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The impact of Hepatitis B Virus (HBV) infection in Some Hematologic Malignancies

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Abstract: ***Background:*** hepatitis B virus (HBV) is suspected to be an etiological agent, which increases the prevalence of hepatocellular carcinoma (HCC) in young non-cirrhotic cases. Recently, much research deal with the positive occurrence of stomach, liver, intrahepatic bile ducts, nasopharynx cancers and diffuse large B cell lymphoma (DLBCL) with H BV infection.

Aim of the work: to estimate the existence of HBV and to clarify the role of HBV regulatory X Protein in subjects with lymphoma.

Subject and Methods: this is a cross sectional study, which carried out on a cohort of 94 subjects with age range 16-50 years. The included subjects were divided into two groups. Group I (HBV negative subjects group): included 78 HBV negative subjects, which were approved by laboratory investigations. They were 39 females and 39 males; their mean age was 45.7 years with SD ±14.7. Group II (HBV positive subjects group): included 16 subjects with HBV positive. They were 9 females and 7 males; their mean of age was 52.5 years with SD ±10.2. RT-PCR and Elisa were used to detect HBV and HBx, respectively.

***Results*:** high prevalence of HBV was observed in older subjects' age. HBx protein concentration was increased in HBV +ve subjects group compared to HBV –ve subjects group, p value was <0.0001. HBx protein concentration was also increased in lymphoma cases compared to cases with reactive lymphadenopathy (*P*= 0.039). In addition, a significant increase of alanine aminotransferase (ALT), aspartate aminotransferase (AST) and LDH activity were detected in the lymphoma subjects' group compared to non- lymphoma subjects' group, *P* value was 0.001, 0.001, and 0.04, respectively.

Conclusion: HBV positive subjects have aggressive lymphoma. HBV infection could have a role in lymphoma development mainly Non Hodgkin lymphoma. It could be related to the HBx protein expression. Therefore, our study suggests that the early treatment of hepatitis virus infection mainly HBV could have an important role in decreasing malignancies development.

keywords: HBV, HBX protein, lymphoma.

1.Introduction

Hepatitis B virus (HBV) is found to be the main cause of liver disease and liver cancer. There are over 250 million people being infected with HBV and an annual death of about 1.5 million globally [1]. HBV is a small DNA virus with unusual features similar to retroviruses which is a member of the Hepadnaviridae family. It replicates through RNA intermediate and can merge into the host genome [2]. Between 2007-2016, there were 1,258 and 1,259 incident diagnoses of acute and chronic HBV infection, respectively [3]. HBs Ag prevalence was 3•61% worldwide with the highest endemicity in countries of the African region (8•83%) and Western Pacific region (5•26%). Furthermore, within WHO regions, the prevalence ranged from 0•20% in Mexico to 13•55% in the Americas [4].

HBV genome has four overlapping open reading frames (ORFs) that are responsible for encoding seven proteins. The HBV regulatory X protein (HBx) is able to trans-activate the expression of all HBV proteins, including the viral core protein HBc [5]. HBx protein (pX) is an HBV genome code for a 16.5-KD and 154 amino acid in size. It is recommended to be expanded from 10,000 to 50,000 copies per cell[6], [7 .[

HBx had a role in the suppression p53 function, which led to ineffective liver cell division and resulting in hepatocellular carcinoma (HCC)[8]. It plays a major role in HBV replication and associated carcinogenesis[9]. Moreover, HBx controls the level of HBV replication [10],[11]. However, immune-mediated liver damage in the infected subjects may cause cirrhosis and HCC [12]. HBx activates src tyrosine kinase pathway which with p53 mutant stimulates tumorigenesis [13]. Moreover, positive correlation is determined between HBx expressions and ecotropic viral integration site 1 (EVI1) transcription factor which intern induced hepatocarcinogenesis [14.[

Lymphoma is a cancer of the lymph system which included two types Hodgkin lymphoma (HL) and non-Hodgkin lymphoma (NHL). Recently, many researchers clarified the prevalence of hepatitis C virus (HCV), and HBV within several hematologic malignancies, mostly B-cell (NHL) [15]. HBV infection conferred resistance to chemotherapeutics that actuated S-phase arrest by HBx specifically inhibiting the enactment of Checkpoint Kinase 2 (CHK2)/ response signaling in diffuse large B cell lymphoma DLBCL [16 . [

APositive association was determined between HBV infection and cancers of the stomach, liver, intrahepatic bile ducts, nasopharynx and diffuse large B cell lymphoma (DLBCL) as well as a myelodysplastic syndrome [17]. Mostly, HBV not only induced tumorigenesis by enhanced cell growth but also by reduced sensitivity to chemotherapy. HBx protein expressed by DLBCL cells reduced sensitivity to methotrexate (MTX) and cytarabine (Ara-C) that induced S-phase arrest [16 .[

The current study aimed to estimate the existence of Hepatitis B Virus (HBV) and to clarify the role of HBx Protein in subjects with lymphoma.

