

Original Article

The role of detection of anti-HBc IgM in HBs Ag negative blood donors

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ABSTRACT

Background: Post Transfusion hepatitis B viral infection is a major problem even after adoption of mandatory screening test, HBsAg by ELISA test in blood banks. In Egypt, HBsAg is the only HBV screening test of blood donors in most bloods banks. However HBsAg negative blood donors does not rule out the risk of transmission of hepatitis B, as the donor may be in the 'window period' or has a mutant strain. During this period, detection of the antibody to the hepatitis B core antigen (anti-HBc) IgM type is a useful serological marker.

Objective: this study aimed to evaluate the significance of screening anti-HBc IgM for HBsAg negative blood donors to reduce the risk of transfusion transmitted HBV infection in Egypt.

Methodology: Four hundred HBsAg negative blood donors were randomly selected from Al-Zahraa University hospital blood bank, for further screening by anti-HBcIgM by ELISA test, then positive samples for anti-HBcIgM were tested for HBV DNA by PCR.

Results: Nine (2.25%) out of selected 400 samples were positive for anti-HBcIgM, and 4 (1%) out of these 9 samples

Conclusion: Anti HBcAg IgM screening test should be implemented as an additional screening test for blood donors in Egypt, to improve transfusion safety as it is an indicator of occult HBV during window period.

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INTRODUCTION

Hepatitis B is a life-threatening liver infection caused by the hepatitis B virus (HBV). It is one of the major global health problems especially in highly endemic areas [1]. It occurs worldwide, up to two billion, approximately 30% of the world's populations, have serological evidence of past or present HBV infection [2]. Despite the availability of a highly efficient vaccine and potent antiviral agents, the burden of the disease is increasing as almost 45% of the global population live in developing regions with high prevalence (>8%) of chronic HBV infection, where vaccination of large populations has not been possible due to economic reasons [3]. HBV infection can induce a wide spectrum of clinical features, ranging from an inactive carrier state, acute to fulminate hepatitis, or chronic infection to cirrhosis or hepatocellular carcinoma (HCC) [2].

Chronically infected people represent about 257 million and about 5 % of these are at risk of developing the sequel of chronic HBV infection [4]. HBV infection is one of the major risk factors for the development of HCC in the world. Most of the burden of the disease (85%) is observed in highly endemic regions. HCC is the sixth most common cancer in the world and the second leading cause of cancer death [5]. Death resulting from HBV, stands for about 1.34 million deaths annually. Most of these due to liver cirrhosis, liver failure and HCC [6]. As blood transfusion is an important route of HBV transmission; it is recommended that all donated blood should be screened for HBV before transfusion to prevent post-transfusion hepatitis. Screening of donated blood is by hepatitis B surface antigen (HBsAg), which was introduced in the 1970s and this greatly reduced post- transfusion hepatitis [7]. However, negative HBsAg of apparently healthy donor's dose not exclude HBV

infection and HBV is still the highest risk among transfusion-transmitted diseases^[8].

There are various clinical conditions at which HBsAg is negative, although there is HBV infection. These conditions include the window period, low viral level after recovery and escape mutants. The presence of HBV-DNA in blood or liver tissues in patients negative for HBsAg with or without HBV antibodies is known as Occult HBV infection (OBI). It represents a carrier state of the disease and a definite hazard of transmission of HBV to recipients^[9]. Finding a marker that can detect HBV infection in HBsAg negative cases is very important to be implemented in blood banks to diagnose these cases. It was found that antibody to the hepatitis B core antigen is more effective for diagnosing HBV in these cases^[10]. Hepatitis B core antigen consists of two classes, IgM and IgG. IgM (anti-HBcIgM) is the first antibody to appear and indicates a recent infection, while the IgG class appears later during the infection and indicates past HBV infection or recovery. Individuals with anti-HBc IgG may not be infectious as they may have high titers of antibodies to HBs Ag, which are protective in nature. So the IgM class of the anti-HBc is more effective marker for HBV infection in HBs Ag -ve cases^[11]. PCR technique is important in diagnosis of these conditions, however if HBV DNA testing is not feasible, detection of anti-HBc, mainly IgM class, is a useful serological marker for HBV infection in these cases^[12].

