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A Study on the Relationship of Hepatitis B Virus with Hepatocellular Carcinoma

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Abstract: *Hepatitis B virus (HBV) has infected approximately 2 billion peo ple worldwide of which more than 350 million are chronically infected life threatening liver disease. This is a study on the relationship of hepatitis B surface antigen (HBsAg) with hepatocellular carcinoma in patients visiting National hospital, Abuja, State house clinic, Abuja and Federal Medical Centre, Keffi, was carried out in 50 liver tissue samples using histochemical reaction of orcein shikata and Haematoxylin and eosin (H and B) staining techniques. Of the 50 samples collected 20 were from National hospital, Abuja, 10 from State house clinic and 20 were from Federal medical centre, Keffi. Sixteen (32%) were diagnosed hepatocellular carcinoma, 10 (20%) liver cirrhosis, 10 (20%) liver hyperplasia and 14 (28%) chronic hepatitis. Out of the 16 hepatocellular carcinoma, 12 (75%) were positive for HBsAg (25%) were negative. For 10liver cirrhosis 7 (30%) were negative. The rest were 8 (80%) positive for hyperplasia out of the 10 samples and all the 14 (100%) cases of chronic hepatitis were positive for HBsAg. Generally, of the 50 samples investigated, 41 (82%) were positive for HBsAg while 9 (18%) were negative. This shows a positive relationship on the pattern of HBsAg amongst the liver diseases under study, and of the two staining techniques employed, orcein shikata gave a better result with ease in recognition and quick screening.*

Keywords: *Hepatitis B, liver diseases, orcein shikata, liver cirrhosis, haematoxylin and eosin.*

I. Introduction

Hepatitis B virus infections are a gastroenterological disease caus ed by hepatitis B virus of the hepadna virus family (Glebe and Urban , 2007). Hepatitis B virus (HBV) has infected approximately 2 billion people worldwide of which more than 350 million are chronically infected life threatening liver disease. (Cirrhosis, r enal failure, and hepatocellular carcinoma occurs in about 40% of patients with hepatitis B (Shuping et al.1999). Hepatitis B viral infection is the tenth leading cause of death worldwide, occurring in up to 1.2 million people per year and Hepatocellular carcinoma is now the fifth Most frequent cancer worldwide causing about 500,000 deaths per year (Vandamine and Herck, 2007). HEPATITIS B VIRUS is a small circular deoxyribonucleic acid virus containing a nucleocapsid and envelope. It neculeocapside contains a relatively small and incompletely double stranded DNA genome, viral polymerase and core protein (Vandamine and Herck, 2007). The agents of hepatitis could be classified according to their biological nature. Hepatitis could be caused by increased alcohol consumption (alcohol induced hepatitis) induced by drugs and also as a result of toxins from drugs (Shapiro, 1993). Hepatitis B virus can cause both acute and chronic infection in man. Viron (viral particle) consist of a protein core particle containing the viral genome in the form of double stranded DNA and an outer lipid (Zouline, 2006). Base envelope is lined with embedded proteins (Taylor, 2006). The viron is 42nm in diameter and possess a 150 metric nucleocapsid of core of 27nm in diameter, surrounded by an outer-coat approximately 4mm thick (Look and Mamahon, 2007). The protein of the viron coat is termed surface antigen or HBsAg. It is sometimes extended as a tubular tail on one side of the virus particle (Lui et al., 1991). The surface antigen is generally produced in the vast excess, and is found in the form of filamentous and spherical particles. Filament particulars are identical to the viron -tail (Lui et al., 1991). They vary in length and have a mean diameter of about 22nm. They sometimes dis play regular, non-helical transverse striation (Lui et al., 1991).

Two recognized analysis are associated with the whole HEPATITIS B VIRUS i.e HBs HBsAg and HBcAg. HBsAG is formed in the liver cell cytoplasm and HBcAg is formed in the hepatocyte nucleus (L ai and Vuen, 2007). These distinct antigens induce specific anti-bodies without cross- reactivity (Lui et al., 1991). HBsAg is generally produced in vast excess and may accumulate in smooth endoplasmic reticulum producing the characteristics ground glass hepatocytes. Hepatitis B surface antigen is always the earliest to appear in acute hepatitis B viral infection (Lui et al.1991).

