV is regarded as the main causative agent of acute viral hepatitis around the world. According to estimates, HEV has infected one-third of the global population. Over 20 million infections are estimated to arise annually around the world, of which 70,000 are fatal [10]. These fatalities predominately occur in Latin American, Asian, and African nations with limited resources [11].
Since HEV was first discovered, contamination of water and food has been the main cause of the majority of HEV infections that have been identified in the general population [12]. However, it is known that blood transfusions in patients with chronic liver disease and immunocompromised individuals are responsible for a small but considerable fraction of HEV infection [12,13]. Whereas many studies suggested that HEV infections could occur from the contaminated blood supply [14,15]. The first conclusive evidence of transmission of HEV through blood transfusion, as confirmed via RNA sequence analysis, was documented in a patient in Hokkaido, Japan, in 2004 [16]. Since then, there have been numerous confirmatory studies of transfusion-transmitted HEV infection published worldwide, emphasizing the demand for universal blood donor screening for HEV [12,13].
To the best of our knowledge, there are currently no national seroprevalence reports for Libyan blood donors. The findings of studies conducted in other nations make it clear that Libyan blood donation safety and the hazard of HEV-contaminated blood need to be researched to determine the clinical significance of implementing safeguards in place to protect blood recipients. Therefore, the current study was conducted to determine the seroprevalence of HEV infection among healthy blood donors of the Central Blood Bank of Tripoli (CBB/T) and to investigate the risk factors associated with HEV infection among these healthy blood donors.

METHODS

Study population and sample collection

The current study was conducted from October to December 2021 in the CBB/T. Two hundred and sixty-seven healthy blood donors who were found eligible as a result of the CBB/T screening process were involved in this study. About 5 ml of venous blood was collected from blood donors and placed in a white tube with no anticoagulant. The clotted blood was centrifuged, the serum was aspirated into Eppendorf tubes, and the tubes were then kept at -20 C until testing.
Blood donors who consented to participate in the study were subjected to a face-to-face interview to complete the questionnaire. The independent variables that were included in the questionnaire are age, gender, marital status, educational level, residence area, family monthly income, technique of washing hands before eating, method of washing vegetables and fruits, source of water drinking, eating outside the home, contact with an animal, travel history, smoking habit, having a blood transfusion.

Ethical considerations

This study was carried out in accordance with the international guidelines that were arranged in the Declaration of Helsinki, and ethical approval was obtained from the CBB/T. In this study, informed consent was obtained from each participant after explaining the objectives of the study, and we have allowed inquiries about any associated questions about this study.

Anti-HEV IgG and Anti-HEV IgM ELISA

HEV ELISA were performed at the Reference Medical Laboratory-Tripoli. The sera of the blood donors were screened for anti-HEV IgM and anti-HEV IgG using ELISA kits (DIA. PRO Srl, Milan, Italy).

Statistical Analysis

Donor data obtained from the questionnaire was entered into a Microsoft Excel 2010 and analysis of the data was performed using SPSS version 26. Descriptive statistics such as frequency and percentage were calculated. HEV IgG

and IgM seropositivity were analyzed as dependent variables and risk factors as independent variables. The statistical significance of the association between dependent and independent variables was evaluated using the chi-square test. A *P*-value < 0.05 was considered an indicator of statistical significance.

Table 1. Univariate analysis of risk factors associated with anti-HEV IgG positivity among blood donors in Tripoli, Libya

