Challenges in the Routine Praxis Diagnosis of Hydatidiform Mole: a Tertiary Health Center Experience

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ABSTRACT

Introduction: Hydatidiform moles (HM), presenting as complete (CHM) and partial (PHM) form, are rare pregnancy disorder. Diagnosis is based on clinical presentation, ultrasound imaging findings and pathological examination of products of conception. Protein p57, encoded by CKDN1C gene, is paternally imprinted and maternally expressed gene and provides quick insight in genetic basis of HM and allows distinction of CHM from all other conceptions. compare the preevacuational and pathohistological diagnosis with outcome of p57 immunostaining. Material and methods: All cases of HM diagnosed between January 2011 and December 2015 were included in this research. Maternal age, gestational age and input diagnosis data were recorded. p57 immunostaining was performed in order to evaluate the diagnosis based on tissue slides examination. Results: There were 198 cases of histologically confirmed HM, 185 PHM, 12 CHM and one case of undefined HM. Mean maternal age in the CHM group was 24.7 and in the PHM group 26.9 years, with no significant differences among these two groups (p>0.27). For CHM mean gestational age was estimated at eight and for PHM 9.2 gestational weeks. Pregnant woman older than 40 years present significant earlier compared with younger woman (p<0.01), and those younger than 20 years tend to present at the beginning of the second trimester more often than older women (p<0.05). In the CHM group, 9 (75%) input diagnoses were mola in obs, and 3 (25%) of them were signed as abortion, unlike the PHM group where 126 (67%) were qualified as abortion, 35 (19%) as blighted ovum, and 26 (14%) were suggestive for molar pregnancy. p57 immunostaining results confirmed all pathohistological diagnosis of CHM whereas 8% of PHM demonstrated divergent p57 expression. Conclusion: PHM, compared with CHM, represent a greater diagnostic challenge for both gynecologist and pathologist even when presenting in more advanced pregnancies.

Keywords: hydatidiform mole, ultrasound finding, p57 immunostaining.

1. INTRODUCTION

Safe Motherhood program was launched in 1987, more than half of a million women living in developing countries died each year. In 2016, the program has reached three decades and yet there have not been changes in developing countries to overcome the postpartum infection. In addition, the economic gap between rich and poor women were also increasingly wider thus it worsened this issue (1). Vaginal epithelium will anatomically and functionally take part in the cycle and depends on age. When reaching puberty, the vaginal epithelium thickness and resistance may increase (2). After giving birth, vaginal epithelial cells will be sensitive to infection. In order to protect the vaginal cells, T cells will secrete IFN-g to capture pathogens (3, 4). In the model of mice vagina that was infected with herpes simplex virus-2, there was an increase in IFN-g secretion caused by the up-regulation of class II MHC antigen. Thereby, postpartum vaginal protection is very important to protect women.

During women fertile phase, vaginal epithelial layer has four layers, they are basal, parabasal or suprabasal layer that actively experiences mitosis, intermediate layer that contains glycogen and the non-cornification superficial layer with pycnoticnucleus. Vitamin A is an immunological therapeutic agent that serves as a differentiation stimulus of various cells in various tissues (6-10). Vitamin A deficiency can lead to squamous metaplasia in uterus epithelium and endocervix. This deficiency can also trigger ectocervical and vaginal epithelial keratinization (8, 11). In the development phase, the A vitamin-deficient mice were characterized by an increasing level of IFN-g indicating the tissue in-
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2. AIM

Aim of study was to identify the contribution of gynecologist in the diagnostic decision of the pathologist in the diagnosis of the CHM and PHM, based on routine microscopic examination of molar specimen, and compare the result with the outcome of p57 immunostaining.

3. PATIENTS AND METHODS

This research included all cases of HMs and supporting data attached to the tissue specimen provided by clinical gynecologist (Clinic for gynecology and obstetrics, University Clinical center Tuzla), at the Pathology department, Policlinic for laboratory diagnostic, University Clinical Center Tuzla, Bosnia and Herzegovina, from January 2011 to December 2015. Following data were analyzed: maternal age, gestational age by last menstrual bleeding (gestational weeks (GW)), input diagnosis and parity.