SUBJECT AND MEDODS

I. Study population

The present study was carried out on four hundred voluntary blood donors, 352 males (88 %) and 48 were females (12 %) with average age from 20 to 50 years old. Samples are collected from the blood bank of Al-Zahra University Hospital from November 2017 to May 2018. The study was held in the microbiology department of Faculty of Medicine (for girls), Al-Azhar University (Cairo, Egypt) and immunity section of clinical pathology department of Al Zahra University Hospital. Verbal informed consent was obtained from all donors. The approval from the Research Ethics Committee of the faculty of medicine, Al-Azhar University was also obtained.

The following was done of all subjects in the study:

1. The blood donors were selected after they fulfilled the mandatory criteria for donation eligibility as guidelines for blood banks (age, sex, and nationality) are recorded.
2. All samples were negative for all screening tests that done in blood banks (HBV, HCV, HIV and malaria).

Inclusion criteria:

Age above 18 years old, both sexes and their blood thought to be safe for transfusion after screening (negative for HbsAg, negative HCV antibodies, negative HIV and malaria).

Exclusion criteria

Age below 18 years old, positive blood samples for HBV, HCV, HIV or malaria.

II. Samples:

1. At the time of blood donation, 5 ml of venous blood was drawn aseptically by venipuncture and collected in a clean sterile glass tube for screening for transfusion-transmitted diseases (HBV, HCV, HIV and malaria).
2. Samples were clearly identified with codes or names in order to avoid misinterpretation of results.
3. All the blood samples were subjected to the mandatory screening tests for detection of transfusion transmitted diseases (HBV, HCV, HIV and malaria) by the ELISA tests for anti-HIV 1 and anti-HCV, HBsAg and malaria.
4. Then negative plasma samples are divided to 3 aliquots and stored at -20°C for our study.
5. Screening for anti-HBcAg IgM by ELISA test for all 400 collected samples.
6. Then positive samples for anti-HBcAg IgM are confirmed by PCR for HBV DNA detection.

III. Methods of study:

(1) ELISA test for detection of anti HBc IgM:

Detection of anti HBV core IgM quantitatively was performed by using the anti- HBc IgM kit manufactured by DIA.PRO Diagnostic Bioprobes Srl Via Columella n° 31, 20128 Milano - Italy.

(2) Detection of HBV DNA by PCR technique for positive anti HBc IgM ELISA test:

DNA extraction protocol:

This protocol is for purification of viral nucleic acids from 200 µl of plasma using the QIAamp MinElute Virus Spin Kit and a microcentrifuge (QIAGEN®, QIAamp®, QIAcube®, BioRobot®, EZ1TM MinElute® (QIAGEN Group); Corex® (Corning, Inc.); Eppendorf® (Eppendorf- Netheler-Hinz GmbH). It was done by using universal primer pairs P1 (sense) ...5'-TCA CCA TAT TCT TGG GAA CAA GA-3' (2823–2845 nt) 1063bp , and S1-2(antisense).....5'CGA ACC ACT GAA CAA ATG GC-3' (704-685 nt). The reaction mixture contains 5 ul of extracted DNA, 25 ul of master mix, 5 Pmol of each primer completed to 50ul with distilled water. The thermal cycler was programmed at 95°C for 10 minutes, followed by 40 cycles at 92°C for 20 sec (denaturation), 56 °C for 20 sec (annealing), and 72°C for 1 min (extension) then at 72°C for 10 minutes. HBV genomic DNA and a negative sample were used as positive and negative controls, respectively from our microbiology lab. For the analysis of the PCR amplification, 10 µL of the amplified DNA were run on 3% agarose gel after addition of 4 µL of loading buffer .The presence of a 1063-bp fragment indicated a positive result. In parallel with samples, a 100-bp DNA ladder

was also run on the gels to estimate the molecular weight of DNA fragments in the gel.

Statistical Analysis

Data were collected, revised, coded and entered to the Statistical Package for Social Science (IBM SPSS) version 22. Qualitative variables were presented as number and percentages. The comparison between groups with qualitative data was done by using Chi-square test. The confidence interval was set to 95% and the margin of error accepted was set to 5%. So, the p-value was considered significant as the following: P > 0.05 was considered non-significant and P < 0.05 was considered Significant.

RESULTS

This study was conducted on 400 donated plasma samples of healthy blood donors and they were negative for all blood screening tests in blood bank (HBV, HCV, HIV and malaria). All donors showed normal blood pressure, normal liver function tests, and no history of jaundice or any liver disease which were done by blood bank. Baseline characteristics of the studied groups presented in tables (1 and 2).