Shortly after the appearance of HBsAg, another antigen named hepatitis B envelope antigen (HBeAg) appears (Oliver et al., 1991). Traditionally, the presence of HBeAg in the host semen is associated with much higher rates of viral replication if the host is able to clear the infection, eventually, the HBsAg will become undetected (Oliver et al., 1991). Anti HBs is detectable shortly after the disappearance of HBsAg. This implies that the acute infection have reached its peak and the disease is on the wane, but overtime chronic HEPATITIS B VIRUS infection can cause hepatic fibrosis and eventually cirrhosis and hepatocellular carcin oma which is the leading causes of cancer death in the world (Pungpapong et al., 2007).

SCOPE

The scope of this work is confined only to the patients whose liver biopsy or autopsy tissue samples have been submitted to the various laboratories between march 2009 to 75 years were included in this work.

AIMS AND OBJECTIVES

1. To determine the relationship between HBsAg with (HCC) hepatocellualr carcinoma.
2. To defect HBsAg in previously para fin embedded liner samples.
3. To compare the orcein shikata and haematoxcyline and eosin staining technique for supportive diagnosis of hepatocellular carcinoma.

II. Materials And Methods

SAMPLE COLLECTION

The specimen use for this research were obtained from formalin – fixed paraffin embedded biopsy and post mortem samples from fifty (50) patients from three hospitals. Out of the 50 samples, 35 samples were obtained from biopsy specimens while 15 were from autopsy.

PREPARATION OF SECTION

The liver samples were processed automati cally using the automatic tissue processor, embedded and sectioned into ribbons with the rotary microtome to a thickness of 4µm. The sections in 20% ethyl alcohol and also in tissue floating water bath, thermostatically maintained at a temperature of 26-48C. The sections were then collected using cleaned numbered Mayer's egg albumenized glass slides. The sections were dried in a hot plate at 30 ^{0}C for two hours to evaporate excess water and also adhere the sections to the slides.

STAINING OF SECTIONS FOR HISTOLOGICAL ASSESMENT

Four (4) micron sections were stained with Haematoxylin and Eosin for liver morphology (structure) and Shikata Orcein technique for hepatitis B surface antigen (HBsAg), sections were viewed at low power magnification light microscope and areas of increasing HBsAg activity were determined usually around the hepatocytes.

PROCEDURE FOR HAEMATOXYLIN AND EOSIN (H/E) STAINING TECHNIQUE

1. Dewax in Xylene and hydrate through descending grades of alcohol (absolute, 95%, 85%, 70%) to.
2. Stain in Ehrlich's haematoxylin for 20 minutes and wash thoroughly in running tap water.
3. Differentiate in 1% acid alcohol for 10 seconds and rinse in water.
4. Blue in Scott's tap water for 5 minutes.
5. Counterstain in 1% Eosin Solution for one minutes and wash in tap water. hydrate through ascending grades of alcohol 70%,80%, 90% and absolute). clear in xylene and mount in DPX (Dibutylphthalate polystrene Xylene).

STAININ G PRO CEDURE FO R S HAK ATA ORCEI N TECHNIQUES

Sections were dewaxed in xylene and hydrated through ascend ing grades of alcohol (absolute, 95%, 85%) to water. Sections were oxidized in 1.5% acidified potassium manganate solution until the section turned bluish black for 5 minutes. Sections were rinsed in water. Permanganate staining were bleached off by ointment ether 1.5% acqueous oxalic acid until sections are clear. Sections were rinsed in water and then in 70% alcohol 5minutes. The sections were dehydrated in ascending concentrations of alcohol (70%, 85%, 95%, absolute). Sections were cleared in Xylene and mounted with DPX.

III. Results

The two staining techniques were taken into consideration and the fifty (50) samples examined. The sections were examined each for tissue morphology, possible spot of hepatitis B Viral inclusion bodies and the nature of the reticulin fibers with using the light microscope.

Histological diagnosis was based on specific pattern of viral inclusion bodies (HBsAg) on liver cells. The hepatitis B virus specific pattern of viral inclusion bodies were observed with magnification power using X400 (See photomicrograph).