Factor	No. tested [1]	No. HEV IgG + [2]	% HEV IgG + [3]	DF [4]	Chi-Square	*P*-value
Age group (years)				4	56.660	0.000
18-26	80	0	0			
27-35	104	1	0.96			
36-44	51	2	3.92			
45-53	20	4	20			
≥ 54	12	5	41.66			
Sex				1	0.000	0.989
Male	134	6	4.47			
Females	133	6	4.51			
Marital status				3	11.217	0.011
Single	178	4	2.24			
Married	82	7	8.53			
Widowed	3	1	33.33			
Divorced	4	0	0			
Educational level				4	8.195	0.085
Non-Educated	1	0	0			
Elementary	20	1	5			
Secondary	86	5	5.81			
College	151	4	2.64			
Postgraduate	9	2	22.22			
Residence area				1	0.591	0.442
Urban	255	12	4.70			
Rural	12	0	0			
Family income				3	1.819	0.611
<500	15	1	6.66			
500-1000	105	5	4.76			
1000-2000	87	2	2.29			
>2000	60	4	6.66			
Hand washing				2	11.029	0.004
water only	29	0	0			
water & soap	236	11	4.66			
without washing	2	1	50			
Washing of v/f [5]				3	1.008	0.799
water only	192	9	4.68			
water & soap	16	0	0			
water & vinegar	56	3	5.35			
without washing	3	0	0			
Drinking water				4	2.935	0.569
Manmade water	3	0	0			
Well water	18	0	0			
Filtered Water	159	7	4.40			
Bottled water	70	5	7.14			
Filtered & bottled	17	0	0			
Eating outside				1	4.819	0.028
Yes	187	5	2.67			
No	80	7	8.75			
Animal contact				1	1.642	0.200
Yes	69	5	7.24			
No	198	7	3.53			

Travel outside				1	0.146	0.702
Yes	191	8	4.18			
No	76	4	5.26			
Smoking habit				1	4.043	0.044
Yes	65	0	0			
No	202	12	5.94			
Blood transfusion				1	0.748	0.387
Yes	15	0	0			
No	252	12	4.76			

[1] No. tested = total number of tested donors; [2] No. HEV IgG + = number of donors tested positive for HEV IgG;[3] % HEV IgG + = percentage of HEV IgG positive donors; [4] DF = degree of freedom;[5] Washing of V/F = Washing of vegetables and fruits.

RESULTS

Characteristics of the study population

There were 267 blood donors who participated in this study. Their ages ranged from 18 to 62 years, with a mean age of 32.82 years. The majority of the blood donors, 104 (39%), were in the age group of 27 to 35, while the minority of blood donors, 12 (4.5%), were in the age group of ≥ 54. Males made up 134 (50.2%) of these blood donors, compared to females, who made up 133 (49.8%) of the total (Table 1).

Seroprevalence of HEV infection

The overall seroprevalence of HEV infection among blood donors enrolled in this study was 5.24%. Of the total study participants, 4.49% tested positive for anti-HEV IgG antibodies. None of the participants tested positive for anti-HEV IgM only. In contrast, 0.74% tested positive for anti-HEV IgM and anti-HEV IgG antibodies (Table 2).

Table 2. Seroprevalence of anti-HEV antibodies among blood donors in Tripoli, Libya

Antibody	Total number	Positive number	Percent
Anti-HEV antibodies (IgM and/or IgG)	267	14	5.24
Anti-HEV IgG	267	12	4.49
Anti-HEV antibodies (IgM and IgG)	267	2	0.74

Analysis of risk factors significantly associated with anti-HEV antibodies (IgM and/or IgG) positivity

The present study found that the highest seroprevalence of anti-HEV IgG was in the age group ≥ 54 years, while all the blood donors included by age group 18-26 years tested negative for anti-HEV IgG. There is a significant association between increased age and the seroprevalence of anti-HEV IgG (Table 1 and Figure 1). Marital status is significantly associated with seroprevalence of anti-HEV IgG and also with the seroprevalence of anti-HEV antibodies (IgM and IgG). The anti-HEV antibodies (IgM and IgG) positivity is significantly associated with marital status only in this study. The highest seroprevalence of anti-HEV IgG was found in the widowed group of blood donors, whereas all blood donors who belonged to the divorced group tested negative for anti-HEV IgG (Table 1). The highest seroprevalence of anti-HEV antibodies (IgM and IgG) was found in the divorced blood donors, while all married and widowed blood donors tested negative for anti-HEV IgG (Figure 2).