The demographic data revealed that the patient ages ranged from 20-50 years. Three hundred and fifty two 352 (88%) were males and 48 (12%) were females, with male to female ratio was 7.3:1 respectively. Concerning age range and ratio between male and female according to this range, Donors of ages between 30-40 years

constituted the largest proportion 172 (43%). 144 (37.25%) were males and 23 (5.25%) were females. While age ranged from 20-30 was 123 (30.75%), 114 (28.5%) were males and 9 (2.5%) were females and age from 40-50 were 105 (26.25%), 89 (22.25%) were males and 16 (4%) were females. The majority of blood donors 312(78%) were from urban area, while the remaining 88(12%) were from rural area.

From a total selected 400 plasma samples of blood donors negative for HBsAg, 9/400 (2.25%) were reactive for anti- HBcIgM, 7 (1.75) males versus 2(5%) females as shown in table (3). Concerning comparison between HBc IgM positive and negative samples from our selected group, 9/400 was positive, and 391/400 were negative. Seven males were positive and 344 were negative, while 2 female were positive and 46 were negative as shown in table (4).

These 9 reactive samples for anti HBcIgM, 6 of them show concentration more than 10 u/ml which indicate positive reaction while 3 was in the gray zone with a conc. between 5-10u/ml, PCR test for HBV(fig.1) was done for 9 anti HBcIgM positive samples for 9 samples were selected randomly from negative anti HBcIgM samples with the same age and sex of positive samples as control group. It was observed that four out of the 9 positive for anti-HBcIgM were positive for HBV DNA by PCR, representing a percentage of 1% while those in the grey zone was negative (table 5).

Table (1): the demographic data of blood donors

Age groups	Total		Male		Female	
	No.	%	No.	%	No.	%
20-30	123	30.75%	114	28.5%	9	2.5%
30-40	172	43%	149	37.25%	23	5.25%
40-50	105	26.25%	89	22.25%	16	4%
Total	400	100%	352	88%	48	12%

Table (2): The Distribution of blood donors according to residence

	No.	%
Urban	312	78%
Rural	88	22%

Table (3): Distribution of positive sero reactive samples for anti HBcIgM among studied group

	IgM	%
Total	9/400	2.25%
Male	7/400	1.75%
Female	2/400	0.5%

Table (4): Comparison between anti HBcIgM positive and anti HBc IgM negative blood donors by sex distribution

	Anti HBc IgM Positive IgM (no.=9)		Anti HbcIgM Negative IgM (no.=391)		Chi square test	
	No.	%	No.	%	X ²	P value
Male	7/9	77.8%	345/391	88%	0.852	0.356
Female	2/9	22.2%	46/391	12%		

Table (5): Results of HBV-DNA by PCR in relation to results of anti-HBC IgM

ELIZA positive samples	HBV-DNA by PCR		Total
	Positive	Negative	
High concentration (More than 10 u/ml)	4/6 (66.7%)	2/6 (33.3%)	6/9 (66.7%)
Gray zone (5-10u/ml)	0.0	3/3 (100%)	3/9 (33.3%)
Total	4/9 (44.4%)	5/9 (55.6%)	9/9 (100%)

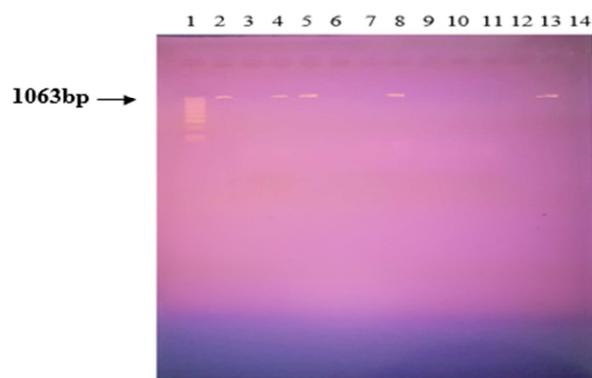


Fig. (1): HBV-DNA PCR An ethidium bromide stained agarose gel showing results of HBV-DNA PCR screening of collected sample which were HBsAg negative and anti-IgMHBc positive.

Lane 1: DNA ladder marker.

Lane 2, 4, 5, and 8 positive samples showing the expected 1063-bp product.

Lane 13: positive control at 1063bp.

Lane 14: negative control.

DISCUSSION

In blood banks of many countries, HBsAg test is the only screening test to indicate HBV infection in donated blood. However, it does not rule out the risk of HBV transmission totally, because during the host serological response there is a phase during which the HBsAg cannot be detected although HBV infection is present. This phase is called the ‘window period’ which represents a carrier state of the disease. In addition, HBsAg is not detected in case of mutant strain therefore, transfusion of blood

collected from donors in these conditions may lead to post transfusion HBV infection in the recipient^[13].