2. Materials and methods

cross sectional study, which carried out on a cohort of 94 subjects with age range 16-50 years attending at Mansoura University Oncology Center (MUOC)- Egypt. The diagnosis of lymphoma was based on the morphological examination and immunohistochemical analysis. Real-time PCR was utilized to detect B virus. Laboratory investigation and clinical examination were done for all subjects. Approval of the ethical committee and written informed consent from all the subjects were obtained. This study was conducted between May 2017 and October 2018.

Inclusion Criteria

Subjects with lymphoma prior to therapy.

Exclusion Criteria

1- Lymphoma subjects who received medical treatments.

2- Coexistence of other malignancy or infection.

Sampling

To estimate hematological and clinical chemistry tests, 5 ml of venous blood was withdrawn from each subject and divided as follow: 1ml was put in a tube Containing EDTA for CBC; 2ml were put in a plain tube then allowed to centrifuge to separate serum for estimation of liver function, kidney function, LDH, HBx Protein (Elisa). In addition, two ml were put in a heparin tube for virology estimation and quantitative HBV DNA by PCR.

Liver function tests were done according to:

- ALT and AST were estimated by kinetic method (SPINREACT, S.A. /S.A.U. Ctra.Santa Coloma) [18, 19].
- S. Bilirubin and S. Albumin were estimated by the colorimetric method as the colored compound measured photometrically[20] (Diamond diagnostics).

Kidney function tests were done according to:

- S. creatinine was detected by fixed rate method[21](Diamond diagnostics).

- S. Uric acid was detected by the enzymatic colorimetric method[22] (Diamond diagnostics).

Subjects were divided into two groups (by RT-PCR results):

- Group I (HBV negative subjects group)
- Group II (HBV positive subjects group)

Subjects were reclassified according to the presence of lymphoma into two groups:

- ***Group A (Lymphoma subjects' group):*** included 53 subjects with lymphoma. They were 24 females and 29 males; their mean age was 47.68 years with SD ± 15.27.
- ***Group B (subjects with reactive lymphadenopathy):*** included 41 subjects.they were 24 females and 17 males; their mean age was 45.8 years with SD ±12.9.

Detection of HBx Protein (by ELISA method):

HBx protein was measured by Hepatitis B Virus X Interacting Protein (HBXIP/EIAAB SCIENCE INC, WUHAN) ELISA method. The color change was found only in wells that contain HBV X-interacting protein, biotin-conjugated antibody and enzyme-conjugated Avidin. The color change was measured spectro-photometrically at a wavelength of 450 nm ± 2 nm (STATE FAX4700). The concentration of HBV X-interacting protein was determined by comparing the O.D. of the samples against the standard curve [23].

Procedure

1-100 µl of Standard, Blank, or Sample was added per well that was covered with the plate sealer and then incubated about 2 h. at 37℃.

2-The liquid was removed from each well, without a wash. 100 µl of Detection Reagent A working solution was added to each well, covered with the Plate sealer and incubated for 1 hour at 37℃. Detection Reagent A working the solution could appear cloudy. Warmed to R.T and gently mixed until solution appears uniform.

3-The process of aspirating each well and washing was repeated three times for a total of three washes. To wash, each well is filled with Wash Buffer (approximately 400 µl) using a squirt bottle, multi-channel pipette, manifold dispenser or auto washer and let it sit for 1~2 minutes. Complete removal of the liquid at each step was essential to good performance. After the last wash, any remaining Wash Buffer was removed by aspirating or decanting. The plate was inverted and blotted against clean paper towels.

4- 100 µl of Detection Reagent B working solution was added to each well, covered with a new plate sealer and incubated for 1 hour at 37℃.

5- The aspiration/wash process was repeated for 5 times as conducted in step 3. 6. 90 µl of Substrate Solution was added to each well, covered with a new Plate sealer and incubated within 15-30 minutes at 37℃. Avoid light.

6- 50 µl of Stop Solution was added to each well. If color change does not appear uniform, the plate is taped gently to ensure thorough mixing.

