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Short Communication

DOI:10.2478/rrlm-2020-0010

HBV genotypes circulation in pregnant women in Romania: a pilot study

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Abstract

***Background**: The risk of mother to child transmission of hepatitis B virus (HBV) is recognized worldwide, a reason for which the World Health Organization aims to reduce this public health issue of major concern in the next ten years. The aim of our study was to detect circulating HBV genotypes in a selected population of pregnant women, as scientific evidence to recommend personalized antiviral therapy and to obtain updated epidemiological information. **Methods**: HBsAg positive pregnant women were selected by the National Institute of Public Health Romania. Blood samples were collected after signing the informed consent. The HBV genotypes were tested by INNO LiPA HBV genotyping method. **Results**. The D genotype was detected in 9/18 (50%) patients, genotype A in 3/18 (16.7%), and genotype F in 3/18 (16.7%) patients. Three patients had double infection, 11 had unique infection, and 4 had no detectable genotype. **Conclusion**. This study confirmed the results of previous studies regarding HBV genotype circulation in our country, with the mention that F genotype was a new one for our area. These data are useful from an epidemiological point of view and also for therapeutical reasons, as it is known that therapy should be genotype guided.*

***Keywors**: HBV, genotype, pregnant women, antiviral therapy*

Received: 11th October 2019; Accepted: 12nd January 2020; Published: 17th January 2020

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Background

The World Health Organisation (WHO) recognizes the risk of maternal transmisson of HBV to newborns, therefore the plan is to reduce this risk by the end of 2030 (1). The *International Agency for Research on Cancer* (IARC) lists HBV as one of the known biological agents involved in human carcinogenesis since 1993 (2). Two hundred and forty million people are known worldwide with chronic HBV infection, with variation between 2 and 8%, acording to each regional endemicity levels. In some countries, HBsAg prevalence is decreasing due to vaccination and probably due to efficient therapy. An actual problem is the migration of population which leads to prevalence changes in countries with known low endemicity (e.g., Italy, Germany) (3).

The estimated age-standardized incidence rate in 2018, for liver cancer, both genders, all ages for Romania was 8.4, value which is one of the highest in Europe (4). A special category of HBV infected patients are the pregnant women, regarding the risk of transmission to new borns. Recently, the seroprevalence of HBV infection markers in this patients category has been evaluated in Romania, and a 5.1% prevalence of HBsAg was detected and a 7.4% for HBeAg among the HBsAg positive ones (5). The proportion of childbearing age women having natural immunity after HBV infection varied between 6.9 and 19.2% and increased with age group (6).

Viral markers such as HBeAg and HBV-DNA are known to influence the risk of hepatocellular carcinoma (HCC). Also, the viral genotype seems to modify the risk of HCC, but this data are difficult to analyze because of the global variation in viral genotypes circulation (2). Ten genotypes of HBV have been identified and labeled A to J. In comparison with genotypes B and D, genotype A is associated with significantly higher rates of HBeAg and HBsAg loss as a result of interferon (IFN) therapy. Differences have beed observed regarding the natural history of genotpes B and C evolution to HCC, in different areas of the world (e.g., Asia, Alaska) (7).

The starting point for our research was the previous studies performed on pregnant women in Romania, aiming to estimate the seroprevalence of HBV markers of infection (5) and also studies which evaluated the HBV genotypes in patients with chronic HBV infection (8,9). As HBV perinatal transmission is a problem of major concern, we thought that it is important to provide scientific evidence for personalized antiviral therapy and for public health interventions.

The aim of this pilot study was to evaluate the HBV genotype circulation in a selected group – pregnant women, from different regions of Romania, in order to establish personalized therapy and to obtain updated epidemiological information.

Material and methods

The selection of HBsAg positive pregnant women was done by the National Institute of Public Health Romania - National Centre for Communicable Diseases Surveillance and Control. The pregnant women were selected as the first who gave their consent to participate in the study. The number of subjects was imposed by the available financial resources. Of all the pregnant women who were tested for HBV markers during routine pregnancy control between May and August 2018, eighteen HBsAg positive pregnant women were selected from 4 different counties of Romania (Iași, Călărași, Sibiu, and Suceava). Sixteen out of 18 pregnant women presented themselves for HBsAg testing at the general practitioner, and only 2 were tested in maternity, immediately after admission to give birth. Blood samples were then sent to the Virology Laboratory of „Grigore T. Popa" University of Medicine and Pharmacy, Iași. The same protocol for HBV genotype detection was used for all samples.

Research ethics

All the patients included in this study signed the informed consent approved by the Research Ethics Committee of „Grigore T. Popa" University of Medicine and Pharmacy Iași. The study follows the international recommendations on human studies as stated by the Declaration of Helsinki.

The INNO LiPA HBV genotyping method followed the next steps: DNA purification, PCR amplification (outer and nested amplification), hybridisation of the amplicons, detection of HBV genotypes by strip hybridisation. All these steps were described in details previously (8,9).