First, we estimated the correlation of referred data and the diagnosis of the pathologist based on pathohistological examination of hematoxylin-eosin (H-E) slides. Then we performed the selection of representative slides (ensuring presence of extravillous trophoblastic columns and maternal decidua) for p57 immunostaining, all CHM and 50 randomly chosen sample of PHM. As recommended by producer, as an external positive control, health placental tissue was applied on every slide. Immunostaining 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 dilutions. 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. Microscope Olympus bx41, magnification 40x, was used for analysis of p57 expression. Interpretation of p57 expression: Expression of p57 was signed as negative when less than 10% of villous cytotrophoblast (CTB) and stromal cells showed nuclear positivity, aiding in the diagnosis of CHM. Diffuse nuclear p57 expression of villous CTB and stromal cells was marked as positive and consistent with the diagnosis of PHM. Clearly positive nuclei of extravillous trophoblastic and maternal decidual cells served as positive internal controls for both CHM and PHM, while consistency of negative stained nuclei of syncytiotrophoblast (SCTB) served as internal negative control. p57 immunostaining results were interpreted regardless of preliminary or diagnosis based on reevaluation.

The earlier verification of pregnancy disorders the higher risk for under or overdiagnosis of HM exists for both gynecologist and pathologist. Significant intra and interobserver variability is well known for all forms of HM. Therefore we performed p57 immunostaining in order to evaluate the results with the data attended with the tissue specimen. Therefore, the purpose of the research was to estimate the contribution of referent gynecologist in the diagnosis of CHM and PHM in routine praxis. Data were analyzed with statistical program Arcus Quicksat Biomedical at the significance level p<0.05.

4. RESULTS

There were recorded 198 cases of HM, 185 (93,5%) was PHM and 12 CHM (6%) and one case (0.5%) of undefined HM. There were two patients with two consecutive PHMs, and therefore 197 women were included in this research. All samples were collected performing surgical uterine cavum evacuation by suction or blunt curette procedure. Clinical data. Mean maternal age in the CHM group was 24,7 (from 17 to 36) and in the PHM group 26,9 (from 18 to 50) years, with no significant differences among these two groups (p=0,27).

For CHM mean gestational age was estimated at eight (from six to 10) and for PHM 9,2 (from six to 14) gestational weeks.

There were no significant correlation of maternal and gestational age, although a trend of earlier presentation in pregnancy was observed among older women (r=−0,281). However, when comparing maternal age, divided into following group: ≤ 20; from 21 to 30; from 31 to 40, and ≥41year, with gestational age (≤8 GW, from 9
to 12 GW, and ≥13 GW), we found that pregnant women older than 40 years present significantly earlier (before or during 8 GW) compared with the other groups (p<0.01), whereas pregnant women younger than 20 years tend to present at the beginning of the second trimester more often than older women (p<0.05). Pregnant women of age between 21 and 30 years, as well as from 31 to 40 years did not differ significantly among other pregnant women.

Outcome of ultrasound examination, carried out by referent gynecologist, was some of the following diagnosis: abortion (missed or incomplete), blighted ovum (anembryonic pregnancy), and finding suggestive for molar pregnancy (mola in obs).

In the CHM group 9 (75%) of all input diagnoses were mola in obs, and 3 (25%) of them were signed as abortion, unlike the PHM where 126 (67%) of specimen were qualified as abortion, 35 (19%) as blighted ovum, and 26 (14%) demonstrated changes suggestive for molar pregnancy. Therefore, only 9% of all molar specimen were correctly diagnosed prior the evacuation. Sensitivity of applied ultrasound diagnostic criteria was estimated at 0.75 for CHM and 0.14 for PHM.