7- The optical density of each well was detected at once, by a microplate reader set to 450 nm

Detection Range

0.56 ng/mL -100 ng/mL

Quantitative determination of HBV (Real-Time PCR)

Polymerase Chain Reaction makes a huge number of copies of a gene through two steps: Extraction and Count the number of HBV (Quantitative HBV)[24] (…….).

☒ **Extraction procedure:**

1-The Kit contents were kept at R.T before using.

2-1.5 tubes were prepared and added 200 µl sample + 300 µl lysis buffer + 10 µl RNA/DNA internal control and shacked for 5sec.

3-400 µl Precipitation was added and vortex 5 sec again.

4-The sample was transferred on a spin filter, incubated for 30 sec and centrifuged for 3 min at 12.000 rpm.

5-The filtrate solution was discarded, 500 µl of Wash 1is added, and centrifuged again for 1 min 12.000 rpm

6.-The filtrate solution was discarded again, 300 µl of Wash 2 is added, and is centrifuged for 1 min at12.000 rpm.

7.-The filtrate solution was discarded and centrifuged for 5 min at 13.500 rpm.

8.-The receiver tube was discarded.

9.-The spin filter was transferred into a dissolving tube and 50 µl of dissolving buffer is added and incubated for 3 min.

10-Then centrifugation for 1 min 11.000 rpm.

11.-The dissolving was transferred into a storage tube which was taken the tube for PCR.

☒ **Quantitation of HBV**

HBV DNA Kit is a specific fragment amplifying kit with specific primers in the PCR reaction. Products are identified by the use of specialized Taqman-MGB Probes. The Taqman-MGB Probes is marked the 5'-end with the FAM report dye and the 3'-end with the NFQ (Non-Fluorescent Quencher)-MGB.

Preparation of the master mixtures:

The volumes shown below focused on a single 35 µl standard reaction, recommended preparing a mixture containing PCR buffer, Taq-Polymerase. In 1.5 ml master mix tube firstly, before dividing this mixture above Paraffin sealed PCR HBV mix tube (Ready to Prep) and adding the extracted DNA Sample.

Component	PCR buffer	Taq-Polymerase
Volume	10 µl	0.5 µl

i.-The collected mixture was mixed gently and then 10 µl of the mixture master mix was added above the Paraffin sealed PCR HBV mix.

ii. 5 µl of the corresponding HBV DNA template was added.

iii. To obtain standard control (1-4); 5 µl was added from each to a separate tube.

iv. Each tube was caped and placed in the Real-Time PCR detection instrument.

Statistical analysis:

Under Microsoft Windows 10 platform, data derived from the current study were calculated utilizing SPSS versions 20 and graph pad 5. Continuous data were expressed in the form of mean ± SD while categorical data were expressed in the form of count and percent. A comparison of continuous data was performed by student t-test, while categorical data were done using Chi-square test. P value less than 0.05 was considered statistically significant, p-value < 0.001 was considered highly statistically significant, p-value < 0.0001 was considered extremely statistically significant and p-value ≥ 0.05 was considered not statistically significant.

3. Results and Discussion

In the current work, there was a significant difference between the two groups regarding age. A high prevalence of HBV positive was observed in older subjects' age. Whereas, no statistically significant differences were estimated between the studied groups regarding sex distribution (HBV -ve group: 39 males and 39 females, HBV + ve group: 9 males and 7 females; p=0.65) and other demographic data (Tab. 1). The ratio of subjects infected with HBV is graphically represented by Fig.1, about 82% of subjects were HBV negative but only 16% were found to be positive HBV depending on RT-PCR.

Table (1) Demographic data of the studied subjects' groups regarding RT-PCR results (N=84)

	HBV negative group (N= 78)		**HBV positive group (N= 6)**		**P value**
Age	45.7	14.7	52.5	10.2	0.04*
	N	%	N	%	
Male (N, %)	39	50	9	56.25	0.2
Female (N, %)	39	50	7	43.75	0.3
Lymphoma patients	46	58.9	7	43.75	0.08
Hodgkin lymphoma	10	12.82	2	29	0.28
Non-Hodgkin lymphoma	36	46.15	5	71	0.058
HBX protein positive	7	8.97	6	37.5	0.23
HCV(+ve)	40	51.28	2	12.5	0.09
HCV(-Ve)	38	48.71	14	87.5	0.12

N: the number of the subject in each group.