PHOTOMICROGRAPHS

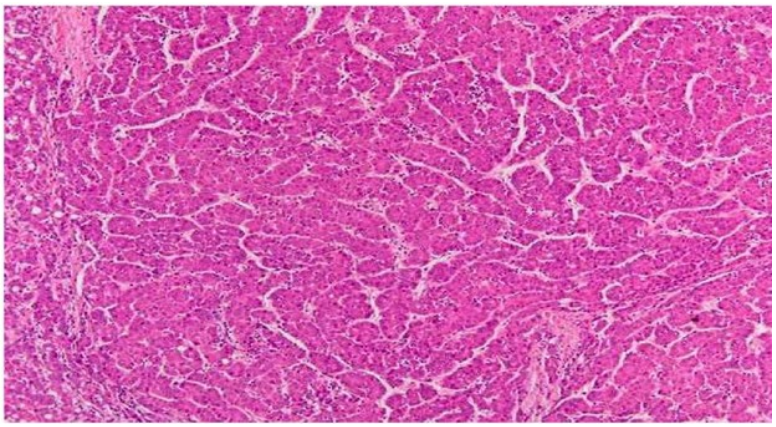

Figure 1: Liver section (H and E X400) showing normal histological features

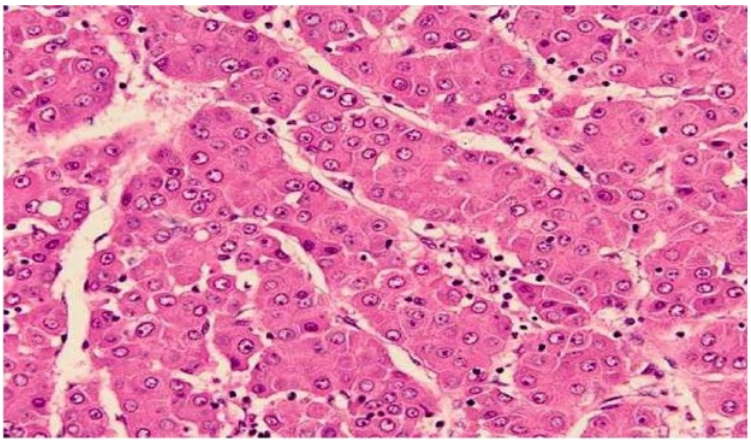

Figure 2

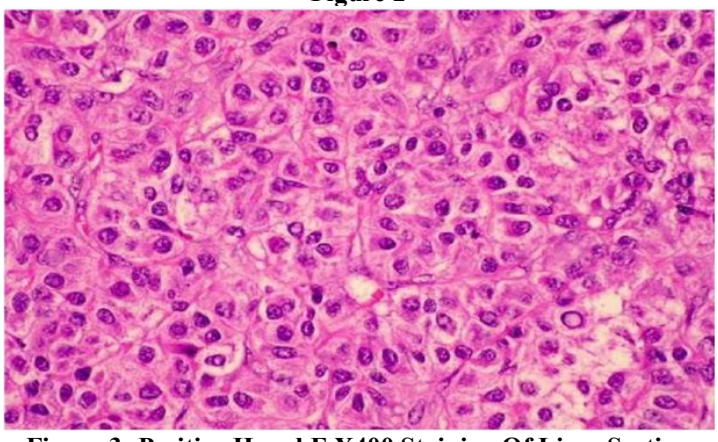

Figure 3: Positive H and E X400 Staining Of Liver Section

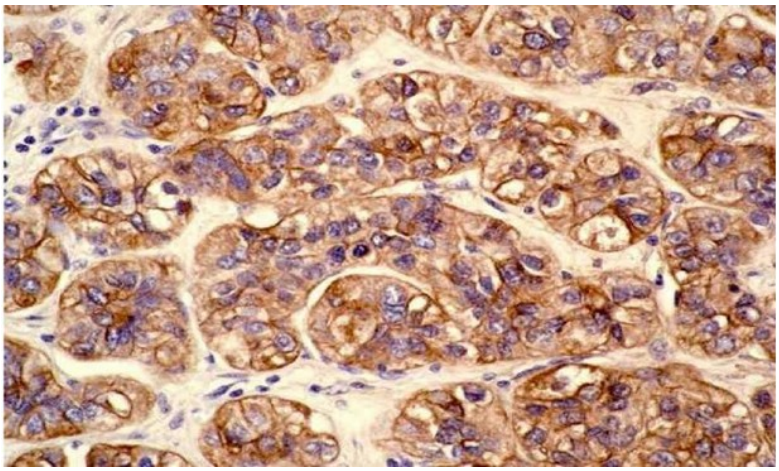

Figure 4: Positive Shikata Orcein Staining Of Liver Section X400

HAEMATOXYLIN AND EOSIN STAINING METHOD

The infected liver cells were found to have granular appearances in part of the endoplasmic reticulum of the cytoplasm. The liver cell appearances show a ground glass appearance indicating the presence of hepatitis B surface antigen (HBsAg). The hepatitis B surface antigen affected hepatocytes were deeply stained (deep purple).