There is a significant association between the technique of hand washing before eating and the seroprevalence of anti-HEV IgG. Blood donors who did not wash their hands before eating had the highest seroprevalence of anti-HEV IgG. In contrast, all blood donors who wash their hands with water only tested negative for anti-HEV IgG. Regarding smoking, the highest anti-HEV IgG positivity was found in the blood donors who did not smoke, whereas all blood donors who practice the smoking habit tested negative for anti-HEV IgG. There was a significant association between smoking habit and anti-HEV IgG positivity (Table 1).

Blood donors who did not eat in restaurants and cafes had the highest seroprevalence of anti-HEV IgG. In comparison, the lowest seroprevalence was found among blood donors who eat in cafes and restaurants. There is a significant association between eating in restaurants and cafes and the seroprevalence of anti-HEV IgG (Table 1).

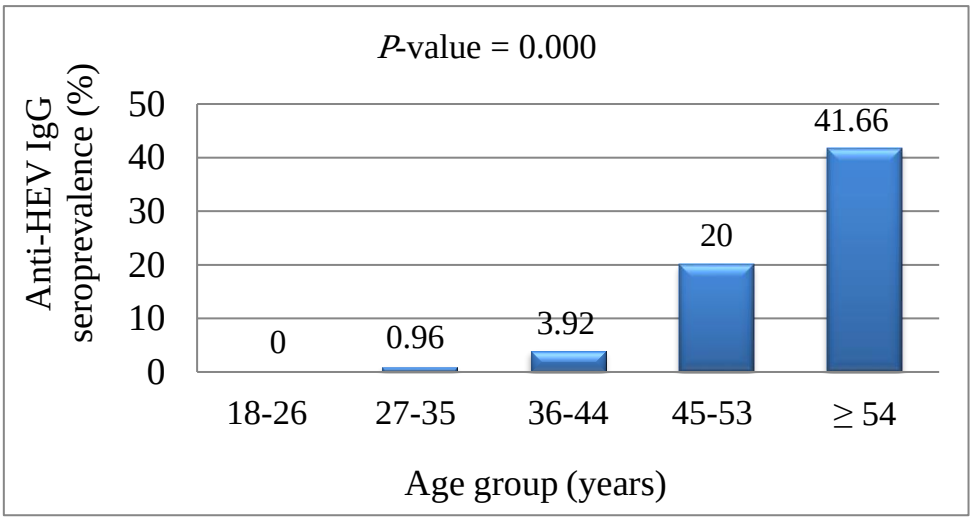

Figure 1. The distribution of anti-HEV IgG positivity among the age group of the blood donors.

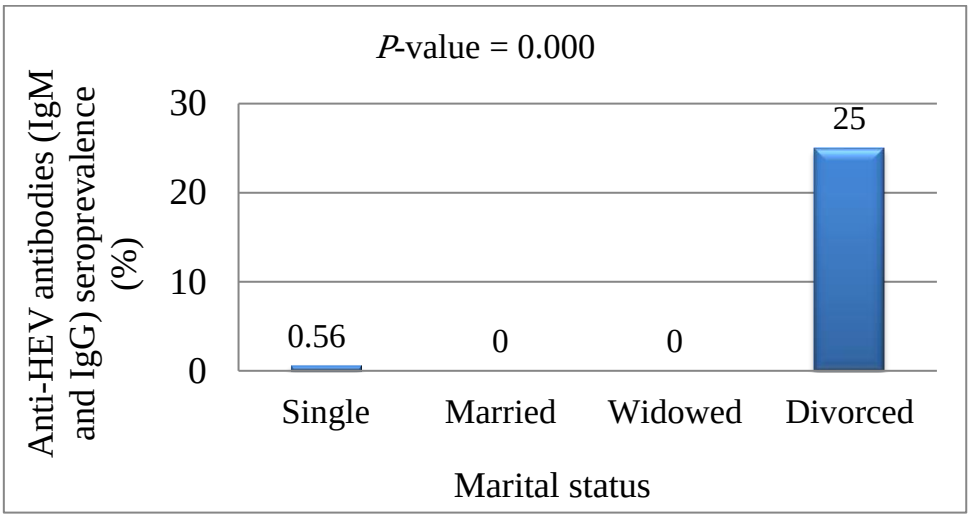

Figure 2. The distribution of anti-HEV antibodies (IgM and IgG) positivity among the marital status of the blood donors.