Results

The median age of pregnant women was 31 years (23-39), and the median age of pregnancy was 28 weeks (9-39). Demographic and laboratory data can be seen in Table I. None of the pregnant women had been vaccinated and none was in antiviral treatment. Three patients had double infection, 11 had unique infection, and 4 had no detectable genotype. The D genotype was detected in 9/18 (50%) patients, genotype A in 3/18 (16.7%), and genotype F in 3/18 (16.7%) patients.

Discussion

In this study we confirmed our previous findings in patients with chronic HBV infections, using the same HBV genotyping method: D is the most frequent HBV genotype in our area, followed by A genotype, as unique or in double infections (8,9).

Many other authors have published their results regarding HBV genotype circulation worldwide, mentioning also the natural history of HBV infection and the role of HBV genotypes.

Table I. Demographic data, age of pregnancy, quantity of purified DNA, purity and the HBV detected genotypes

Crt. number	County	Age of pregnant woman (years)	Age of pregnancy (weeks)	DNA quantity ng/µl	DNA purity A 260/280	HBV genotype
1	Iasi	30	36	33,4	2.000	D, F
2	Iasi	32	35	42,9	1,955	D, F
3	Iasi	37	39	89,3	1,904	A, F
4	Iasi	34	16	54,4	1,946	D
5	Iasi	23	32	76,3	1,866	D
6	Iasi	23	13	31,9	1,939	D
7	Iasi	35	16	25,9	1,857	D
8	Iasi	32	15	90,3	1,885	D
9	Iasi	28	36	57,4	1,885	D
10	Iasi	29	37	28,9	1,933	D
11	Iasi	31	32	20,5	2,05	D
12	Iasi	30	9	17	2,1	A
13	Iasi	31	39	11,0	2,000	*
14	Iasi	23	31	13,0	2,167	*
15	Calarasi	27	24	19,5	2,167	A
16	Calarasi	39	22	24	2,087	*
17	Suceava	32	18	29,4	2,107	*
18	Sibiu	28	18	64,4	1,738	D

* undetectable

A research team from Fundeni Clinical Institute, Bucharest, detected the same two genotypes D and A in Romanian chronic infected patients (10, 11). In a recent review, Kmet Lunaček et al. confirmed the A and D genotype distribution in Europe, but also mentioned that 41% of non–A-D genotypes were identified in some European countries (12). Thereby, we mention the study of Toy M et al. in which the Dutch chronic B hepatitis (CHB) patients were detected positive for genotypes B, C, and G besides the known A and D genotypes (13). Also, in a study conducted on French population, Moussa S et al. detected genotype D, followed by E (14). Ghany MG et al. identified the distribution of HBV genotypes in a North American cohort (A 18%, B 39%, C 33%, D 8%, E 3%, and other 1%) and correlated the genotype with Asian, White, and Black races. Interestingly, among Whites they found genotypes A (55%) and D (33%) and this distribution was also correlated with the place of birth - Europe (15). A recent review highlights the relations between HBV genotypes and mutants, and hepatitis B vaccine failure, acute and chronic HBV infection, HBeAg seroconversion and HBsAg seroclearance, with the purpose to implement individualized management for HBV infected patients (16).

Some papers mention the subtypes of HBV and their role in influencing the response to antiviral therapy (17, 18), a relevant reason to test chronic infected patients. In a comprehensive review, Pourkarim MR et al. showed the role of the almost forty subgenotypes of HBV in the natural history of HBV infection. Also, the authors suggested the introduction of the term "immigro-subgenotype" to distinguish exotic (sub) genotypes from local known genotypes in each area of the world. The hope of the authors is that by vaccination, accurate diagnosis method, and monitoring therapy, elimination of HBV could be achieved (17). In another review of Rajoriya N et al., the genotype of HBV was mentioned as the first viral factor involved in a personalised medicine approach for the future treatment of hepatitis B infection (18).

Compared with the previous regional studies carried out in Romania, this study is also mostly a regional one, as 78% of tested pregnant women were from the North-East region, and only a few patients belonged to other counties (Calarași, Sibiu, and Suceava). Detection of F genotype of HBV in 3 cases, in double infection with A and D genotypes, was an unexpected result given the known global geographical distribution of HBV genotypes. We reviewed the literature and we found in a recent review that F genotype of HBV is usually present in South and in Central America, but it was also detected at the Arctic Circle and in Spain (19). Another recent review (Velkov S et al., 2018) established the following genotype distribution within chronic HBV infections: 0.86% for F genotype, 22.1% for D, and 16.9%, for A genotype. The authors of this paper analyzed the genotyping results of 26,319 HBV-infected individuals from 125 countries. 75% of studies analyzed the HBV genotype by sequencing and the rest by PCR – based methods. These authors also mentioned that genotypes F - I together account for only 1.3% of all infections, mostly in Latin America (20). There are not many studies published about F genotype, but we can mention that it was detected the second as prevalence in different regions of Brasil (21). Regarding its role in pathogenesis, Marciano S et al. specify that genotype F is correlated with a higher risk of HCC and mortality, and the respose to interferon is similar with that of genotype A (22). As a support for our findings is the study of Hirzel C et al. which detected genotype F in 5 cases (1.1 %) in a retrospective cohort study on Swiss patients having different countries of birth, using the same genotyping method (e.g., INNO LiPA) (23).

