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Original Article

Seroprevalence and Potential Risk Factors of Hepatitis E Virus Infection Among Pregnant Women in Tripoli, Libya

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ABSTRACT

Background and aims. *Hepatitis E virus (HEV) is considered one of the public health important zoonotic diseases and is well-documented to cause significant risk overall the pregnancy terms vary from self-limiting to life-threatening for pregnant women and their offspring. This study was conducted to investigate the seroprevalence and potential risk factors associated with HEV infection among pregnant women in Tripoli, Libya.* ***Methods.*** *A total of 180 serum samples were collected from pregnant women from March to October 2022 at Reference Medical Laboratory in Tripoli, Libya. Serum samples were screened by Enzyme Immunosorbent Assay against anti-HEV IgG using commercially available ELISA kits (DIA.PRO HEV IgG ELISA, Italy). Statistical significance associations between dependent and independent variables were evaluated using the Chi-square test. P-value < 0.05 was considered an indicator of statistical significance.* ***Results.*** *The overall seroprevalence rate was estimated to be 10% (18/180); CI 95%= 5.62%-14.38%). The highest seroprevalence of HEV was 60 (23.33%; 95% CI= 12.63%-34.04%) among pregnant women 35-45 years of age, the results revealed a statistically significant among age groups (P=.0001). Women in the third trimester reported the highest HEV seroprevalence (11.18%; 95% CI= 6.02%-16.36%).* ***Conclusion.*** *The present results highlighted a potential risk of HEV infection among pregnant women in the country and an increased risk of potential exposure to HEV infection. Further national studies should be conducted to evaluate the seroprevalence and determine the molecular epidemiological patterns, and phylogenetic analysis should be considered.*

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INTRODUCTION

Hepatitis E is considered one of the important public health infectious diseases causing acute viral hepatitis [1]. Hepatitis E is caused by the hepatitis E virus (HEV), a member of the genus Orthohepevirus within the Hepeviridae family, a genus of single-stranded positive-sense RNA virus [2,3]. HEV is a zoonotic disease and is capable of infecting a wide variety of animal species including pigs (swine HEV), chickens (avian HEV), rabbits, rodents, sheep, camel, and cattle [4-6]. HEV is classified into four genotypes, these four genotypes infect humans, and however, different animal species are considered the main reservoir for genotypes III and IV [4, 6]. The first epidemic outbreak of HEV was back in 1955

when 29,000 cases of icteric hepatitis occurred in Delhi due to poor sanitation and sewage contamination of the city's drinking water supply [7,8]. According to the World Health Organization (WHO) data, every year there are approximately 3 million symptomatic cases of hepatitis E and 44,000 hepatitis E-related deaths in the world [9,10]. The clinical manifestation of HEV infection is variable from an acute ending to an asymptomatic course within the host with a fast-self-limiting disease or might develop into acute, icteric hepatitis or might have extra-hepatic manifestations and cause chronic hepatitis [9].

HEV infection is severe in patients with pre-existing liver disease, pregnant women, and immunosuppressed people, especially in recipient organ transplants in developing countries [11]. Thus, numerous epidemiological data across the world have shown a high incidence of HEV infection in pregnant women which has been reported to be associated with maternal morbidity, fulminant hepatitis, and acute liver failure [4]. Vertical transmission has been documented often leading to a high risk of some obstetric complications like premature rupture of membranes, preterm labour, intrauterine growth restriction, neonatal death, and stillbirth [4,9]. HEV is transmitted primarily by the fecal-oral route, and fecal-contaminated drinking water is the foremost frequent reason behind the transmission, especially in developing countries where poor sanitation water sources supply [12,13]. However, alternative fewer common routes are direct person-to-person transmission, blood transfusion, and organ transplantation [9, 12]. The incubation period between infection and clinical signs is variable, usually between 2 and 8 weeks with an average of 40 days [14]. The IgM anti-HEV response is speedy, occurring about a month after infection and peaking at the time of onset of biochemical abnormalities and/or symptoms [13]. Anti-HEV IgG increased shortly after detection of IgM but anti-HEV IgG peaks many weeks later and can be detected several months and years the initial after infection [15]. The most reliable serological test is the Enzyme immunoassay (EIA), which is characterized by highly sensitive and specific diagnostic tools for the detection of anti-HEV antibodies [14, 15].