Table (2) Hematological, and clinical chemistry data based on HBV infection

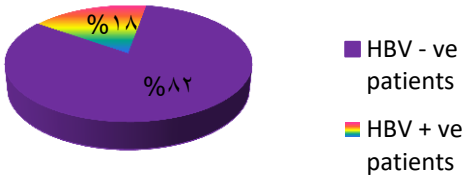
	HBV negative (N= 78)		**HBV positive (N= 16)**		**P value**
	mean	SD	Mean	SD	
Hemoglobin concentration(g/dl)	11.7	1.793	10.5	1.794	0.016*
RBCs(×10^9 /l)	4.31	0.7	4.36	0.63	0.8
WBCs(×10^3 /l)	6.36	2.7	7.08	4.12	0.19
PLT(×10^3 /l)	183.2	101.4	205.7	178	0.2
ALT(U/L)	40.1	22.6	91.02	50.7	0.0001***
AST(U/L)	43.54	22.84	90.9	47.2	0.0001***
S.Bilirubin(mg/dl)	0.98	1.5	1.45	0.64	0.12
S. Albumin(g/dl)	3.93	0.78	3.47	0.61	0.014*
S. Creatinine(mg/dl)	0.87	0.25	1.07	0.38	0.004**
S.Uric acid(mg/dl)	5.04	1.84	4.79	0.86	0.3
LDH(U/L)	425	213.3	476	138.7	0.18
HBX protein(ng/ml)	0.30	0.16	32.67	107.9	0.004**

*P < 0.05, significant & **P <0.001, highly significant and ***P< 0.0001extremally significant.

Table (3). Demographic and clinical data of based on lymphoma

Varities	**Lymphoma patient (53)**		**Non lymphoma (41)**		**P value**
Age (mean, SD)	47.68	15.27	45.8	12.92	0.41
	No	**%**	**No**	**%**	
Male	29	54	17	41	0.08
Female	24	46	24	59	
PCR HBV Positive	5	9.4	3	7.3	0.57
PCR HBV (Negative)	48	91.6	38	92.7	0.6
HBX (Positive)	12	22.6	3	7.3	0.039*
HBX(Negative)	41	77.4	38	92.7	0.09

P* > 0.05, significant



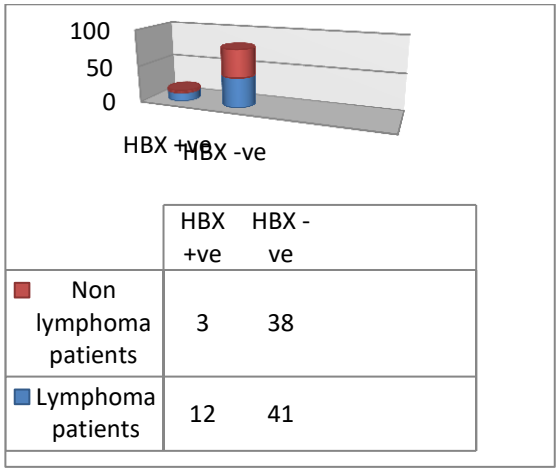
Fig(1). Ratio of HBV –ve and HBV +ve cases

RBCS: red blood cells & WBCS: white blood cell count & PLT: platelet count

ALT: alanine aminotransferase & AST: aspartate aminotransferase & LDH: lactate dehydrogenase

According to laboratory data, our results revealed that HBV positive group had lower Hb concentration (*P*, 0.016), lower serum albumin level (*P*, 0.014), higher creatinine (*P*, 0.004), higher HBX protein concentration (*P*, 0.004), and increased, ALT and AST activity (*P*, 0.0001) compared to HBV negative group, Tab.2.

The current study has reclassified the subjects according to the presence of lymphoma cancer into two groups. The lymphoma subjects group included 53 patients with lymphoma cancer. They were 24 females and 29 males; their mean of age was 47.68 years with SD ± 15.27. The non-lymphoma subjects group included 41 subjects who have not lymphoma cancer, they were 24 females and 17 males and their mean age was 45.8 years with SD ±12.9. Only HBx protein concentration in lymphoma subjects was found to be higher than in non-lymphoma subjects (***P*= 0.039), represented by Fig (2).



Fig(2). HBx protein based on lymphoma in two studied group

Table (4) deals with laboratory data in the study groups. A significant increase of ALT, AST and LDH activity were estimated in the lymphoma subjects' group compared to the non- lymphoma subject's group, *p* value was 0.001, 0.001, and 0.04, respectively. Regarding the other parameters, no significant differences were found between the two groups.