In the CHM group, three women (25%) had positive anamnestic data on earlier positive pregnancy outcome, for the rest of the women this was the first pregnancy.

In the PHM group, there were 43 women (20%) with positive anamnesis earlier pregnancies favorable outcome. For 127 (60%) of patients with PHM this was the first conception event, and there were 42 (20%) of positive history on earlier spontaneous abortion in the PHM group.

4.1. IMMUNOSTAINING RESULTS FOR P57

Performed immunostaining for p57 achieved satisfactory staining result for distinguishing CHM and PHM. Extravillous trophoblastic columns, as an internal parenchymal positive control, disposed a great variability of percentage of p57 positive nuclei, showing strong positivity regardless of the type of the mole. In comparison, higher percentage, but lower intensity, of p57 positive nuclei was observed among maternal decidua cells.

Out of the 12 samples diagnosed and confirmed as CHM, satisfactory staining results were obtained for 11 cases and one sample was signed as unsatisfactory. Clearly negative CTB and stromal cell's nuclei confirmed the diagnosis of CHM, and neither divergent nor doubtful staining result was recorded (Figure 1a). Out of the 50 samples of PHM, 46 (92%) displayed expected p57 expression (more than 10% of positive nuclei among referent cellular population) (Figure 1b). Four samples (8%) indicated the presence of divergent p57 expression, positive stromal but negative CTB nuclei and vice versa or presence of two villous populations with positive CTB but positive or negative stromal cell (Figure 1c and 1d).

In the CHM group, there were no differences between the result of pathohistological and diagnosis based on the results of p57 expression.

Among PHM samples with divergent p57 staining result there were three samples with input diagnosis of abortion and one sample with the input diagnosis suggestive for molar pregnancies. No significant differences have been observed among input diagnosis of the samples with expected and divergent p57 expression (p>0.05). Gestational age was not in significant relation with outcome of p57 immunostaining for PHM (p>0.05).

5. DISCUSSION

Early recognition of complicated or failed pregnancies increases the risk of error in diagnosis for both the gynecologist and pathologist. Well defined differences in post-evacuation management and prognosis underline the importance of clear distinction of PHM from CHM as well as true moles from molar mimics. With only seven cases evacuated at the beginning of the second trimester (majority diagnosed before the onset of typical symptoms and clinical findings) this study accentuate the diagnostic challenges that gynecologists face with and the importance of the pathologist in the diagnosis of molar pregnancy. Routine H-E slides examination of products of conception disclosed that significant proportion/portion of the first trimester PHM remains an underdiagnosed early pregnancy condition for gynecologist. More specific ultrasound presentation as well as the morphological features were seen in the first trimester CHM, where p57 immunostaining results confirmed all ultrasound and morphologic diagnoses.

The diagnosis of HM is based on clinical finding/presentation, ultrasound imaging findings and pathohistological analysis of a product of conception. Gestational age highly correlates with the accuracy of the pre-evacuation diagnosis of HM, particularly considering the clinical presentation with symptoms typical for molar pregnancy, such as vaginal bleeding, hyperemesis gravidarum and uterus enlarged for gestational age (18). Systematic use of ultrasound examination in follow-up of pregnancy significantly decreased the proportion of HM with advanced clinical symptoms (19, 20). Several studies confirmed the importance of pre-evacuation ultrasound imaging in the diagnosis of different forms of GTD as pregnancy advances (12, 14). Lindholm et al, reported that ultrasound finding and macroscopic examination are sufficient for the diagnosis of 80% of CHM and 30% of PHM (21). Since the beginning of ultrasound era, there were reports on hydropic abortion that imitate the molar changes, and such reports became common finding, even nowadays (22-24). The ultrasound finding of failed or pregnancies with signs unusual for gestational age during the first trimester of pregnancy, suffers from possible under or sometimes, over-diagnosis of molar pregnancies (25). In consideration to the potential for malignant transformation, some researchers believe that unrecognition of CHM is less favorable compared with the incorrect diagnosis of the CHM (26). However, such opinion is unacceptable for population of older women, whit physiological reduced possibilities for conception. Compared with the results provided by Sebire et al, (27), our results suggests significant decrease of ultrasound finding suggestive for mole among all cases of HM (34% vs 9%). Separating CHM and PHM disclosed that the PHM is the leading cause of misdiagnosis. The same
Improvement of ultrasound equipment helps in reaching the finding similar with the Benson et al. (28). Improvement of ultrasound equipment helps in reaching the correct diagnosis of CHM, where marked cystic formation becomes relatively easy to identify (23). Pathohistological analysis of the product of conception is known as the "gold standard" in the diagnosis of molar pregnancies (29). However, numerous studies reported on significant intra- and inter-observer variability of the final diagnostic decision based on tissue slides examination for both partial and complete forms of molar pregnancies even among experienced pathologists (30).

