HEPATITIS VIRUS MARKERS OF THE POPULATION IN THE SOUTHERN PLAIN REGION OF HUNGARY; PERINATAL AND/OR TRANSPLACENTAL TRANSMISSION OF VIRUSES

PhD THESIS

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ABSTRACT

HEPATITIS VIRUS MARKERS IN THE POPULATION OF THE SOUTHERN PLAIN REGION OF HUNGARY; PERINATAL OR TRANSPLACENTAL TRANSMISSION OF HUMAN VIRUSES

Nation-wide programmes have been initiated in Hungary for the prevention of perinatal transmission of hepatitis B virus (HBV) in 1995 and mandatory vaccination has been initiated in 2001.

The prevalence of hepatitis virus markers have been determined in the population of the Southern Plain Region of Hungary in sera collected before 2001. The behaviour of the hepatitis B X-protein and antibodies as well as HBV genotypes were also examined. These data can be used later for the control of efficacy of preventive programmes.

Recently conflicting results have been published on the perinatal and transplacental transmission of viruses, therefore a systematic molecular examination of healthy pregnants and amniotic fluids taken at term were completed.

Results confirmed that the preventive measures will reduce the occurrence of HBV prevalence in the age group below 20 years of age to very low level (< 1 %). It has been observed, that of X-antigens and antibodies form probably complexes in the plasma similar to other HBV-coded viral proteins.

One third of the amniotic fluids at term were found to contain DNA of herpesvirus types 4, 5, 6, 7 and 8. Low levels of endotoxin and papillomaviruses could be also detected. The comparison of virus content of maternal blood and amniotic fluid suggested unequal transplacental transmission of viruses.

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INTRODUCTION

Intrafamily transmission of hepatitis B virus (HBV) and the possibilities of non-parenteral and perinatal transmissions have been recognized early. The clinical course and the acute and chronic consequences of the disease have been published and reviewed .

Prevention of perinatal transmission of HBV have been proposed early. Transplacental transmission had not been taken into account at all, since the postnatal active-passive immunisation could prevent 99% of vertical transmission. Because of the low prevalence of symptomless HBV-carrier mothers in Hungary, HBV cannot be used as a model to test possible relationships of transplacental or perinatal transmission.

Herpesviruses, papillomaviruses have been shown to be transmitted transplacentally when the pregnants suffered from primary infections. Transplacental transmission to the fetus of HIV/AIDS positive mothers has been proven. Recently the DNA of several viruses including HBV has been found in the blood of the neonates. In such cases the maternal contamination during delivery can be excluded. Systematic tests performed using different latently harboured human viruses had to be planned to resolve the problem.

AIMS OF THE WORK

1.) The original aim of the work was to perform serological screening of the population for the presence of hepatitis virus markers in the population of the Southern Plain of Hungary (Ref. 1). Serum samples have been collected and stored between 1993 and 2001. Since the mandatory HBV vaccination began in 2001 the data obtained may be used later to assess the efficacy of the vaccination.

2.) The systematic collection of blood samples from HBV-carrier persons the genotypes of the viruses present in the Region could be also determined in association with the virus detected in a hospital epidemic and characterised by nucleotide sequencing (Ref. 2).

3.) Selected blood samples taken from symptomless HBV-carrier persons, and from patients suffering from acute and chronic HBV hepatitis have been planned to be examined by new techniques for the quantitative measurement of hepatitis B oncogenic protein X (HBxAg) and for its specific antibodies using recombinant X-protein, developed by Joseph Pál in the frames of a cooperation of teams from Szeged and Pécs (Refs. 3 and 4).

4.) HBV carrier pregnants have not been available for the differentiation of transplacental and perinatal virus transmission. The question has been formulated as follows: “Is there a possibility for virus-transmission through the placenta to the fetus when the mother is only latently carrying the viruses? Amniotic fluid and blood samples (106 each) have been collected exclusively from healthy pregnants at term in connection with the arteficial induction of the delivery.