This study highlights for the first time the F genotype circulation in Romania, which is why these

3 cases have been thoroughly investigated from an epidemiological point of view. No direct or indirect link(s) with the above-mentioned geographical regions/country have been established. One of them could have been exposed, through a transfusion, 20 years before, and another one mentioned a long stay of her husband for work, in another European country.

The risk of mother-to-child transmission of HBV is correlated with an elevated risk of developing CHB, cirrhosis and HCC, and therefore, many professional associations have developed guidelines for the diagnosis and monitoring of pregnant women in order to reduce this transmission. One of them is the 2018 Guide of American Association for the Study of Liver Diseases (AASLD) which recommends the counseling of pregnant women for HBV vaccination, breast feeding, monitoring of HBsAg positive women, the risk of mother to child transmission, the necessity of testing the sexual partners for HBV. The same guideline suggest that HBsAg positive pregnant women should be tested for liver transaminases, HBV - DNA and assessed for the utility of antiviral therapy (7). The EASL (European Association for the Study of the Liver) 2017 Clinical Practice Guidelines suggest that in pregnant women with high HBV- DNA viral load or high levels of HBsAg, antiviral therapy should be initiated (3). The guidelines recognize the utility of HBV genotyping, as different responses to peg-IFN therapy were seen for different genotypes of HBV. Perinatal transmission from HBsAg positive pregnant women with high level of HBV-DNA could be reduced by antiviral therapy (7).

HBV genotyping in pregnant women was also performed by other authors. In a similar study to ours, 21 HBsAg positive out of 1489 Japanese pregnant women were detected positive for genotype C in 14 cases, D in six cases, and undetermined in one case (24). In a Chinese pregnant women study group, genotypes B and C of HBV were detected, genotype C being considered a risk factor for mother to child transmission (25). Denmark is another country which introduced HBV genotyping for pregnant women, genotype C being the most prevalent in their population (26). The pregnant women from an antenatal clinic in UK were detected positive for genotypes E (13/40, 32.5%) and B (10/40, 25%) as predominant, and for genotypes A (6/40, 15%), C (9/40, 22.5%), and D (2/40, 5%) in lower proportion (27). In a study performed in France, the author did not find any relation between the HBV genotype or origin of the patients and the risk of mother to child tranmission (28).

We are confident in our HBV genotyping method, as it is an optimized assay which has amplification, conjugation positive and negative controls. Four patients known to be HBsAg positive had no HBV genotype detectable. We analysed the literature and we found at least two other articles which reported the same untypable HBV genotypes. Possible explanations could be some immune escape mutants or the lower sensitivity of our genotyping method, so the untypable genotype can be completely identified only by sequencing and phylogenetic analysis of the S gene (29, 30) which was not the case in this study. A recent study from Norway supports this information, as the authors mentioned that their molecular epidemiology analysis indicated a geographical clustering of sequences depending on their geographical origin (31). Still, a comparison was performed between the Sanger sequencing assay and INNO Lipa method and the authors found a very good correlation of the results of both assays (32).

Another application of INNO LiPA is to test antiviral resistance. A multidisciplinary team concluded in a clinical trial that TDF (tenofovir) alone is safe and effective for the treatment of patients with lamivudine-resistant, chronic HBV infection (33).

From our point of view, the major disadvantage of INNO LiPA assay is that it is time consuming

(14 hours all procedure), and secondly, the costs which cannot be supported by patients. More developed techniques have beed used by other authors for HBV genotyping: Real Time PCR or sequencing (32, 34). The future of HBV testing regarding the genotypes worldwide distribution and antiviral resistance is by far the sequencing analysis, which, in countries such as Romania, requires larger studies. Due to financial constraints, this study was limited to only 18 pregnant women.

To achieve the elimination goal (1), building on such pilot studies that bring valuable data on circulating genotypes, personalized therapy, combined with public health actions at national level, could be keys to success.

Conclusions

In this study, we detected the genotypes of HBV in a group of pregnant women. The most frequent genotype was D, followed by A and F. The last one has never been described in our country to date. These data are important from an epidemiological point of view, regarding the HBV genotype circulation, and also for guiding a personalized antiviral therapy in case of chronic hepatitis B.

Abbreviations

AASLD - American Association for the Study of Liver Diseases
CHB - chronic B hepatitis
EASL - European Association for the Study of the Liver
HBV - hepatitis B virus
HCC - hepatocellular carcinoma
IARC - International Agency for Research on Cancer
IFN – interferon
WHO - World Health Organisation

Acknowledgments

We would like to thank the colleagues in the 4 participating county public health authorities (CPHA), with a special mention for Dr. Elena Duca, epidemiologist in Iasi CPHA.

Authors' contributions

O.P. coordinated the study, contributed to the methodology, collected the data, undertook the data analysis and participated in drafting the manuscript.

R.G.U. contributed to the methodology, performed the genotyping procedure, participated in drafting the manuscript.

D.A. and L.S.I. were involved in revising the article critically for important intellectual content; and final approval of the version to be published.

Conflict of interest

None to declare.

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