Table (4). Hematological and clinical chemistry data of the lymphoma subjects group compared to subjects with reactive lymphadenopathy

Varieties	**Lymphoma subjects group (53)**		**Subjectswithreactivelymphadenoy group (41)**		
	Mean	SD	Mean	SD	
Hemoglobin concentration	11.62	2.13	11.33	1.38	0.45
Red blood cells (RBCS)	4.2	0.7	4.4	0.69	0.25
White blood cells (WBCS)	6.26	6.7	3.09	2.8	0.22
Platelets (PLT)	207.6	137	198	77.2	0.09
Alanine aminotransferase(ALT)	65.2	42.6	36.06	19.6	0.001**
Aspartate aminotransferase (AST)	68.07	40.4	38.8	18.6	0.001**
S. Bilirubin	2.2	0.54	1.24	0.49	0.51
S. Albumin	3.96	0.8	3.72	0.72	0.14
S. Creatinine	0.88	0.2	1.02	0.3	0.06
S. Uric acid	4.99	1.77	4.98	1.66	0.98
LDH	465.7	233.2	392.3	147.9	0.04*
HBX protein	10.04	59.8	0.33	0.41	0.3

0.001 was highly significant< **0.05** was significant **& **P** <*P **Discussion**

Hepatitis is a basic global health problem, which causes the death of 1.5 million people every year **[25]**. Hepatitis B is found to be the most common form of hepatitis. Approximately 57 million persons over world have been infected with the HBV **[26]**. It is the prototype of the *Hepadnaviridae* which has different strategies to evade the immune system. It replicates through reverse transcription after its genomes are archived in the nucleus of hepatocytes as episomal DNA**[27]**. Recently, the Global Health is trying to eliminate viral hepatitis by 2030 by utilizing different Sector Strategies. So, the current study deals with the clinical effect of HBV infection. Also, risk assessment of HBV in influencing lymphoma has been approved in this study.

A significant difference between mean ages of our study groups was determined which appeared that higher infection with HBV was in older subjects. This is in agreement with Abdullah[25] who reported that age of study group 54years. Yewande Nejo *et al.*, **[28]** found the highest predictor of HBs Ag was aged 31-40 years and older age was associated with HBV exposure **[29]**.

There were no statistically significant differences between sex distributions in our study. However, higher prevalence of HBV infection (HBs Ag) was found in males, but this is statically not reached. Where, it was recorded that among positive subjects 30 % were males and 25% were females **[30]**.

HBV is suspected to have a significant role in progress of lymphoma diseases. So, our study evaluated the onset of lymphoma between the studied groups. About 44% of HBV positive subjects had lymphoma. 71% of these subjects with lymphoma were non-Hodgkin's lymphoma type. This is constituted with previous study; HBV carriers had higher risk for B-cell NHL onset **[31]**.

HBsAg-positive members had an increased risk of NHL overall compared with those who were HBsAg-negative. According to NHL sub types, HBs Ag positive was associated with increased risk of diffuse large B-cell lymphoma**[32].** HBV may play a critical role in development of NHL **[33].** Also, Fwu *et al.*,**[34]**. estimated an etiological role of HBV in the development of some NHL subtypes. Women infected with chronic HBV had an increased risk of DLBCL. Moreover, a significantly increased NHL risk in HBV-infected individuals with higher incidence in B-cell NHL was reported. HBV can infect B

lymphocytes and a correlation between HBV infection and DLBCL development has been determined. The study of Wang et al., **[35]** revealed that HBV plays a fundamental role in the development of B-cell NHL**.**

It is observed that nearly 40% of HBV +ve subjects were positive for HBx protein that is not found in HBV –ve subjects. HBx had a solid upgrading impact on HBV transcription and replication which was affected by host cell type. It has a main role in HBV transcription and replication in hepatocytes *in vivo* **[36]**. The proapoptotic action of X protein may have a role in tha viral spread during the acute phase of HBV infection **[37].**

The HBV +ve subjects occasionally presented with lower Hb concentration compared to HBV –ve case group. This is in accordance with many previous studies. The majority of the subjects had decreased Hb level which may be due to iron deficiency **[38].** Moreover, increased ALT and AST levels were found in infected cases with HBV **[29].** Infected cases with HBV had abnormal liver tests with mostly elevated liver enzymes**[39]** and these previous data are comparable to our results.

The current study determines the coexistence of HBV infection with lymphoma. There were 53 cases with lymphoma and 5 of these cases had +ve lymphoma cancer +ve HBV.