HEV is highly prevalent in most African and Asian continents, and Latin America, where large epidemics and sporadic outbreaks are frequently reported [8]. Conversely, HEV is considered an emerging zoonotic disease in some developed countries, and the prevalence of anti-HEV antibodies within the general population is lower in Europe and the United States than in Asia and Africa [16]. HEV is highly endemic in many African countries with a high seroprevalence rate among pregnant women [8]. However, there continues to be a paucity of information about the seroprevalence of HEV among pregnant women in Libya, and the epidemiological situation is also underestimated. Therefore, the present study was conducted to assess the seroprevalence of HEV infection in asymptomatic pregnant women and the potential risk factors associated with increased risk of exposure to infection.

METHODS

Study design and setting

A total of 180 study participants were enrolled from Reference Medical Laboratory, Tripoli. They were asked to participate in the study. Following their consent, each was interviewed individually and the blood sample was taken. Five ml of blood sample was collected in a white tube and sera were separated by centrifugation at 4000 rpm for 20 min and kept at 20^{o}C until used.

Ethical Consideration

Ethical approval was obtained from the scientific council of the Microbiology Department of the Libyan Academy and informed consent was obtained from each pregnant woman after describing the objectives of the study.

Questionnaire Survey

Pregnant women who consented to participate were subjected to a face-to-face interview, and then the questionnaire was filled in to obtain information on relevant and the time sampling was completed for each participant. A questionnaire composed of three parts, part I: socio-demographic characteristics (age, place of residence, occupation, monthly income, family members, traveling out of Libya, education levels,); Part II: healthy status (stage of gestation, history of abortion, Blood transfusion); part III: variables based on household characteristics (source of drinking water, method of washing vegetable/ fruit, ownership animals, type of meat consumption, eating in restaurant).

Laboratory Diagnosis

Serum samples were screened by Enzyme Immunosorbent Assay against anti-HEV IgG using commercially available ELISA kits (DIA.PRO HEV IgG ELISA, Italy).

Statistical Analysis

Statistical significance associations between dependent and independent variables were evaluated using the Chi-square test. P-value < 0.05 was considered as indicator of statistical significance.

RESULTS

Characteristics of the Study Population

The study population was 34 (18.9%) aged between 15- 24 years, 86 (47.8%) aged between 25-34 years, and 60 (33.3%) aged between 35-45 years. The mean age ± SD of participants was 27.93±5.7 years, and the majority of pregnant women were in the age groups of 25-34. The highest seroprevalence rate of HEV was estimated to be (23.33%; 95% CI= 12.63%-34.04%) among pregnant women (35-45 years of age). The result reported statistical significance with age groups (P=.0001) (Figure-1 & Table 1). The study reported no statistical significance between educational level and HEV (P= .266), Anti-HEV reactivity among pregnant women in primary school 5 (20%), was higher than that of their counterparts in secondary 49 (4.1%), and high schools 120 (12.5%).

Table 1. Univariate analysis of risk factors associated with anti-HEV IgG seropositivity

Risk Factors (Variables)	Samples Tested	Seropositive (%)	DF	X^2	p-value
Age group					
≤ 24 years	34	0%	2	18.363	.0001
25-34 years	86	4.65%			
35-45 years	60	23.33%			
Education level					
Primary school	5	20%	3	3.963	.266
Secondary school	49	4.1%			
High school	120	12.5%			
University	6	0%			
Occupation level					
House wife	117	4.3%	5	19.081	.002
Student	5	0%			
Employee	28	25%			
Teacher	8	37.5%			
Medical staff	21	14.2%			
lawyer	1	0			
Monthly income					
<500 LD	4	0%	4	9.877	.043
500-1000 LD	96	9.37%			
1000-2000 LD	64	7.81%			
>2000 LD	11	36.41%			
Refuse	5	0%			
Travel abroad					
yes	71	8.45%	1	.313	.576
no	109	11.01%			
Total	180	10%			

