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ORIGINAL PAPERS

HYDATIDIFORM MOLE IN TUZLA CANTON

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ABSTRACT

Aim: Hydatidiform mole is a rare pregnancy disorder, with wide variety of reported incidence. The aim of the study was to estimate the incidence of hydatidiform moles (HM) in Tuzla Canton, specifically partial (PHM) and complete (CHM) forms.

Material and methods: All cases of HM that have been diagnosed at the University Clinical Center, Tuzla, between January 2011 and December 2015 were registered. The overall incidence of HM, as well as the incidence of PHM and CHM was calculated using the Tuzla Canton's live birth rate during the study period. A second review of tissue slides and p57 immunohistochemistry (IHC) staining was performed to determine the validity of the criteria for the diagnosis and distinction of the molar specimens.

Results: There have been 256 cases of HM, 243 cases of PHM, 12 cases of CHM, and one case of unspecified HM. The average incidence of PHM was estimated at 11.03/1,000 and CHM at 0.54/1,000 live births. A second pathologist review revealed one PHM as a non-molar specimen, confirmed all CHM and identified an unknown HM as a PHM. Out of the 50 randomly chosen samples of PHM, p57 expression confirmed the diagnosis in 48 cases, disclosed one case as unrecognized CHM, and one sample showed discordant staining. IHC staining for p57 approved the diagnosis for all cases of CHM.

Conclusion: HM incidence reporting remains a challenge due to the study design. p57 immunohistochemistry confirmed the strong validity of histopathological criteria in the diagnosis of CHM.

Keywords: hydatidiform mole, partial mole, complete mole, incidence, immunohistochemistry

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INTRODUCTION

A hydatidiform mole (HM) is the most common form of the gestational trophoblast disease (GTD) that develops as a result of viable conception failure with a disrupted ratio of paternal and maternal genes. Domination and overexpression of paternal genes that enhance trophoblast growth and invasion may be relative or absolute, with subsequent development of partial (PHM) or complete (CHM) hydatidiform moles, respectively. Although HMs are benign disorders, the risk of developing gestational trophoblastic neoplasia is significantly higher after CHM as compared to PHM, for both single and twin pregnancy with coexisting fetus. Post-evacuation treatment planning therefore requires a clear distinction of these conditions [1–5].

The variation of geographic distribution is one of the most interesting characteristics of GTD and has been recognized for several decades. Clear differences in incidence are observed in different parts of the world, but the results are difficult to compare due to differing methodologies and population selections. GTD incidence is low in Europe and North America, at approximately 1.22 to 2,08 per 1,000 deliveries, while in some Asian countries the relevant figure is seven to 10 times higher. HM incidence worldwide is estimated at 2.4 per 1,000 live births, with the proportion of CHM at between 25 and 55% of that figure [6–11].

Pathohistological examination of hematoxylin-eosin (HE) slides of the products of conception is a gold standard in the diagnosis of HM. Several pathological characteristics contribute greatly to the diagnosis of molar pregnancies and help in their classification as either CHM or PHM. Criteria such as trophoblast proliferation and central cistern formation are considered to be principal histological features, along with the absence or poor development of fetal vessels and two populations of villi. However, de-

spite the well-known histopathological characteristics of both CHM and PHM, several genetic studies have reported interobserver variability and suboptimal accuracy of diagnosis based on HE slides alone [12–15]. The diagnosis is refined with the use of several ancillary techniques, and studies confirm the strong value of immunohistochemistry (IHC) analysis of p57 (or $p57^{Kip2}$) expression for diagnosis and distinction of CHM and PHM. The protein p57 is a product of the cyclin-dependent kinase inhibitor 1C gene (CKDN1C), located on chromosome 11 pl5.5, which is a paternally-imprinted and maternally-expressed gene. Without the maternal chromosome, androgenetic conceptions do not express positive nuclear staining among referral population of cells [16–18].

This retrospective study was performed with the aim of estimating the incidence of HM based on pathohistological reports from the Department of Pathology at the University Clinical Center, Tuzla, which provides healthcare for approximately 450,000 residents of the Tuzla Canton in Bosnia and Herzegovina. Most of the samples were provided by the Clinic of Gynecology and Obstetrics, University Clinical Center, Tuzla, while a small number were collected from two local hospitals and private practices, due to the rule of pathohistological examination of all products of conception. IHC analysis of staining for p57 expression was used to assess diagnostic accuracy based on HE slides examinations.