Most cases occasionally presented with weight loss, splenomegaly, lymphadenopathy and hepatomegaly. This is in agreement with **Lettieri, and Berg** study**,** who found that lymphoma cases share common clinical features, including hepatomegaly, lactic acidosis, and death shortly after the onset of symptoms**[40].**

Regarding HBX protein, lymphoma subjects were found to have higher HBx protein-positive than in non-lymphoma. This supported the possibility that infection with HBV could influence the development of lymphoma. This is in line with many studies which deal with the role of infection with HBV and the development of lymphoma types. HBV-infected individuals had increased affinity for developing NHL**[41].** HBx protein possesses the most pathogenic potential. It can accompany many proteins to transactivate, intracellular signal transduction, genotoxic stress response, protein degradation, cell cycle control and apoptosis **[42]**. Current published data shows HBx protein triggers epigenetic abnormalities and disrupts cellular signaling pathways which results in uncontrolled hepatocyte proliferation, development of HBV-induced inflammation, fibrosis and cancer **[43]**. Furthermore, the interaction of HBx protein and p53 mutant enhances progressive tumors formation by the activation of Proto-oncogene MYC, vascular endothelial growth factor (VEGF) and phosphatase and tensin homolog (PTEN) *via* Phosphatidylinositol 3-kinase/ Serine–threonine protein kinase pathway (PI3K/AKT) **[13]**.

Nagral *et al.,* **[36]** clarified that about 70 % of subjects with lymphoma had irregular liver function enzymes and 30 to 80% had elevated LDH**.** Lymphoma subjects initially suffered from elevated LDH, total bilirubin, and liver function enzymes **[44]** which also constituted our results. This could be explained by the possibility that hematological malignancies frequently affect the liver and high-grade lymphoma could penetrate the liver **[40]**. It is evident over the past decade that HBV-infected

subjects have a 2-3-fold greater chance of developing NHL than non - diseased ones **[45]**. The increased HBx concentration is due to HBV. Modification of cytosolic calcium was a fundamental requirement for HBV replication and was mediated by HBx protein [46].Hence, hypercalcemia which has been reported to occur in 10-20% of subjects with malignancies may be also enhancing HBX protein**[47]**. Mostly, HBx initially induces DNA damage and then disrupts nucleic acid metabolism, which in turn blocks DNA repair and induces the occurrence of cancer **[48].** This is may be

Conclusion

HBV infection could have a role in lymphoma development mainly NHL type. It could be related to the HBx protein expression. HBV positive cases also have aggressive lymphoma. Therefore, our study suggests that early treatment of hepatitis virus infection mainly HBV has an important role in decreasing malignancy developmentone of the

causes which led to increased HBx in HBV +ve lymphoma +ve subjects.