As post transfusion HBV infection is a major problem especially in developing countries, finding a marker which would be indicative of HBV infection in HBs Ag negative cases is very important in blood

banks. It is shown that HBc Ab and nucleic acid tests are more effective in these cases. Since nucleic acid tests are expensive, anti- HBc antibodies has been found to be more suitable indicator in these conditions^[14].

The IgM class of the anti-HBc is the first to appear even late in incubation period and indicates a recent infection. So anti-HBc IgM is an excellent marker for HBV infection in HBsAg negative blood donors. While the IgG class of anti-HBc appears later and indicates a past infection. Individuals with anti-HBcIgG may not be infectious and their blood is suitable for transfusion as they may have sufficiently high titres of anti- HBs which are protective in nature^[10].

The prevalence of total anti-HBc in blood donors is proportional to the incidence of HBsAg in the general

population. It is low in the Western countries as these countries have low incidence of HBsAg so, screening of donated blood in these countries by total anti-HBc is practical and they can discard such positive blood units without wastage^[15].

Several studies reported that detection of total anti-HBc had contributed significantly in reduction of the incidence of post transfusion HBV infection amongst donors^[16]. On the other hand, blood banks in medium or high- endemic areas cannot depend on total anti HBc as a screening test for donated blood, as these countries have high prevalence of total anti-HBc. The reactive blood units for total anti HBc in these areas may not be infectious and these blood units are suitable for transfusion. Anti-HBc IgM seems to be more practical in these countries to see the infectivity status of HBs Ag negative blood donors^[17].

In our study we aimed to evaluate the role of anti-HBc IgM in blood donors negative for HBsAg in order to increase safety of donated blood. In this study, 400 blood donors negative for all routine screening tests in blood banks (HBsAg, HCV antibodies, HIVAbs and Treponema Abs), 352 (88%) were males and 48 (12%) were females with age ranges from 20-50 years. Quantitative analysis of anti-HBc IgM was done by "capture" enzyme immunoassay using DIAPRO Diagnostic Bioprobes kits. Positive samples for anti-HBc IgM were further tested for HBV-DNA by PCR.

This study revealed the following results, 9/400 (2.25%) were reactive for anti-HBc IgM, 7 (1.75) males versus 2 (.5%) females. The quantitative analysis for HBc IgM revealed that 6 donors had antibody levels greater than 10.00 Paul Ehrlich International units per mL (PEI U/mL); these were considered positive. However, 3 donors in the grey area within the 5- to 10-PEI U/mL were considered border line. All these 9 HBc IgM reactive samples were tested for DNA by PCR, 4/9 (1%) were positive. The other 2 +ve samples and all 3 samples which were in gray zone were negative for PCR

There was a close data to our study reported by a previous study in South Egypt Cancer Institute conducted on 180 HBsAg negative blood specimens, were selected randomly for further testing for (anti HBcIgM, anti HBs antibody and HBV DNA testing). 7/180 (3.8%) were positive for anti- HBcIgM., While positive donors for anti-HBs antibody were 34/180 (18.8%). Two specimens (1.1%) out of 7 anti-HBcIgM positive samples were positive for HBV DNA by PCR^[18].

Another study that conducted on 760 Egyptian blood

donors were routinely screened for (HBsAg, HCV-Ab, HIV and Syphilis), accepted blood units (712) for donation were further tested for the presence of anti HBc-IgM and HBV-DNA. These results were (0.13%) HBc-IgM positive unit and 2/30 HBV DNA positive unit. Antibody should be tested routinely on all donated blood units as well as sensitive methods for detection of HBV (e.g. PCR) may be recommended in screening donated blood^[19].