MATERIAL AND METHODS

All cases of molar pregnancies that have been diagnosed at the Pathology Department (Laboratory Diagnostic Clinic of the University Clinical Center, Tuzla) between January 2011 and December 2015 were registered.

The incidence of HM was calculated according to the overall live birth rate in the Tuzla County (data provided by the Clinic of Gynecology and Obstetrics, University Clinical Center, Tuzla, and two local hospitals). The incidence of CHM and PHM, more specifically, were also determined.

Second review by a single experienced pathologist and the selection of representative slides for IHC staining for p57 expression was performed. Out of the total PHM cases, 50 randomly chosen samples of PHM, all samples of CHM and unspecified HM were prepared for IHC.

Immunostaining

IHC staining was performed on formalin-fixed, paraffin-embedded tissue samples, cut on 4μm, using rabbit polyclonal antibody (ThermoFisherScientific, Rockford, Illinois, USA, PA5-32532) with 1:100 dilution. Prior to staining, 1mM citric buffer (pH 8.0 at 100°C, 10-minute duration) was used for antigen retrieval. A Shandon Sequenza Immunostaining Center was used for all incubation stages. After 30 minutes of incubation with the primary antibody, samples were treated with the secondary antibody, signed with biotin, streptavidin and peroxidases. Mayer's hematoxylin was used for nuclear counterstaining and Canada balsam was used for mounting the slides. Placental tissue applied on every slide, and treated with the same procedure, served as an external positive control. Microscope Nikon ECLIPSE E400, magnification 40x, was used for analysis of p57 expression.

Interpretation of p57 expression: Diffuse nuclear p57 expression of villous cytotrophoblast and stromal cells were marked as positive and such expression was consistent with the diagnosis of PHM. Expression of p57 was signed as negative when less than 10% of villous cytotrophoblast and stromal cells showed nuclear positivity, aiding in the diagnosis of CHM. Clearly positive nuclei of extravillous trophoblast cells served as positive internal controls for both PHM and CHM. The results of IHC staining were compared with the pathohistological diagnosis based on examination of HE slides.

RESULTS

During the study period, 256 pathohistological diagnoses of molar pregnancies were verified, of which 243 were PHM, 12 were CHM and one was an unspecified HM incident. All samples were collected by suction curettage.

In the same period, 22,022 live births were registered in the Tuzla Canton.

The average incidence of HM for the period 2011-2015 was estimated to be 11.80/1,000 live births; with an average PHM incidence estimated at 11.03/1,000 and CHM at 0.54/1,000 live births. Table 1 presents the annual incidence of PHM and CHM, calculated per live birth rate per year.

Table 1. Observed incidence of CHM and PHM between January 2011 and December 2015

	2011	2012	2013	2014	2015
Live birth rate/Year	4577	4636	4436	4327	4046
CHM total/ Incidence	1/0,21	-	3/0,67	2/0,46	6/1,48
PHM total/ Incidence	51/11,14	50/10,78	26/5,86	61/14,09	55/13,59

A surprisingly high incidence of PHM was observed and, consequently, an unexpected ratio of PHM to CHM was found (20:1 on average). Further analysis showed a great variation in incidences calculated per year, with the lowest incidence identified being 2013, which was approximately half that of other observed incidences. As presented, the decreased live birth rate was not accompanied by a reduction of PHM incidence.

Second pathologist's review disclosed one PHM specimen as non-molar, confirmed all CHM diagnosis while unspecified HM was determined as partial form.

Analysis of IHC staining for p57 expression

IHC staining for p57 expression was performed and satisfactory staining results allowed the distinction of p57 negative and p57 positive molar pregnancies.

Out of the 50 randomly chosen PHM cases, clear p57 positivity was identified in 48 samples. One sample of PHM expressed discordant staining which was presented as follows: diffuse positive nuclei of villous cytotrophoblast with low or negative nuclear staining among stromal cells. The remaining sample previously diagnosed as PHM showed diffusely negative nuclear staining for p57, and was therefore found to represent an unrecognized case of CHM. All 12 studied cases of CHM were p57 negative. The sample of unspecified HM diagnosis showed diffusely positive nuclear staining of villous cytotrophoblast and stromal cells, indicating PHM. p57 immunostaining confirmed the efficiency and accuracy of the diagnosis for both PHM and CHM based on HE slides, leaving the overall incidence insignificantly changed.