ORCEIN SHIKATA METHOD

Sections show distinctive cytoplasmic inclusion bodies of hepatitis B surface antigens on affected hepatocytes. The hepatitis B virus affected cells were found singly or in group and are stained medium brown to black, while background of uninfected hepatocytes shows a clear to pale brown colour agreeing with the demonstration of Bruss 2007.

Table 1: Staining reaction of two staining techniques used as a case study.

LIVER DISEASE	S.ORCEIN	H/E
Hepatocellular carcinoma	++++	++
Liver cirrhosis	+++	+
Hyperplasia liver cells	+++	++
Chronic Hepatitis	++++	++

LEGEND:

S/ORCEIN = Shikata Orcein Method
H/E = Haematoxylin and Eosin Method
\+ = Positive

Table 1 shows a summary of the staining reactions to Hepatitis B surface antigen (HBsAg). Orcein Shikata staining techni que demonstrated the presence of abnormality clearly than Haematoxylin and Eosin technique.

Table 2: Distribution of liver diseases amongst the 50 subjects studied.

LIVER DISEASE	NUMBER OF CASES	OBSERVATION IN ORCEIN	
		% POSITIVE	% NEGATIVE
Hepatocellular Carcinoma	16	12 (75%)	4 (25%)
Liver cirrhosis	10	7(70%)	3 (30%)
Hyperplasia of the liver cells	10	8(80%)	2 (20%)
Chronic hepatitis	14	14(100%)	0 (0%)
Total	50	41 (82%)	9 (18%)

Table II shows the distribution of pathologic liver tissues in Shikat a orcein stain. Out of the 50 liver samples, 16 cases were hepatocellular carcinoma. Of this 16, 12 (75%) were positive for HBsAg, while 4 (25%) were negative. Of the 10 liver Cirrhosis, 7 (70%) were positive for HBsAg, while 3 (30%) were negative. In

hyperplasia of the liver, 10 cases were observed of which 8 (80%) were positive for HBsAg and 2 (20%) were negative, while in chronic hepatitis, 14 cases were observed and all (100%) were positive for HBsAg.

IV. Discussion

The result of this study demonstrates th at hepatitis B surface antigen can be clearly demonstrated in both formalin fixed and paraffin embedded liver specimens by histochemical techniques.

This work agrees with the findings of Aoki et al, and Bancroft Stevens (1996) who documented a ground -glass appearance which affirm the accumulation of HBsAg in rough and endoplasmic reticulum of hepatocytes.

The bodies of the histochemical technique for HBsAg is that Shikata Orcein method employs selectivity for disulphide (S.S) bond linkage which predominates in hepatitis B surface antigen (HBsAg) coat.

In this study, chronic hepatitis shows 100% presence of HBsAg followed by hyperplasia of the liver and hepatocellular carcinoma which shows 80% and 75% respectively and liver cirrhosis being the least with 70%.

Therefore this appears that HBsAg remains well preserved not only after fixation in formalin but also after subsequent processing through alcohol and xylene.

V. Conclusion

This study has demonstrated that proper screening of conventionally stained liver sections may help to suggest the presence of hepatitis B surface antigen.

The cytoplasmic staining pattern observed with modifying Shikata orcein is easily recognized with additional advantages of quite screening of sections being made possible by the good contrast staining. Though the exact basis of the apparent preferential selectivity for HEPATITIS B VIRUS by orcein is not yet understood, it is probable that this stain under the circumstance is fairly specific for the coat material (HBsAg).

All patients confirmed of primary liver disease showed the presence of HBsAg with various degree of infectivity. According to Cooper et al, 2003, the presence of HBsAg on serum indicates the presence of hepatitis B virus infection.

In conclusion, modified Shikata orcein has been confirmed as being suitable for routine use in demonstrating HBsAg. However, the method should not be used for primary diagnoses of hepatitis B virus in patients, it value this in the screening of suspected cases of hepatitis B virus infections.