There were two cross-sectional studies in Nigeria supporting the fact that screening blood donors for HBsAg does not rule out the risk of post transfusion HBV infection. The first one was in 2011, 92 blood donors were enrolled for this study screened for 5 different markers of HBV (HBsAg, anti HBsAg, HBeAg, anti HBeAg and anti HbcIgM). HBsAg was detected in 18 (19.6%), anti-HBs in 14 (15.2%), HBeAg and anti-HBe were detected in 4(8.9%) and 12(26.7%) respectively from 45 donors sampled. Anti- HBc IgM was found in 12 (13.0%) cases, 7 of them sharing with other markers, while 5 (5.4%) of the 92 donors had anti-HBcIgM as the only serological evidence of HBV infection, which represents high numbers from all infected cases^[11]. The other study was conducted on 200 HBsAg- negative blood donors, then tested for HBV markers (HBeAg, anti-HBeAg, anti-HBs, total anti-HBc and anti- HBc IgM). only 5 (2.5%) were positive for anti-HBs. Sixty- five participants (32.5%) were positive for total anti-HBc, indicating a past exposure to HBV. Overall, 8 (4.0%) of the donors were found to be positive for anti-HBc IgM alone. Five (2.5%) out of the 200 HBsAg-negative blood donors were positive for anti-HBs (2.5%)^[20].

Another study by *Lavanya* of a total 200 blood donors were screened for the presence of HBsAg. Total anti-HBc, anti-HBc IgM and anti-HBs were done for HBsAg negative cases. The prevalence of HBsAg was 3.5% (7 cases) and HBsAg negative cases were 193, total anti-HBc 10.9% (22 cases), anti-HBc IgM 5.7% (11 cases) and anti-HBs 3% (6 cases). All the 6 anti-HBs positive donors were also found to be positive for total anti HBc indicating past infection, but the result of anti-HBc IgM indicates recent infection^[21]. An Indian study support the above results, 12232 healthy blood donors negative for HBsAg were screened for anti-HBc IgM, this study revealed a percentage of (0.12%) reactive for anti-HBc (IgM). This low percentage in comparison to other studies due to high number included in this study^[22].

According to study in India, a total of 2552 voluntary blood donors were studied for (HBsAg and anti HBcIgM) of which 47 (1.84%) cases were HBsAg positive and 11 blood units were anti-HBcAg IgM

positive, only one of these positive IgM was HBsAg positive and 10 were negative, giving a positivity rate of 0.39% amongst the 10 HBsAg negative and anti-HBcAg IgM reactive blood donors [23]. El-Zayadi and his coworkers study was conducted on 760 Egyptian healthy blood donors were screened according to routine practice for the presence of (HBsAg, HCV antibodies, HIVAbs and Treponema Abs). They reported that 48/760 units (6.3%) were rejected. The accepted blood units for donation were tested for the presence of total anti- HBc Abs. Positive units for total anti-HBc were further tested for HBV-DNA by PCR. Among the accepted blood units (712) for donation, prevalence of total anti-HBc was 78/712 units (10.96%). HBV-DNA was detected in 9/78 (11.54%) of the total anti-HBc-positive units and thus, occult HBV infection was detected in 9/712 (1.26%) of the accepted blood donations [24].

Another cross sectional study reported lower data, a total of 1026 HBsAg-negative Egyptian healthy blood donors were tested for the presence of total anti-HBc . Anti- HBc-positive samples were subjected to PCR to confirm the presence of HBV DNA. They reported that 80/1026 (7.8%) blood samples were found reactive to total anti-HBc and HBV DNA was detected in five (4%) of these samples [25]. Another study in El Fayoum conducted on 800 voluntary blood donors, negative for HBsAg, HCVAb and HIV Ab. They were further screened for total anti HBc antibodies, then anti-HBc-positive samples were tested for anti-HBs and HBV DNA for total anti HBc only. Their result was 99/800 (12.37%) anti-HBc-positive including 78 anti-HBs positive. The remaining 21 donors were anti-HBc alone, 2 of which (9.52%) were HBV DNA-positive [26].

Tamer and his coworkers in Al-Gharbia governorate, Tanta University reported 2 recent studies for high risk group of HBV transmission and reported higher prevalence data than our study. The first one included 90 regular Hemodialysis patients negative for HBsAg and anti-hepatitis C virus. Patients were investigated for anti- HBc and samples of anti-HBc-positive patients were tested for HBV-DNA PCR. The reported results were 17 /90 (18.9%) +ve anti-HBc. Eleven anti-HBc-positive patients were anti-HBs-positive. HBV-DNA was detected in seven of those 17 total anti-HBc-positive patients (7.8% of all patients) [27]. The second study included 79 poly transfused patients negative for HBsAg, HBsAb, and anti HBC Ab. Patients were investigated for total anti-HBc and positive samples of total anti-HBc were tested for HBV-DNA PCR. Among the 79 HBsAg-negative sera, total anti-HBc was detected in 12 of 79 (15.19%) cases. All total anti-HBc-positive sera were anti-HBs-negative. HBV-DNA was detected in five of 12 (6.3% of all patients) [28].