The representative cases of PHM and CHM confirmed with p57 immunostaining are presented in Figure 1.

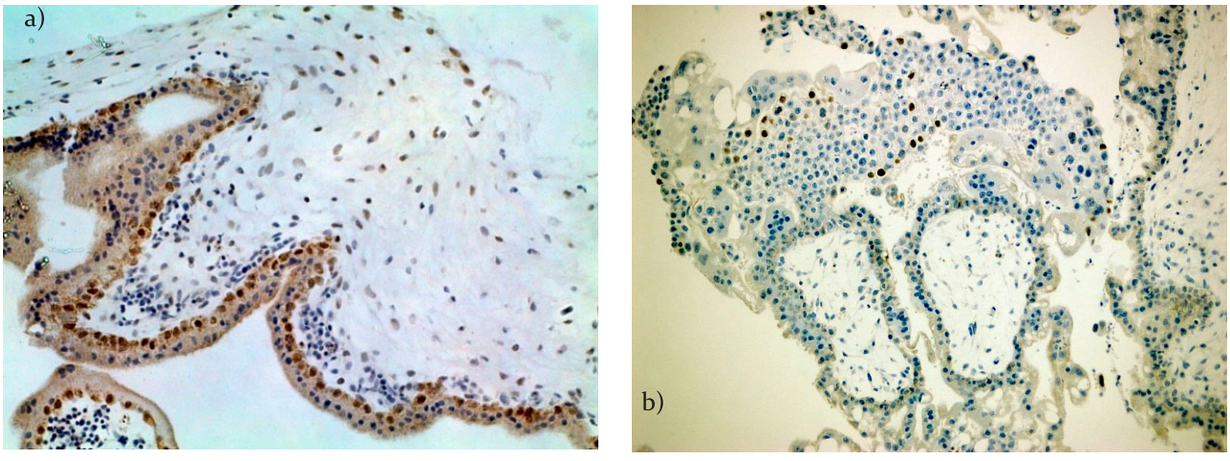

Figure 1. IHC staining for p57, 40x. a) Strong nuclear expression both villous cytotrophoblast and stromal cells consistent with the diagnosis of PHM. b) Typical CHM lacks the expression among villous cytotrophoblast and stromal cells (positive internal control were nuclei of extravillous trophoblast).

DISCUSSION

In this five-year long study, we observed an unexpectedly high incidence of PHM, but not CHM, considering the geographic localization of our country (Southeastern Europe). Wide incidence variation of PHM between the studied years was observed – for example, the incidence in 2014 was twice as high as in 2013. The primary diagnosis, based on pathohistological examination of HE slides, was reappraised during a second review by a referent pathologist, who excluded one partial form non-molar specimen. The results of p57 expression showed that the accuracy of diagnosis of PHM and CHM based on morphology is very high and satisfactory.

The reporting and interpretation of GTD incidence data pose a great challenge due to several issues. The rarity of this specific group of pregnancy disorders, reporting based on different incidence denominators (number of pregnancies, deliveries, live births) and populations (hospital-based vs. community) are recognized as possible causes of under- or overestimation of GTD incidence [6]. Ethnicity and race are the most commonly referenced factors that determine the risk of developing HM and gestational trophoblastic neoplasia. The available data report a higher incidence among Asian women, while the lowest incidence is found in North America and Europe. Additionally, we noted inconsistent data regarding the GTD incidence trend. An increasing incidence is observed, for example, in the Netherlands and China. Improvement in socioeconomic conditions and diet have been recognized as potentially factor that contribute with declining the incidence of HM worldwide [8,10,19–24]. A study in the USA regarding a racially/ethnically heterogeneous population of patients referred to single health center showed that Asian women are at an increased risk of developing CHM, and black women of PHM, as compared to white women [25].