VI. Recommendation

The work done on the course of this research project is limited to histochemical technique and light microscopy. Histochemical technique (Shikata Orcein stain) permits the detection of hepatitis B viral inclusion in hepatocytes but does not allow the recognition of hepatitis B core antigen (HBcAg) distribution within the cell and nucleus.

Therefore to achieve better sensitivity and specificity in identification of viral inclusion, immunoperoxidase and immunoflurescence technique using electron microscopy should be employed.
All persons suspected of various liver diseases should undergo serum hepatitis screening.

References

[1]. Alter, H. J. Blumberg (1966): Further Studies on a New Human in precipitation system (Australia antigen): Blood 27 **(3):** 297-3 09.
[2]. Alter, M. (2003): Epidemiology and Prevention Hepatitis B. Seminars in liver disease 23 **(1):** 39-46.
[3]. Barker, L. F. et al. (1996): Transmission of serum hepatitis 1970. JAMA: **276:** 841
[4]. Beck, J; Nassal, G. (2007): Hepatitis B virus replication. World Journal of gastroenterology; 13 **(1):** 48-64.
[5]. Bell, S. J and Nyuyen, T. (2009): The management of hepatitis B. Aust presser 23 **(4):** 99-104.
[6]. Bonacini, D. and Manizio, M. (2009): Hepatitis B reactivation. University of Southern California Department of Surgery. Retrieved 2009-01-24.
[7]. Borino, D., Chiaberge, F., Maran, N. and Piantino, O. (1987): Serological markers of HEPATITIS B VIRUS infectivity. 24 **(2):** 21-23.
[8]. Bouchard, M., Schrieder, R. (2004): The enigmas gene of hepatitis B. virus morphogenesis. World Journal of gastroenterology; 13 **(1):** 65-73.
[9]. Chang, M. (2007): Hepatitis B virus infection. Seminars in fetal and neonatal medicine 12 **(3):** 160-167.
[10]. Chu, C; Liaw, R. (2007): Prediction factors for reactivation of hepatitis B following hepatitis B antigen seroconversion in chronic hepatitis B. Gastroenterology 133 **(5):** 1458-1465.
[11]. Custer, K. and Gullivan, K.V. (2004): Global epidemiology of HEPATITIS B VIRUS. Journal of clinicial gastroenterology; 38 (10 suppl 3): 515-868.
[12]. Dane, D. (1970): Virus-like particles in serum of patients with Australia-Antigen-Association hepatitis. The lanlet 295: 695 -698.
[13]. Dienstag, J. L. (2008): Hepatitis B Viral Infection. The New England Journal of Medicine 359 **(14):** 1486-1500.
[14]. Glebe, D. and Urban, T. (2007): Viral and cellular determ inants involved in hepadna viral entry. World Journal of gastroenterology; 13 **(1):** 22-38.
[15]. Galibert, F.J Mandert, N., Fitoussi, B., Toillais, I. and Charnay, V. (1979): Nucleotide sequence of the hepatitis B virus genome (subtype ayw) cloned in E. coli. Nature 281: 646.
[16]. Howard, C. R. (2006): The biology of hepadnaviruses. Journal of genral virology; **67:** 12-15.