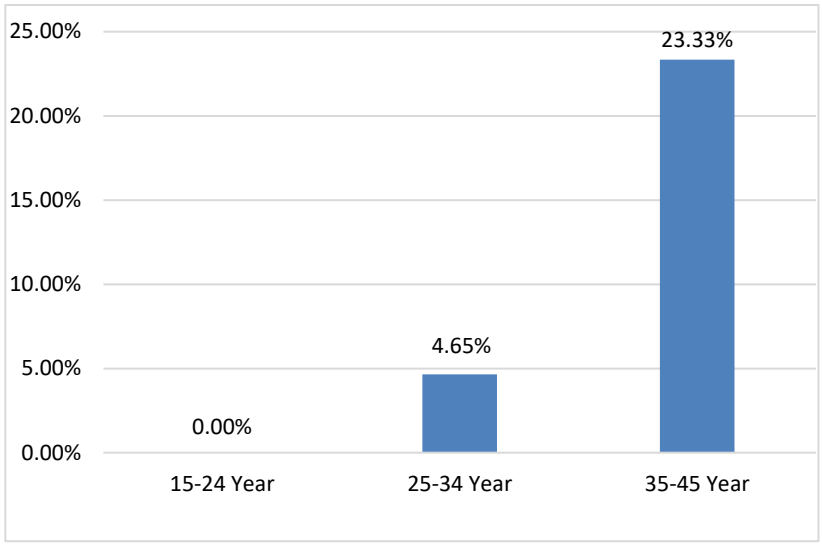

Figure 1. Seroprevalence rate of HEV IgG among age groups of pregnant women

The study revealed that there was a statistical association between occupational level and HEV seroprevalence (P=.02). The highest rate of HEV was 8 (37.5%) in teachers, followed by 28 (25%) in the employee, 21 (14.2%) in medical staff and only 117 (2.77%) in housewives, while (0%) in both lawyers and students. Regarding the monthly income, the highest seroprevalence of HEV was estimated to be 11 (36.41%) in the high-income group, while, relatively low 64 (7.81%) and 96 (9.37%) in the medium and low income respectively (Figure 2).

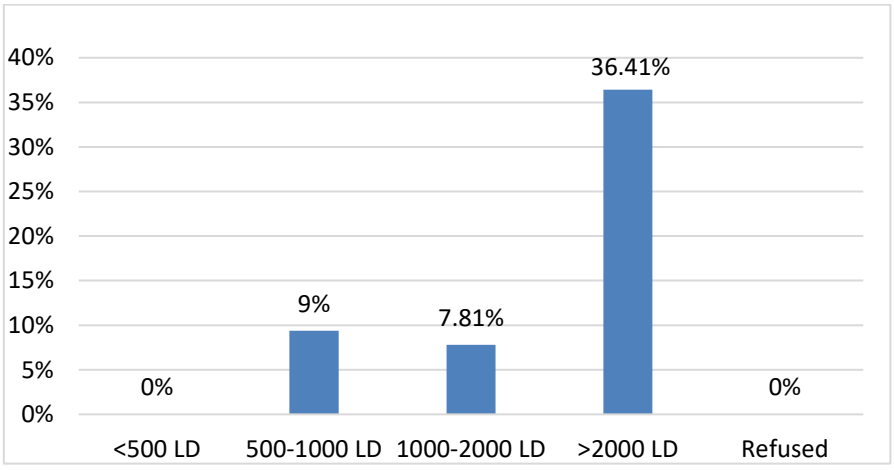

Figure 2. Seroprevalence of HEV according to monthly income

A significant statistical association exists between monthly income and HEV seroprevalence (p =.043). The number of positive cases varied in family member groups, the highest rate was estimated to be 66 (16.66%) in 5-6 family members and were 39 (2.56%) in 1-2 family members, 56 (5.35%) in 3-4 family members, and 19 (15.78%) in >7 family members, there was no statistical association between the family member and HEV seroprevalence (p=.054). Also, the seroprevalence was high 109 (11.01%) in pregnant women who did not have a history of traveling abroad (out of Libya), while 71 (8.45%) in pregnant women who had a history of traveling abroad. Hence, there was no statistically significant association between HEV seroprevalence and traveling out of Libya.

The result reported variability in positive cases in different periods of pregnancy. The highest seroprevalence was reported in the third trimester of pregnancy 143 (11.18%), followed by women in their first and second trimesters of pregnancy 16 (6.25%) and 21 (4.76%) (Figure 3).