DISCUSSION

To our knowledge, this is the first study to investigate the seroprevalence of HEV infection and associated risk factors among healthy blood donors in Libya. This study revealed that the seroprevalence for anti-HEV IgG is (4.49%), while the seroprevalence of anti-HEV antibodies (IgM and IgG) is (0.74%). The seroprevalence of anti-HEV IgG in the present study is lower than the anti-HEV IgG seroprevalence (10%) found among 180 pregnant women in Tripoli, Libya [17]. A study conducted by Khuroo *et al*. showed that hepatitis E developed in 36 (17.3%) of 208 pregnant women, as compared to 71 (2.1%) of 3350 non-pregnant women and 107 (2.8%) of 3822 men [18]. In another study, anti-HEV IgM positivity was identified in (46%) and (57.5%) of non-pregnant women and pregnant women, respectively [19]. This study revealed that the incidence of hepatitis E was higher in pregnant women than in non-pregnant women and men. These findings are partially consistent with the difference between our seroprevalence and the seroprevalence of the Libyan study [17]. The anti-HEV IgG seroprevalence in this study is consistent with the anti-HEV IgG seroprevalence in some neighboring Middle Eastern nations like Tunisia (5.4%) [20] and Saudi Arabia (3.2%) [21]. In addition to the similarities among the study participants employed in these studies, homogeneity in cultural and socioeconomic conditions also seems to be present. In contrast, the anti-HEV IgG seroprevalence of the present study is lower than the seroprevalence reported in other studies of neighboring Middle Eastern nations like Egypt (17.7%) [22], Sudan (36.3%) [23], Egypt (84.3%) [24], and Qatar (20.5%) [25].

The difference in seroprevalence of HEV infection may be caused by variations in the demographics of the population studied and in the HEV antibody detection assays used [26]. The anti-HEV seroprevalence of the current study is lower than the seroprevalence (84.3%) among 2428 pregnant women in Egypt [24]. In addition to dissimilarities between

study participants, this discrepancy may be caused by the fact that all Egyptian study participants live in rural areas, compared to the present study, where the majority of participants live in urban areas. The discrepancy in seroprevalence may also be partially explained by the fact that the in-house ELISA used in the Egyptian study was less sensitive and specific than the DIA.PRO ELISA was used in this study. The anti-HEV IgG seroprevalence of the current study is also lower than the anti-HEV IgG seroprevalence of Sudan (36.3%) [23]. According to many Sudanese studies, insufficient safe water supply is the main risk factor for HEV infection in Sudan [23, 27]. This may help to explain why the seroprevalence of the present study is different from that of Sudan. The anti-HEV seroprevalence of this study is additionally lower than that of Qatar (20.5%). The high seroprevalence in the Qatarian study may be attributed to the mixed nationalities of the participants, where a significant proportion of the participants come from hyperendemic nations like Egypt and the nations of the Indian subcontinent [25].

When compared to the anti-HEV IgG seroprevalence of most studies conducted in developed nations like France (56.1%) [28] and Italy (49%) [29], this study represents a low anti-HEV IgG seroprevalence. The HEV infection in developed nations is mainly a zoonotic disease transmitted primarily through contaminated meat and in underdeveloped nations through contaminated water, which may reflect the differences in seroprevalence in different nations [30]. Many studies carried out in European nations revealed that the main risk factor for HEV infection in Europe is the consumption of pig meat or its derivatives [29, 31, 32]. However, eating pig meat is forbidden in the Islamic religion and therefore in Libya. This may explain the differences in seroprevalence rates between the current study and most studies of developed nations. Nevertheless, the anti-HEV IgG seroprevalence of this study is consistent to the seroprevalence from other developed nations, such as the USA (7.3%) [33], Canada (5.9%) [34], New Zealand (8.1%) [35], and Australia (5.9%) [36]. The anti-HEV IgG seroprevalence of the present study was lower than the anti-HEV IgG seroprevalence reported in Asian developing nations such as India (60.5%) [37], China (32.6%) [38], China (13.36%) [39], and Bangladesh (35%) [40]. This difference in seroprevalence may be attributed to poor sanitation and water quality in several areas in these nations compared to Libya.