[17]. Hollinger, F; Lau, D. (2006): Hepatitis B: the pathway to recovery through treatment Gastroeterology clinics of North America 35 **(4):** 895-931.
[18]. Kats, L, Fraser, A., Gafter-Gvilli, A., Leiboviu, E. and Turkaspa, R. (2008): Launiviudine presents reactivation of hepatitis B and reduces mortality in immuno suppressed patients: Systematic review and meta-analysis. Journal of viral hepatitis 15 **(2):** 89-102.
[19]. Kay, A; Zoulum, F. (2007): Hepatitis B virus genetic variability and evolution. Virus research 127 **(2):** 164-176.
[20]. Kerkar, N. (2005): Hepatitis B in children: complexities in management Pediatric transplantation 9 **(5):** 685-691.
[21]. Kranvis, A.; Kew, M; Francis, G. (2005): Hepatitis B virus genotypes. Vaccine 23 **(19):** 2409-2423.
[22]. Lai, C. L and Vuen, D. (2007): The natural history and treatment of chronic hepatitis B: a critical evaluation of standard treatment criteria and points 3(2): 58-61.
[23]. Lannacone, M, Sitia, G; Rugger, Z., Guidotti; L. (2005): hepatitis B virus pathogenesis in animal models: recent advances on the role of platelets. Journal of hepatology 46 **(4):** 719-726.
[24]. Lannacone, M., Sitia G., Isogawa, M., et al (2007): Platelets mediate cytotoxic T.hympho cyteindicued liver damage. Nature medicine 11 **(11):** 1167-1169.
[25]. Lui, A.U., Li, K., Tam, R., Tong, J. and Lai, O. (1991) Membranous nephropathy related HEPATITIS B VIRUS in adults. The New England Journal of medicine 324 **(21):** 145-763.
[26]. Libbus, M. K and Philips, L. M. (2009): Public health Management of perimatal HEPATITIS B VIRUS. Public health nursing 26 **(4):** 353.
[27]. Locarnin, S. (2004): Molecular Virology of Hepatitis. B. Virus. Seminars in Liver Disease 24 Suppl **1:**310.
[28]. Look, A and Mamahon, B. (2007): Chronic hepatitis B. Hepatitis (Baltin Ore, Md.) 45 **(2):** 50-75.
[29]. MacCallum, F. O. (1947): Homologous Serum Hepatitis. Lancet 2, 691
[30]. Magnius L. O. Norder (1995): Subtypes, genotypes and molecular epimiology of the hepatitis V virus as reflected by sequence intervirology 38 (1-2): 24-34.
[31]. Martin - Ancel, A, Cases, M; Bonet, B. (2004): Implication of postv.accination hepatitis B surface antigenemia in the management of exposures to body fluids. Infection control and hospital epidemiology: the official journal of America; 25 (7): 611-613.
[32]. Mortality and Burden of disease estimates for WHO member statess in 2022 (XLS): World health organization 2004-12.
[33]. Ng, F; Cohang M. Chan, Cheng, C; Leung, H; Cheng, N; Ren, C. (Jul 2005), Host heterogeneous ribonucleoprotein K (hnRNK) as a p otential target to suppress hepatitis B-replication PLOS medicine 2 **(7):** 163.
[34]. O' Connor John (2008-10-03): Hepatitis B prevention of the body. Body health Resources Corporation.
[35]. Oliver, F; Brunetto; Acts; Bonino (1991): Pathobiology of chronic hepatitis virus infection and hepatocellualr carcinomia (Hcl): The Halian Journal of gastroenterology 23 **(8):** 498-502.
[36]. Peterson, N. J; Barret, Bind; Berquist; Fauero; Bender; Maynard (1976): Hepatitis B surface antigen in S.aliua, impetiginous lesions, and the environment in two remote Alaskan villages. Applied and environmental microbiology 32 **(94):** 24-57.
[37]. Pramoolsinsup, C. (2002): Management of viral hepatitis B. journal of gastroenterology and hepatology; 17 suppl: 5 12-545.
[38]. Pungpapong, S; Klin, w. Poterucha, J. (2007): Natural History of hepatitis B virus to infection; an update for uinicians. Mayor clinic proceedings 82 **(8):** 9-67.
[39]. Red, J; Baumbac, J; John, W. Naman O; Khristoua M; Williams, I. (2007): Patient to patient transmission of hepatitis B virus associated with oral surgery (PDF): The journal of infectious disease 195 **(9):** 131-134.
[40]. Shapino, C. N. (1993): Epidemiology of hepatitis B. The Pediatric infectious disease journal 12 (5): 43-37.
[41]. Shuping, Tony, Jisu Li and Jack R. wands (1999): Carboxypeptidase D is an avian hepatitis B virus receptor. Journal of virology 73 **(10):** 8696-8702.
[42]. Taylor, J. (2006): Hepatitis Delta virus. Virology; 344 (1) 7 1-76.
[43]. Vandamine, P. and Van Herck, K. (2007): A review of the long - term protection after hepatitis A and B vaccination.
[44]. Zouline, F. (2006): New nucleic acid diagnostic tests in viral hepatitis. Seminars in liver disease 26 **(4):** 309-3 17.
[45]. Zuckerman, J. (2006): Accination against hepatitis A and B: Current Opinion in infectious disease 19 (5): 456-459.