BACKGROUND

1.) Hepatitis B preventive active-passive immunisation of newborn babies born from hepatitis B carrier mothers was initiated in Hungary in 1995. The seroepidemiological survey revealed in 2000, that the vaccination has been successful. Less than 1 % of the treated neonates remained HBV-carrier.

According to the new vaccination system, the mandatory vaccination of school-children has been introduced in 2001. Historical serum samples were examined from the Southern Plain Administrative Region of Hungary, to obtain serological data from the years before vaccination. In addition to this examination of genotypes of HBV in the region was also planned, and see the possible applications of new serological tests for the examination of the oncogenic protein of hepatitis B virus.

2.) The group of late Ferenc D.Tóth reported from Debrecen in a series of good publications, that the **explants of human syncytiotrophoblast cells** can be infected and are able to support the growth of different viruses: human cytomegalovirus (HCMV), . Epstein-Barr virus (EBV), Human T-cell leukemia-lymphoma virus type 1 (HTLV-I), human immunodeficiency virus type 1 (HIV-1) and human herpesvirus type 6 (HHV6). Co-infections with different viruses support and accelerate the replication of at least one of the viruses. Interleukins 6 and 8, tumor necrosis factor α and transforming growth factor β enhance replication of HIV-1 and HCMV in the syncytiotrophoblast cultures.

Cellular receptor molecules of different herpesviruses, can be detected on the membranes of different fetal tissues (CD21, CD46, CD98, ICAM-I and integrins) at different times of fetal development.

Disintegrin like domains are present on the glycoprotein "B" of herpesviruses which play an important role in both the formation of syncytiotrophoblast and glycoprotein B induced fusion of the envelope and host cell membranes.

Direct contact of maternal monocytes, macrophages with microvilli and transfer of maternal lymphocytes into the fetal parts of the placenta offer yet unidentified mechanisms for the transfer of latently harboured viruses into the fetal organism.

Endotoxins and cytokines in addition to co-infections might facilitate transplacental transport of viruses even in the presence of maternal antibodies. HBV-carrier mothers could not be tested, therefore healthy pregnants were

examined at the end of normal pregnancies for the presence of lymphotropic herpesviruses and human papillomaviruses.

MATERIAL and METHODS

Diagnostic samples have been collected by many clinical laboratories. Sera of chronic symptomless carriers were taken from women by the obstetricians. Special care was introduced in order to prevent external or retrograde contamination of the amniotic fluid samples. All samples were stored at -20 ^{o}C.

The serological tests were performed using commercial ELISA and EIA kits for the detection of hepatitis markers. Results regarding HB-X antigen and HB-X-specific antibodies were obtained by sandwich type and inverted ELISA tests developed in Szeged and Pécs (Pál et al. 2005 and 2006).

The genotypes of HBV DNA was measured by PCR products sequenced using automatic DNA sequencing (Szomor et al. 2006).

The DNA of herpesviruses was detected by nested PCR (Csire et al. 2006). The papillomavirus DNA was detected by a technique developed at the firm Genoid (Jeney et al., 2007) and typed by nucleotide sequencing. The endotoxin was measured by the semiquantitative Limulus (LAL) test.

RESULTS AND DISCUSSION

1.) The data of markers indicating acute hepatitis revealed, that the different cohorts have been affected unequally (Table I) by the classical hepatitis viruses. The number of acute hepatitis A virus (HAV) infections was the lowest in all age groups except in the youngest one. The number of acute and chronic HBV infections was the highest below 40 years of age. The number of HCV positive patients increased with age. The mandatory

vaccination and the preventive treatment against perinatal transmission is supposed to reduce the proportion of HBV infections below 20 years of age around zero % within a decade (Seress et al. 2003).