4. Reference

1. Velkov, S., et al., (2018) The Global Hepatitis B Virus Genotype Distribution Approximated from Available Genotyping Data. Genes (Basel),. **9(10).**
2. Liang, T.J., Hepatitis B: (2009) the virus and disease. Hepatology,. **49**(5 Suppl): p. S13-21.
3. Stahlman, S., V.F. Williams, and A.A. Oetting, Viral hepatitis B, (2017) active component, U.S. Armed Forces, (2007)-(2016). MSMR,. **24(5)**: p. 6-11.
4. Schweitzer, A., et al., (1965) and (2013). Estimations of worldwide prevalence of chronic hepatitis B virus infection: A systematic review of data published between Lancet (London, England), (2015). **386**.
5. Lamontagne, R.J., S. Bagga, and M.J. Bouchard, Hepatitis B (2016) virus molecular biology and pathogenesis. Hepatoma Res, **2**: p. 163-186.
6. Dandri, M., P. Schirmacher, and C.E. Rogler, (1996) Woodchuck hepatitis virus X protein is present in chronically infected woodchuck liver and woodchuck hepatocellular carcinomas which are permissive for viral *replication. J Virol*,. **70(8)**: p. 5246-54.
7. Ali, A., et al., (2014).Hepatitis B virus, HBx mutants and their role in hepatocellular carcinoma. *World J Gastroenterol*, **20(30)**: p. 10238-48.
8. Dewantoro, O., R.A. Gani, and N. Akbar, (2006) Hepatocarcinogenesis in viral Hepatitis B infection: the role of HBx and p53. Acta Med Indones,. **38(3)**: p. 154-9.
9. Slagle, B.L., et al., (2015). Technical standards for hepatitis B virus X protein (HBx) research. Hepatology, **61(4)**: p. 1416-24.
10. Yao, Y., et al., (2018) RBM24 stabilizes hepatitis B virus pregenomic RNA but inhibits core protein translation by targeting the terminal redundancy sequence. Emerg Microbes Infect,. **7(1)**: p. 86.
11. Lee, H., et al., (2019). Hepatitis B Virus X Protein Stimulates Virus Replication Via DNA Methylation of the C-1619 in Covalently Closed Circular DNA. Mol Cells, **42(1)**: p. 67-78.
12. Karayiannis, P., Hepatitis B (2017).virus: virology, molecular biology, life cycle and intrahepatic spread. Hepatol Int, **11(6)**: p. 500-508.
13. Lu, J.W., et al., (2013) Liver-specific expressions of HBx and src in the p53 mutant trigger hepatocarcinogenesis in zebrafish. PLoS One,. **8(10)**: p. e76951.
14. Huang, J.F., et al., (2016) EVI1 promotes cell proliferation in HBx-induced hepatocarcinogenesis as a critical transcription factor regulating lncRNAs. Oncotarget,. **7(16)**: p. 21887-99.
15. Marcucci, F. and A. Mele, (2011) Hepatitis viruses and non-Hodgkin lymphoma: epidemiology, mechanisms of tumorigenesis, and therapeutic opportunities. Blood,. **117(6)**: p. 1792-8.
16. Zhao, X., et al., (2018) HBV infection potentiates resistance to S-phase arrest-inducing chemotherapeutics by inhibiting CHK2 pathway in diffuse large B-cell lymphoma. Cell Death Dis,. **9(2)**: p. 61.
17. Mahale, P., E.A. Engels, and J. Koshiol, Hepatitis B (2019) virus infection and the risk of cancer in the elderly US population. *Int J Cancer*,. **144(3)**: p. 431-439.
18. Huang, X.-J., et al., (2006) Aspartate Aminotransferase (AST/GOT) and Alanine Aminotransferase (ALT/GPT) Detection Techniques. Sensors (Basel, Switzerland),. **6(7)**: p. 756-782.
19. Kihara, K., et al., (1984) Determination of glutamate-pyruvate transaminase activity in blood serum with a pyruvate oxidase/poly(vinyl chloride) membrane sensor. Analytica Chimica Acta,. **159**: p. 81-86.
20. Garber, C.C., (1981) Jendrassik--Grof analysis for total and direct bilirubin in serum with a centrifugal analyzer. Clin Chem,. **27(8)**: p. 1410-6.
21. Murray, R.L., (1984) Creatinine In: Clinical Chemistry; Theory, Analysis and Correlation, Kaplan, L.A. and A.J.CV Mosby Co., St. Louis, (Pesce (Eds.): p. pp: 1247-1253.
22. Fossati, P., L. Prencipe, and G. Berti, (1980). Use of 3,5-dichloro-2-