CONCLUSIONS

To reach a completely safe blood transfusion; It is recommended that anti HBc mainly IgM class need to be implemented in Egypt as primary test in blood banks for detection of HBs Ag negative blood donors. Also, HBV DNA need to a confirmatory test for anti HBc IgM positive cases and if they were positive regardless of anti-HBs titer, the blood should be discarded.

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Conflicts of interest

The authors declare that there is no conflict of interest regarding the publication of this paper.

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الملخص العربي

دور الكشف عن الجلوبيولين المناعي م لمستضد فيروس ب اللبي لمتبرعي الدم سالبني المستضد السطحي لفيروس ب

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ملخص البحث:

الخلفية: إن العدوي بفيروس ب بسبب قل الدم يمثل مشكلة كبيرة وذلك حتي بعد تطبيق الفحص الالزامي في كل بنوك الدم لكل متبرعي الدم لكشف عن المستضد السطحي لفيروس ب بطريقة الاليزا وكمن لك المشكلة في الحصول علي نتائج سلبية لهذا المستضد لبعض المتبرعين بالرغم من اصابتهم بالفيروس حيث يعتبر هذا المتبرع حاملا للمر؛ ومن ثم فان هذا التحليل غير كاف للتأكد من عدم إصابة متبرعي الدم بفيروس ب وسمي الحالة التي ظهر فيها نتيجة تحليل مستضد فيروس ب السطحي سلبية مع وجود المادة الوراثية لفيروس ب بالالتهاب الكبدي ب المتخفي حيث قد يكون المتبرع في "فترة النافذة" أو يعاين من سلالة متحولة و خلا هذه الفترة، يعد الكشف عن الجلوبيولين المناعي م لمستضد فيروس ب اللبي للكشف عن الإصابة بفيروس ب لمتبرعي الدم سالبني المستضد السطحي لفيروس ب علامة مصلية مفيدة.

الهدف: تعيين كفاءة الجلوبيولين المناعي م لمستضد فيروس ب اللبي للكشف عن الإصابة بفيروس ب لمتبرعي الدم سالبني المستضد السطحي لفيروس ب وذلك لزيادة سلامة قل الدم.

الطرق: شملت لك الدراسة ٤٠٠ عينة لمتبرعي الدم والتي أظهرت جميع حاليهم التي جري بشكل روتيني في بنوك الدم للكشف عن الأمرا التي تنقل بالدم (فيروس ب؛ فيروس سي؛ فيروس قاص المناعة المكتسبة والملاريا) نتيجة سلبية. بعد ذلك قمنا بإجراء تحليل الكشف عن الجلوبيولين المناعي م لمستضد فيروس ب اللبي لكل العينات عن طريق فاعل الاليزا. ثم بعناه بتحليل فاعل البلمرة المتسلسل للكشف عن الحمض النووي لفيروس ب للعينات الموجبة لتحليل الجلوبيولين المناعي م لمستضد فيروس ب اللبي.

النتائج: أثبتت هذا الدراسة وجود بعض الحالات المصابة بفيروس بي من بين متبرعي الدم والتي أظهرت نتيجة الكشف عن فيروس ب عن طريق المستضد الفيروسي السطحي للفيروس نتيجة سلبية مسبقا. لقدم الحصول علي نتيجة ٩ عينات موجبة للجلوبيولين المناعي م لمستضد فيروس ب اللبي من بين العينات الاربعمائة المشمولة في البحث بنسبة ٢,٢٥%. وبعد إجراء تحليل فاعل البلمرة المتسلسل للكشف عن الحمض النووي لفيروس بم التأكد من وجود ٤ عينات إيجابية لوجود الحمض النووي لفيروس ب.

الاستنتاجات: لذلك؛ فان من التوصيات الناتجة عن هذا البحث هي ادراج تحليل الكشف عن الجلوبيولين المناعي م لمستضد فيروس ب اللبي في بنوك الدم المصرية لاكتشاف الحالات التي لايمكن شخيصها عن طريق المستضد السطحي للفيروس ثم التأكد عن طريق تحليل فاعل البلمرة المتسلسل للكشف عن الحمض النووي لفيروس ب للعينات الموجبة لهذا التحليل.

الكلمات المفتاحية: الجلوبيولين المناعي م، المستضد السطحي لفيروس الكبد ب، الحمض النووي لفيروس الكبد ب، الفترة النافذة.

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