A great variations between samples has been recognized as a possible cause of misdiagnosis even when morphological features are well developed (17). Significant genetic differences between CHM and PHM underlies the different behavior of CHM and PHM and potential for development persistent gestational trophoblastic disease or neoplasia. According to data, abnormal trophoblast persistency follows between 15% and 33% of CHM and 5% to 12% of PHM, whereas metastatic disease occurs in approximately 4% CHM (31-33). These observations, considering diagnostic dilemmas as well as prognostic implications, resulted with the application and introduction of several ancillary techniques in the diagnostic procedure for molar specimen. The protein p57, product of CKDN1C gene (creatkinase dependent inhibitor 1c) is paternally imprinted and strongly maternally expressed gene. Therefore its expression allows distinction of typical complete molar pregnancies and all other product of conception. Rare and unusual cases of CHM with retention of maternal chromosome 11 demonstrates p57 positivity, such observations are usually reported as case report and unexpected finding. Presence of maternal set of genes in cases of hydropic abortion and trisomy conception disallows the distinction of PHM from these conditions (15-17). Significant correlation of p57 expression with high demanding diagnostic procedure, i.e. molecular genotyping, appoints the importance of p57 immunostaining in the diagnostic algorithm of molar pregnancies (34). p57 immunostaining result in our study points that the diagnosis of CHM in the first trimester does not represent significant diagnostic problem, and, when excluding confirmation of all CHM diagnosis. Histopathological presentation of PHM provides a wide variety of morphological characteristics, from two populations of villi, where some of the villi with morphology regular for gestational age to largely extended hydropic villi, with or without trophoblastic pseudoinclusions (29).

Some studies reported that systematic reevaluation or ancillary technique application significantly decrease apparently high incidence of the PHM (35, 36). Romaguera et al, indicated that p57 is valuable in the differentiation of PHM and hydropic abortion (37). However, other researchers reported that PHM represents a greater diagnostic problem compared with the CHM, when distinguishing from molar mimics, particularly hydropic abortion (38, 39). Unexpected and divergent expression of p57 have been previously reported in case of complex pregnancy, such as retention of maternal chromosome 11, mosaic/chimeric conceptions, as well as in case of unrecognized twin pregnancies, where divergent p57 expression cannot exclude the presence of a molar population of villi (17, 40, 41). Similar to earlier reports, a discordant staining pattern in our study was noted among the population of stromal cells as well as villous cytotrophoblast cell line. Unchanged potential for the development of the persistent gestational trophoblastic neoplasia in such cases with divergent p57 expression, highlights the importance of ancillary techniques in the diagnosis of such confounding cases (42).

6. CONCLUSION

Although PHM represents a form of gestational trophoblastic disease with more favorable outcomes, considering prognosis and management, compared with the CHM, in the majority of cases they represent a greater diagnostic challenge even in more advanced pregnancy. Divergence of p57 expression in failed pregnancies diagnosed as PHM, indicate that more complex genetic basis of the conception may underlie the histomorphological features indicative for molar pregnancy.

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