In this study, anti-HEV IgM was detected in two blood donors; both donors were also positive for anti-HEV IgG antibodies, suggesting the presence of acute HEV infection at the time of donation. In the current study, the seroprevalence of anti-HEV antibodies (IgM and IgG) was found to be 0.74%. The seroprevalence of anti-HEV antibodies (IgM and IgG) of the current study is inconsistent with that of the UK (21%) [41] and Nigeria (3.8%) [42]. But it is consistent with that of Italy (0.6%) [29], the USA (0.58%) [33], and Qatar (0.30%) [25].

This study showed that age was a significant risk factor for past HEV infection, with seropositivity increasing significantly with age. This finding is consistent with other studies carried out in Libya [17], Tunisia [20], Qatar [25], Nigeria [42], France [28], the UK [41], Canada [34], the USA [33], New Zealand [35], and Australia [36]. The increase in positivity with age seen in this study may be attributed to the cumulative exposure to HEV throughout life. However, the current study disagrees with other studies conducted in Sudan [23], Saudi Arabia [21], and Italy [29].

The current study found a significant association between marital status and anti-HEV IgG positivity. Also, marital status is the only factor that has been found to be significantly associated with anti-HEV antibodies (IgG and IgM) positivity. These results are consistent with the findings of an Iranian study which revealed that married individuals had a significantly higher seroprevalence of anti-HEV IgG than single individuals [43]. A logical explanation for why marital status increases the risk of HEV infection suggests person-to-person transmission of HEV among individuals who share intimate personal contacts. Married individuals may have a higher susceptibility to HEV infection compared to unmarried individuals, due to both person-to-person and sexual transmission, as well as the sharing of household items like utensils, toilets, drinking water, and food from the same source. However, the findings of this study are inconsistent with studies done in China [39] and Nigeria [42], which found no association between marital status and the seroprevalence of anti-HEV IgG.

This study also found that there is a significant association between smoking habit and seroprevalence of anti-HEV IgG, in which 5.94% of the blood donors who had positive anti-HEV IgG tests did not smoke, whereas all smoker blood donors tested negative for anti-HEV IgG. This suggests that non-smoking blood donors have a higher probability of contracting a HEV infection. This finding agrees with that of a study carried out in Iran, which revealed that the seroprevalence of anti-HEV IgG in participants who were smokers (23.7%) was significantly lower than in those who were nonsmokers (25.2%) [43].

The seroprevalence of anti-HEV IgG positivity has a significant association with the technique of washing hands before eating. A study from India found that the seroprevalence of anti-HEV IgG was significantly lower in the participants who regularly wash their hands with soap before eating food than in the other group, who do not regularly wash their hands with soap before eating food [44]. This is partly in agreement with the current study. The results of the present study are inconsistent with those of studies from Nigeria [42] and Turkey [45], which found no association

between a technique of hand washing before eating and the seroprevalence of anti-HEV IgG positivity.
Regarding eating outside the home, this study found that blood donors who eat in cafes and restaurants had a significantly lower seroprevalence of anti-HEV IgG than blood donors who do not. This finding may be attributed to the fact that the restaurants and cafes in Tripoli have good food hygiene standards, such as the use of disposable cups and tools. According to a study conducted in the Netherlands, the seroprevalence of anti-HEV IgG decreased significantly from 35.1% in blood donors who visited restaurants less than once per month to 28% in blood donors who visited restaurants more than once per month [46]. This finding is partly in agreement with our study.