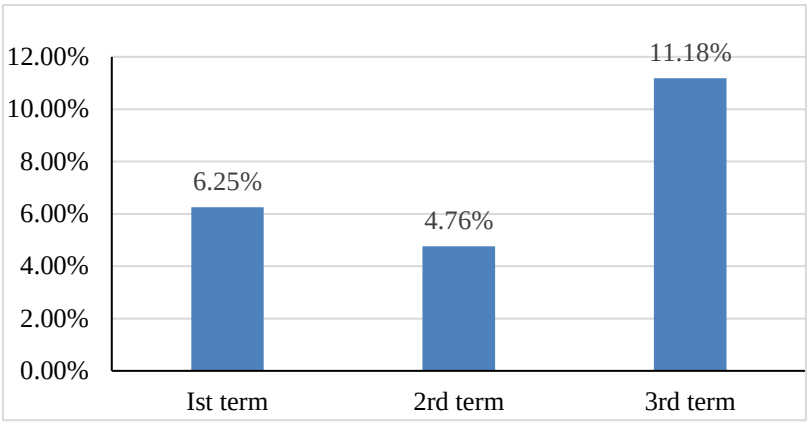

Figure 3. Seroprevalence of HEV according to period of pregnancy

The highest seroprevalence was 120 (10%) in pregnant women who did not have a history of abortion. None of the positive HEV had a history of blood transfusion. However, anti-HEV reactivity was not significantly associated with health status (the pregnancy duration, history of abortion, and blood transfusion) (Table 2).

Table 2. The Univariant analysis of HEV seroprevalence based on health status.

Risk Factors (Variables)	Samples Tested	Seropositive (%)	DF	X^2	p-value
Pregnancy duration					
Frist trimester	16	6.25%	2	1.231	.573
Second trimester	21	4.76%			
Third trimester	143	11.18%			
Abortion					
Yes	60	10%	1	.000	1.000
No	120	10%			
Blood transfusion					
Yes	6	0%	1	.690	.406
No	174	10.34%			
Total	180	10%			

The seroprevalence of HEV according to the household characteristics of the study population, the highest seroprevalence of pregnant women for HEV was recorded among those that used desalinated water 122 (10.65%) as the source of water intake, followed by Wells 11 (9.1%). Seroprevalence of HEV in those who did not have animals at home was higher 146 (10.27%) than 34 (8.82%) in those who had animals at their homes. Also, the highest seroprevalence was 44 (11.36%) in pregnant women who consume red meat, followed by 60 (10%) in pregnant women who consume white meat and 75 (9.33%) in pregnant women who consume red & white meat, while (0%) was reported in vegetarian pregnant women. As well as the positive participant who ate in restaurants had a low seroprevalence of HEV 151 (9.93%) as compared to 29 (10.34%) seroprevalence of HEV in pregnant women who do not eat outside the home.

DISCUSSION

HEV is considered one of the public health important diseases and is well-documented to cause significant risk overall the pregnancy terms vary from self-limiting to life-threatening for pregnant women and their offspring [17, 18]. It is well-known that HEV is endemic in most North African Countries [18]. Libya is one of the North African Countries, and the Country is bordered by the Mediterranean Sea to the North, on the West by Tunisia and Algeria, and to the South, by Niger and Chad, Sudan to the Southeast, and Egypt to the East. In Libya, data are scarce about the epidemiological situation of HEV among pregnant women. To the best of our knowledge, this is considered the first study in Libya to investigate the seroprevalence of HEV among pregnant women in Tripoli, Libya. Viral hepatitis in Libya is well documented and reportable, and various reports have described an epidemic outbreak of different viral hepatitis [19, 20]. Nonetheless, little is known about the seroprevalence and epidemiological patterns of HEV in the Libyan general population, and the seroprevalence rate seems to be underestimated because of the lack of serological

testing. In 1975, Christie and colleagues reported an epidemic outbreak of non-A, non-B hepatitis with a high case fatality rate (CFR) among pregnant women in Libya, suggesting that the epidemic might be attributed to HEV infection [21]. The present study reported a relatively high seroprevalence of HEV among pregnant women. Comparatively, the seroprevalence of HEV reported among the pregnant women in this study was lower than that reported in neighboring countries, in Sudan (61.2%, 41.1%) and Egypt (84.3%) [22-24], Ethiopia (31.6%) [25], Ghana (28.66%) [26], the United Arab Emirates (20.0%) [27]. Also, lower than that reported in the recent study in Sudan (40%) [28].