Surprisingly all HBsAg-positive sera of acute, chronic hepatitis patients and symptomless carriers were found to be positive for IgG specific to HB-X (Table II). The X-Antigen concentration was above the level of detection (62.5 ng/ml) in all, but 38 sera of patients suffering from chronic hepatitis (Pál et al, 2005 and 2006).

.Table I. Distribution (%) of markers of acute or active classical hepatitis virus infections in the age groups indicated in 583 patients.

Age groups(years)	0-19 y	20-39 y	40-59 y	> 60 y	Total
Anti-HAV-IgM %	34.9	13.7	8.1	6.3	13.0 (76)
HBsAG %	57.1	62.9	30.2	21.1	45.1 (263)
Anti-HCV %	7.9	23.4	61.7	72.4	41.9 (244)
Total	99.9	100	100	99.8	100 (583)

Patients	X-Ag Ng/ml	IgG OD_{420}	IgM OD_{420}
Acute hepatitis (14)	300	0.700	0.450
Chronic hepatitis (80)	370*	0.8	0.5
Symptomless Carrier (12)	100	0.45	0.31

Table II. Mean concentrations of HB-X protein (ng/ml) and relative concentrations of HB-X-specific IgG and IgM in the three groups of patients tested

* The level of detection was 62.5 ng/ml of the X-protein. 38 of the patients suffering from chronic hepatitis were found to carry less or undetectable amounts of HB-X protein.

IgM was also present in a low proportion of the symptomless carrier persons. Indicating that sometimes liver cell destruction occurred in these persons, too. In the patients suffering from chronic hepatitis the presence of high antibody titres in the absence of circulating antigen, one may conclude, that X-antigen/antibody complexes are formed, similar to the behaviour of HBeAg and HBsAg . The genotype analysis of the HBV viruses present in the Southern Plain Administrative Region of the country detected, that both "A" and "D" genotypes are present within this population, too (Szomor et al. 2006).

2.) The examination of amniotic fluids taken in connection with the arteficial induction of delivery resulted in surprising results. Fifteen of 50 samples contained detectable amounts (0.03 – 3.0 Endotoxin U/ml) of endotoxin. This concentration, however, is very low. It was thirty-three times less, than approved by the FDA in parenteral vaccines (i.e. influenza). This is probably the reason, that the detected concentrations were found not to influence either the appearance of viruses or the development of the fetus.

The DNA of lymphotropic herpesviruses was detected in 26 of 106 amniotic fluid samples. Only 13 of the 106 whole blood samples of the same mothers contained herpesviruses. It has to be emphasised, that the DNA could be detected either in the amniotic fluid or in the blood of the pregnants with two exceptions (samples # 28 and # 97), but 2 different viruses were present in each of the two samples indicating that it was not the consequence of laboratory contamination.

The herpesviruses could be detected either in the supernates or in the cellular sediments of amniotic fluids with the exception of HCMV, where 3 samples labelled with asterisks (*) were present in both fractions of the amniotic fluid (Table III).

The observation, that the relative number of viruses was lower in the blood samples than in the amniotic fluid indicates, that the virus reactivation occurred earlier. Subsequently they were transported into the amniotic fluid. The viruses landed in the precipitate and in the meconium sediment, when they were reactivated and passed the placenta earlier. The reactivation of HHV6 and 8 (bold numbers in Table III) occurred probably just before delivery, or their passage through the placenta was slow, since the amniotic fluid/blood ratio of these differed significantly from the others (Pearson Chi-square test: $p < 0.001$).

Table III. Physical distribution of viruses in the supernates, cellular sediments and blood samples.