hydroxybenzenesulfonic acid/4-aminophenazone chromogenic system in direct enzymic assay of uric acid in serum and urine. Clin Chem, **26(2)**: p. 227-31.
23. Hwang, G.-Y., et al., (2003). Detection of the hepatitis B virus X protein (HBx) antigen and anti-HBx antibodies in cases of human hepatocellular carcinoma. *Journal of clinical microbiology*, **41(12)**: p. 5598-5603.
24. Liu, C., et al., (2017) Real-time PCR assays for hepatitis B virus DNA quantification may require two different targets. *Virology Journal*,. **14(1)**: p. 94.
25. Abdullah, S.M., (2018). Prevalence of Hepatitis B and C virus infection and their co-relation with hematological and hepatic parameters in subjects undergoing Premarital Screening in the Jazan Region, Kingdom of Saudi Arabia. *Pak J Med Sci*, **34(2)**: p. 316-321.
26. Spearman, C.W., et al., (2017) Hepatitis B in sub-Saharan Africa: strategies to achieve the 2030 elimination targets. Lancet Gastroenterol Hepatol,. **2(12)**: p. 900-909.
27. Glebe, D. and A. Konig, (2014) Molecular virology of hepatitis B virus and targets for antiviral intervention. Intervirology,. **57**(**3-4**): p. 134-40.
28. Nejo, Y., et al., (2018) Hepatitis B virus infection among sexually active individuals in Nigeria: a cross-sectional study. *Pan Afr Med J*,. **30**: p. 155.
29. Shankar, H., et al., (2016) A Novel Collaborative Community-Based Hepatitis B Screening and Linkage to Care Program for African Immigrants. Clin Infect Dis,. **62 Suppl 4**: p. S289-97.
30. Yan, X., et al., (2018) Diffuse large B-cell lymphoma with concurrent hepatitis B virus infection in the MabThera era: Unique clinical features and worse outcomes. *J Cancer Res Ther*,. **14**(Supplement): p. S248-S253.
31. Kim, J.H., et al., (2002) Hepatitis B virus infection and B-cell non-Hodgkin's lymphoma in a hepatitis B endemic area: a case-control study. *Jpn J Cancer Res*,. **93(5)**: p. 471-7.
32. Engels, E.A., E.R. Cho, and S.H. Jee, (2010). Hepatitis B virus infection and risk of non-Hodgkin lymphoma in South Korea: a cohort study. Lancet Oncol, **11(9)**: p. 827-34.
33. Kim, Y.M., et al., (2011) Chronic hepatitis B, non-Hodgkin's lymphoma, and effect of prophylactic antiviral therapy. *J Clin Virol*,. **51(4)**: p. 241-5.
34. Fwu, C.W., et al., (2011) Hepatitis B virus infection and risk of intrahepatic cholangiocarcinoma and non-Hodgkin lymphoma: a cohort study of parous women in Taiwan. Hepatology,. **53(4)**: p. 1217-25.
35. Wang, Y., et al., (2018). Capable Infection of Hepatitis B Virus in Diffuse Large B-cell Lymphoma. *J Cancer*, **9(9)**: p. 1575-1581.
36. Gong, D.Y., et al., (2013) Role and functional domain of hepatitis B virus X protein in regulating HBV transcription and replication in vitro and in vivo. Viruses,. **5**(**5**): p. 1261-71.
37. Pathak, R.K., et al., (2014).Virtual screening of natural inhibitors to the predicted HBx protein structure of Hepatitis B Virus using molecular docking for identification of potential lead molecules for liver cancer. Bioinformation, **10**(**7**): p. 428-35.
38. Matsumoto, N., et al., (2016) Hemoglobin Decrease with Iron Deficiency Induced by Daclatasvir plus Asunaprevir Combination Therapy for Chronic Hepatitis C Virus Genotype 1b. PLoS One,. **11**(3): p. e0151238.
39. Puri, P., et al., (2017) Liver Function Tests Abnormalities and Hepatitis B Virus & Hepatitis C Virus Co-infection in Human Immunodeficiency Virus (HIV)-infected Patients in India. *J Clin Exp Hepatol*,. **7**(1): p. 1-8.
40. Lettieri, C.J. and B.W. Berg, (2003) Clinical features of non-Hodgkins lymphoma presenting with acute liver failure: a report of five cases and review of published experience. *Am J Gastroenterol*,. **98**(7): p. 1641-6.
41. Dalia, S., et al., (2013) Hepatitis B infection increases the risk of non-Hodgkin lymphoma: a meta-analysis of observational studies. Leuk Res,. **37**(**9**): p. 1107-15.

42. Peng, Z., et al., (2005) Integration of the hepatitis B virus X fragment in hepatocellular carcinoma and its effects on the expression of multiple molecules: a key to the cell cycle and apoptosis. *Int J Oncol,*. **26(2)**: p. 467-73.
43. Kgatle, M.M., M. Setshedi, and H.N. Hairwadzi, (2016) Hepatoepigenetic Alterations in Viral and Nonviral-Induced Hepatocellular Carcinoma. Biomed Res Int,. **2016**: p. 3956485.
44. Ramai, D., et al., (2018) Primary hepatic peripheral T-cell lymphoma associated with Epstein-Barr viral infection. *World J Hepatol,*. **10(2)**: p. 347-351.
45. Marcucci, F., et al., (2012) The association of hepatitis B virus infection with B-cell non-Hodgkin lymphoma - a review. *Am J Blood Res,*. **2(1)**: p. 18-28.
46. Bouchard, M.J., L.H. Wang, and R.J. Schneider, (2001) Calcium signaling by HBx protein in hepatitis B virus DNA replication. Science,. **294(5550)**: p. 2376-8.
47. Malangone, S. and C.J. Campen, (2015) Hypercalcemia of Malignancy. *J Adv Pract Oncol,*. **6(6)**: p. 586-92.
48. Dan, Y., et al., (2016) Hepatitis B virus X protein (HBx)-induced abnormalities of nucleic acid metabolism revealed by (1)H-NMR-based metabonomics. Sci Rep,. **6**: p. 24430.