In line with several studies reported seroprevalence of HEV infection among pregnant women, in Tunisia (12.1%), Burkina Faso (11.6%) Gabon (14.2%) [6, 29-31]. In contrast to other studies, the seroprevalence of HEV was higher than that reported in the following countries, Morocco (3.96%) [32], Lebanon (0.22%) [33], Spain (3.6%) [34], France (7.74%) [35]. There is a shortage of data about the seroprevalence of HEV among pregnant women in most African Countries, as well as infection rate varies, and is inconsistent, consequently, the real situation is unpredictable and very critical to be estimated especially in the resource-limited Countries. Therefore, this variability in the seroprevalence of HEV among pregnant women on the regional level and even within the same geographic area is attributed to many factors that might be influenced by the infection rate of HEV among pregnant women [31]. The reason for this variability might be due to differences in hygienic measurement, health care facilities, sanitation standards, risk factors, and vaccination policy. Also, the specificity and sensitivity of HEV detection diagnostic laboratory techniques are considered potential factors that influence seroprevalence rates reported from different countries [31].

Regarding the age groups, HEV seroprevalence is significantly influenced by age groups (p=.0001), HEV was more prevalent in women in the old age group (35-45 years) as compared to young age women, and that was constant in another study [24]. Therefore, in this study, pregnant women in this age group showed a significant association with HEV seroprevalence due to cumulative exposure to the virus infection over a long period. And this might be attributed also to a decrease in the immunity status with the increased age of pregnant women. In contrast, other studies reported higher HEV seropositivity in young women [36].

Regarding the education level, our results reported variable differences in HEV seroprevalence among pregnant women, where, higher seroprevalence in lower-level educated pregnant women as compared to higher-level educated, however, our results reported no significant difference (P=.266) among different education levels. In agreement with other studies that reported a higher seroprevalence among uneducated pregnant women [36, 37].

In spite, of the frequency of the highly educated group being higher than other groups, however, the results reported a high frequency of exposure to HEV infection in the lower-educated pregnant women, therefore, this difference might be attributable to more exposure for lower-educated women to the infection with HEV than the higher level of educated women. The occupation level significantly influences HEV infection among pregnant women, the seroprevalence was higher (37.5%) in teachers as compared to other occupational levels, and however, the result reported no statistical significance.

In the present study, the seroprevalence rate of HEV among pregnant women was potentially influenced by the monthly income factor. Comparatively, High-income pregnant women showed a higher seroprevalence of HEV among pregnant women, however, HEV seroprevalence is significantly influenced by the economic status of pregnant women, therefore, the high-income groups pose more risk of exposure to HEV infection as compared to other groups [38]. The present study results showed that pregnant women with a history of traveling abroad were less likely to be at risk of exposure to HEV infection. Pregnant women who had traveled to the endemic area showed a higher seroprevalence rate of HEV as compared to had not traveled. Consequently, travel abroad to those endemic areas is considered one of the potential risk factors that emit and increase the possibility of exposure to infection [38, 39].

HEV seroprevalence was higher in the third trimester than in the first and second trimesters. Despite this, there was no statistically significant difference reported in the seroprevalence of HEV over the pregnancy trimester (p=.602), however, the women in the third trimester showed high seroprevalence of HEV. In agreement with other studies were is a high seroprevalence of HEV in the third trimester of pregnancy [22, 23, 26]. And in contrast, other studies showed variable results of HEV seroprevalence during the gestation period [25]. Frequently, many epidemiological studies showed that HEV seropositivity tends to be higher in the third trimester of pregnancy [23, 25].

Regarding the source of drinking water, the highest seroprevalence of HEV in pregnant women was reported among those who used desalinated water, and to less extent in those who irregularly drank bottled water. The drinking water source is considered an important route of HEV infection, especially in endemic regions where contamination of water sources frequently occurs [28]. However, the present study reported a high seroprevalence of HEV among pregnant women who were drinking desalinated water as compared to those who were drinking from other water sources. In contrast, other studies reported high seroprevalence of HEV among pregnant women who were drinking from wells, and that might be frequently associated with fecal contamination of the water supplement system, especially in developing countries [28]. The results reported no statistical association (p=.906) between HEV infection and the source