Viruses	Supernate of AF	Cellular sediment of AF	Total No. in AF	No. of viruses in blood samples
HCMV	1+3*	5+3*	9+3*	1
HHV7	0	8	8	1
EBV	1	3	4	1
HHV6	**0**	**1**	**1**	**3**
HHV8	**5**	**0**	**5**	**7**
Total	7+3*	17+3*	27+3*	13

Human papillomaviruses (HPV) were tested in 96 samples and 8 of them were found to be positive. The results are shown by arrows in Figur 1. The data of the Hungarian HPV genotypes are also plotted, to show, that the genotypes present in the amniotic fuids are different of those identified in tumours or in vaginal secretions. This is a second evidence, confirming the

absence of retrograde contamination, during sampling. More than half of the HPV types in clinical samples is genotype 16, but in the amniotic fluid no one of this type could be identified (Figure 1).

CONCLUSIONS

1.) The first part of this work characterized the hepatitis viruses in the Southern Plain Region of Hungary, before the onset of the mandatory HBV vaccination. New serological technique have been applied for the detection of HBxAg and for the detection of its specific antibodies.

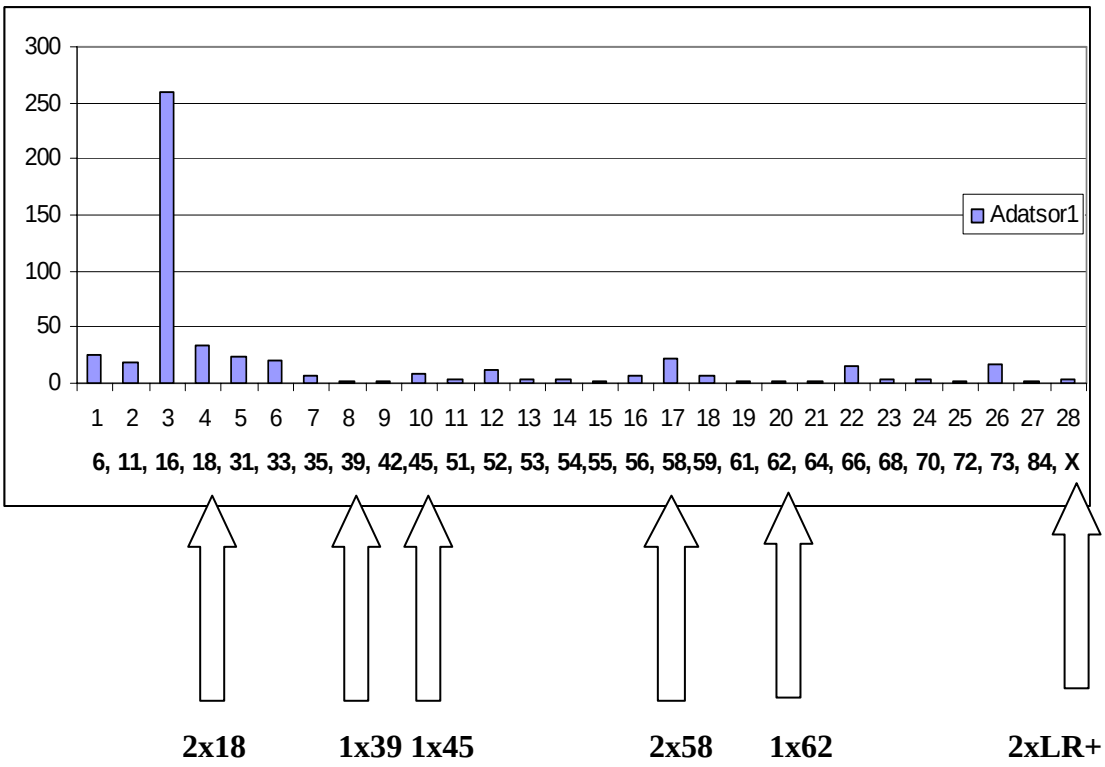

Figure 1. HPV genotypes identified in Hungarian patients (arrows: HPV genotypes in AF samples)

2.) The systematic virological screening of amniotic fluids revealed, that lymphotropic herpesviruses, papillomaviruses and endotoxin penetrate the placenta before delivery probably at differen rates, but no harmful effect of these to the development of the fetus could be detected.

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