Most reports of high HM incidence are based on hospital or tertiary center studies, despite the observation that such approaches may lead to under- or (more often) overdiagnosis of HM. Institutional incidence, therefore, has become a well-known and widely used term [26–29]. Systematic analysis and multicenter studies report significantly decreased incidences of HM [19], but population study in Japan provides diver-

gent data on molar incidence through several decades [20]. Recent researches reported delay of medical care as well as medical abortions and selected forwarding of products of conception as a substantial cause of changes in incidence of molar pregnancy [30,31].

Although all cases were confirmed by the Department of Pathology at the University Clinical Center, Tuzla, ours is not a typical single-center study. Following the principle of obligatory pathological examination of all products of conceptions, all specimens from the Clinic of Gynecology and Obstetrics, two local hospitals and several private practices were addressed to referral center. The classification of this study is thus uncertain, as it could be designated as a small community-based study, though the population of Tuzla Canton represents approximately one-eighth of the population of Bosnia and Herzegovina.

Among other possible factors that contribute to the incidence of HM, only low beta-carotene and animal fat intake are recognized as consistent environmental etiological factors for CHM, though not for PHM [32]. We have no exact data regarding undernutrition among our studied population, which could partly explain the observed HM incidence. Nevertheless, we believe it is important to note that Bosnia and Herzegovina, based on socioeconomic data, the high rate of unemployment, is a developing country. This might be an indicator for planning future research regarding HM involving data related to nutritional habits, healthcare and socioeconomic status, thereby helping us to understand the etiological factors that potentially contribute to the incidence of HM.

From the beginning of the present study, we became aware of the divergent incidence of PHM as compared to CHM. Moreover, in one year alone (2012), 50 cases of PHM and none case of CHM were identified. Researchers report divergent data on the PHM to CHM ratio, from 3:1 (similar to the results of countries with low HM incidence), to as much as 1:50 [26–28]. Our results demonstrate a PHM to CHM ratio that is divergent to an extent that is difficult to explain, especially as compared to the earlier reported data [8–10]. However, reports of extremely high incidence ratios are not supported by either second pathologist reviews or ancillary technique applications that would support the primary results, as were performed in our study.

Intra-observer and inter-observer variability in diagnosis and the differentiation of HM from non-molar pregnancies, which comprise a substantial proportion of post-conceptional specimen, are well known. There are some evidences regarding existing significant differences of histological features of partial mole and molar mimics, such as digynic triploidy, that could help to differentiate these conditions [33]. A significantly higher risk for developing persistent GTD and gestational trophoblast neoplasia makes underdiagnosis of CHM a less favorable outcome than overdiagnosis. Nevertheless, the exact cause of insufficient diagnostic accuracy based solely on HE slides remains unknown [4,5,7,15,16,34].

In this study, a second review was performed by a pathologist with many years' experience in gynecological pathology. During the study period, the incidence of unspecified HM was calculated at 0.004/1,000 live births. Discovery that one sample of PHM was in fact non-molar specimen left the overall incidence of HM insignificantly changed. This result is similar to earlier finding [8] and leads to the conclusion that the existing pathohistological criteria are reliable for diagnosis based on HE slides. IHC staining for p57 performed in our study demonstrated a high level of efficiency of diagnosis based on HE slides and agreement between primary diagnoses, second slide review and the results of p57 expression, and for CHM the accuracy of this method reaches 100%. Confounding results of staining were observed in one case of PHM. Genotyping identifies mosaicism, multiple pregnancies and trisomy of chromosomes as possible causes of divergent p57 expression. Less than 1% of HMs show an unusual genetic basis that makes p57 expression difficult to interpret and potentially leads to incorrect diagnosis. However, the high correlation of p57 expression results with genotyping makes p57 staining a reliable ancillary diagnostic procedure [4,14,18,19,35,36].

The incidence of PHM observed in our study may be considered to be confined to this specific dataset given the population included in the study, even though we calculated the incidence according to the live birth rate, as is recommended. We believe that this consideration gives good grounds for planning inter-institutional collaboration with other health centers in Bosnia and Herzegovina. Further, clinical characteristics and potentially repetitive HM are yet to be analyzed and discussed.

In conclusion, the results reported in our study request a large retrospective study in order to identify the factors that contributed unusually high incidence of PHM but not CHM. p57 IHC confirmed the validity of histopathological criteria in the diagnosis of CHM. Although satisfactory results were achieved for PHM, other ancillary techniques are recommended in order to obtain absolute diagnostic accuracy.

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