of drinking water. Our study showed a relatively higher seroprevalence of HEV among pregnant women who kept animals at their homes and frequently came in contact with animals. Inconsistency, another study reported high seroprevalence of HEV associated with individuals who kept animals at their homes [40]. Also, the highest seroprevalence was reported in pregnant women who consume red and white meat. As well as the positive participant who does not eat in restaurants had a high seroprevalence of HEV. However, there was no statistically significant association between HEV infection and household characteristics (source of water intake, method of washing vegetables and fruits, washing hands before eating, ownership of animals, degree of cooking, type of meat consumption, drinking raw milk, and eating outside). Conversely, the results of the present highlighted a potential risk of HEV infection among pregnant women in the country and an increased risk of potential exposure to HEV infection. However, the real epidemiological situation of HEV among the general population as well as pregnant women in the country is difficult to estimate, still, the present study results are valuable and impressive on the potential risk of HEV for pregnant women, especially for those high-risk groups.

CONCLUSION

This study highlighted a relatively higher seroprevalence of HEV among pregnant women. Subsequently, our results suggest an urgent need for education of pregnant women to raise their awareness and attention for good hygiene to reduce the chances of infection, and more extensive studies should be conducted to provide more reliable data about HEV seroprevalence, and also to determine the molecular epidemiological patterns and phylogenetic analysis should be considered.

Authors' Contributions

ASM: Conceived the design, review and drafted the manuscript. AA and KAA: performed Lab work, AA, LK ad AN: Performed the literature search, provided critical comments, and feedback, and helped in drafting the manuscript. All authors read and approved the final manuscript.

Conflict of Interest

There are no financial, personal, or professional conflicts of interest to declare.

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الانتشار المصلي وعوامل الخطر المحتملة لعدوى فيروس التهاب الكبد E بين النساء الحوامل في طرابلس، ليبيا

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المستخلص

الخلفية والأهداف. يعتبر فيروس التهاب الكبد E (HEV) أحد الأمراض الحيوانية المنشأ المهمة للصحة العامة وهو موثق جيدًا لأنه يسبب مخاطر كبيرة بشكل عام، وتختلف فترات الحمل من الحد الذاتي إلى تهديد الحياة للنساء الحوامل وذرياتهن. أجريت هذه الدراسة لاستقصاء الانتشار المصلي وعوامل الخطر المحتملة المرتبطة بعدوى فيروس التهاب الكبد الوبائي بين النساء الحوامل في طرابلس، ليبيا. **طُرق الدراسة.** تم جمع ما مجموعه 180 عينة مصل من النساء الحوامل في الفترة من مارس إلى أكتوبر 2022 في المختبر الطبي المرجعي في طرابلس، ليبيا. تم فحص عينات المصل بواسطة مقايسة الامتصاص المناعي الإنزيمي ضد IgG المضاد لـ HEV باستخدام مجموعات ELISA المتوفرة تجاريًا (DIA.PRO HEV IgG ELISA)، إيطاليا .(تم تقييم الارتباطات ذات الأهمية الإحصائية بين المتغيرات التابعة والمستقلة باستخدام اختبار مربع كاي. واعتبرت القيمة P <0.05 مؤشرا ذا أهمية إحصائية. **النتائج.** تم تقدير معدل الانتشار المصلي الإجمالي بنسبة 10% (180/18)؛ .(CI 95% = 5.62%-14.38% كان أعلى معدل انتشار مصلي لفيروس التهاب الكبد الوبائي 60 (23.33%؛ فاصل الثقة 95%= 12.63%-34.04%) بين النساء الحوامل بعمر 35-45 سنة، وكشفت النتائج عن وجود دلالة إحصائية بين الفئات العمرية .(P=.0001) أبلغت النساء في الثلث الثالث من الحمل عن أعلى معدل انتشار مصلي لفيروس التهاب الكبد الوبائي (11.18%؛ فاصل الثقة 95% = 6.02%-16.36%). **الخاتمة.** سلطت النتائج الحالية الضوء على الخطر المحتمل للإصابة بفيروس التهاب الكبد الوبائي بين النساء الحوامل في البلاد وزيادة خطر التعرض المحتمل للإصابة بفيروس التهاب الكبد الوبائي. وينبغي إجراء المزيد من الدراسات الوطنية لتقييم الانتشار المصلي وتحديد الأنماط الوبائية الجزيئية، وينبغي النظر في التحليل التطوري.

الكلمات الدالة. فيروس التهاب الكبد الوبائي، الانتشار المصلي، عامل الخطر، ليبيا.