CONCLUSION

According to the present study, HEV is not currently prevalent among healthy blood donors of CBB/T and poses very little threat to the national blood supply. Therefore, current blood donor screening protocols are sufficient. Similar seroprevalence studies should be performed periodically to assess whether this will change in the future. The seroprevalence of anti-HEV IgG in this study was significantly associated with increased age, marital status, the technique of washing hands before eating, and smoking habit, and it was significantly low in blood donors who eat in cafes and restaurants. However, the seroprevalence of anti-HEV antibodies (IgM and IgG) was significantly associated with marital status only. There were no significant differences between other HEV risk factors and anti-HEV IgG positivity or anti-HEV antibodies (IgM and IgG) positivity.
We recommend conducting an awareness program about HEV infection, the route of transmission, and the risk of HEV to blood transfusion. Additionally, further studies should be conducted to fully understand the epidemiology of HEV infection among blood donors in the rest of the country. As well as to characterize the circulating genotypes of HEV in Libya.

Conflicts of Interest

The authors declare no conflicts of interest.

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الإنتشارالمصلي وعوامل الخطرالمرتبطة بعدوى فيروس الإلتهاب الكبدي هـ بين المتبرعين بالدم في طرابلس، ليبيا

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المستخلص

الالتهاب الكبدي هـ هو مرض فيروسي ناشئ ينتقل عن طريق الدم و يسببه فيروس التهاب الكبد هـ. لا توجد حاليًا تقارير وطنية عن الانتشار المصلي لعدوى فيروس الالتهاب الكبدي هـ، للمتبرعين بالدم الليبيين. لذلك هدفت هذه الدراسة إلى تقييم خطر فيروس الالتهاب الكبدي هـ، على سلامة نقل الدم بطرابلس، ليبيا، من خلال تقييم انتشار الأجسام المضادة لفيروس الالتهاب الكبدي هـ (الغلوبولين المناعي ج والغلوبولين المناعي م)، بين المتبرعين بالدم في طرابلس، ليبيا. تم جمع 267 عينة مصل من المتبرعين بالدم. تم جمع العوامل الاجتماعية والديموغرافية، وعوامل الخطر المرتبطة بفيروس التهاب الكبد هـ، لجميع المتبرعين بالدم باستخدام نموذج إستبيان،و بإستخدام تقنية الإليزا، تم فحص عينات المتبرعين بالدم، للكشف عن وجود الأجسام المضادة لفيروس الالتهاب الكبدي هـ (الغلوبولين المناعي ج والغلوبولين المناعي م). الأهمية الاحصائية للارتباط بين المتغيرات التابعة والمستقلة، تم تقييمها باستخدام اختبار مربع كاي. القيمة الاحتمالية أقل من 0.05، أعتبرت مؤشرا للأهمية الإحصائية. بلغ معدل الانتشار المصلي الإجمالي لعدوى فيروس التهاب الكبد هـ 5.24% (267/14). من بين 267 متبرعًا بالدم، كان اختبارالغلوبولين المناعي ج ايجابي لعدد 12 (4.49%) متبرعا بالدم. لم تكن نتيجة اختبار أي من المشاركين إيجابية بالنسبة لإختبار الغلوبولين المناعي م. في المقابل، كانت نتيجة إختبارالأجسام المضادة لفيروس التهاب الكبد هـ (الغلوبولين المناعي ج والغلوبولين المناعي م) لعدد 2 (0.74%) من المتبرعين بالدم إيجابية. ارتبط الانتشار المصلي للأجسام المضادة لفيروس التهاب الكبد هـ (الغلوبولين المناعي ج) ارتباط مهم إحصائيا مع زيادة العمر والحالة الاجتماعية وتقنية غسل اليدين قبل الأكل وعادة التدخين، وكان منخفضًا بشكل مهم إحصائيا في المتبرعين بالدم الذين يتناولون الطعام في المقاهي والمطاعم. كشفت الدراسة الحالية، ان معدل الانتشار المصلي الإجمالي لعدوى فيروس التهاب الكبد هـ منخفضا في ليبيا. عدوى فيروس الاتهاب الكبدي هـ، ليست منتشرة في المتبرعيين بالدم في طرابلس، ليبيا، وتشكل خطرا بسيطا على نقل الدم بداخل الوطن. وبالتالي فان بروتوكولات فحص المترعين بالدم الحالية، تعتبر كافية.

الكلمات الدالة. فيروس الإلتهاب الكبدي هـ، الانتشار المصلي، ليبيا، المتبرعين بالدم.