ABSTRACT

Introduction: Hydatidiform mole is a gestational trophoblastic disease characterized by a range of disorders of abnormal trophoblastic proliferation.

Methods: This was a retrospective study of 70 singleton pregnancies until the 12th week of gestational age diagnosed with hydatidiform mole or spontaneously aborted physiological pregnancy. The pregnant women had almost similar demographic features and were divided into two groups. 35 pregnant women with a molar pregnancy were included in the study group; while 35 pregnant women with physiological pregnancy spontaneously aborted were included in the control group. Analyzed parameters included a pregnant woman’s age, blood type, parity and previous pregnancies (course and outcomes).

Results: In the study group 11.43% of cases had hydatidiform mola during previous pregnancies as well as the advanced average gestational age of an ongoing pregnancy (9.63±1.83 in contrast to 8.25±2.03 in the control group). The pregnant women with the hydatidiform mole were reported to have statistically significantly greater number of irregular villous borders (71.43%); slightly enlarged villi (54.29%); moderated presence of cisterns (65.71%) as well as mild avascularisation of villi (57.14%).

Conclusion: It was concluded that a previous molar pregnancy represents the highest risk for hydatidiform mole and the pathomorphologic analysis of vilous changes can be a reliable parameter for establishing proper diagnosis of partial hydatidiform mole.

Keywords: hydatidiform mole, epidemiologic factors, resorption villi

INTRODUCTION

Hydatidiform mole is a gestational trophoblastic disease characterized by a range of disorders of abnormal trophoblastic proliferation. Based on differences in morphology, histopathology, karyotype and clinical features, hydatidiform mole can be categorized into partial and complete moles. A partial mole (25-45% of all molar pregnancies) is characterized by a partial degeneration of villi while trophoblast proliferation is focally pronounced (1).

Molar pregnancy incidence varies across countries. The incidence of molar pregnancy in Asia is 1:100-300, choriocarcinoma 1:1000-2000 of live born children; while the incidence of molar pregnancy in the countries of America and Europe is 1:1500-
2000, and choriocarcinoma 1:2000-40000 of live born children (2). However, the precise incidence date cannot be reported for Bosnia and Herzegovina. Limited data are available regarding risk factors for the occurrence of partial mole associated with oral contraception, irregular menstrual cycles, higher educational levels, smoking, history of molar pregnancy resulted in a live born male infant (3). Hydatidiform mole risk is more likely for pregnant women aged over 35 (4) and in women with the blood type A (5). Recurrent hydatidiform mole occurred in approximately 0.5-2.5% women in a subsequent pregnancy. The risk of invasive mole or choriocarcinoma is substantially increased in those women (6). Hydatidiform mole was benign in 75-80% patients and was spontaneously reduced after the uterine evacuation. The molar pregnancies, however, are regarded as premalignant lesions as well. Approximately 15-25% of moles developed into invasive moles, and 3-5% into a choriocarcinoma (8). Gestational choriocarcinoma was preceded by hydatidiform mole in 30-60% of cases which was 1000 times greater than after a normal pregnancy (4).

**METHODS**

This was a retrospective study which included 70 pregnant women diagnosed with hydatidiform mole or with physiological pregnancies spontaneously aborted. Based on survey results and a patient’s file, diagnostic test data were processed while findings were pathohistologically verified. The pregnant women were reported to have almost similar demographic characteristics and were included in two groups. 35 pregnant women with a molar pregnancy diagnosed during the first trimester treated by evacuating the molar tissue by uterine suction or curettage were included in the study group. 35 pregnant women with physiological pregnancy spontaneously aborted during the first trimester treated by uterine suction or curettage were included in the control group. Inclusion criteria: 1) singleton pregnancy, 2) gestational age until the 12th week, 3) reliable gestational age (exact date of the last menstrual period, early ultrasound examination), 4) molar pregnancy diagnosis (history, gynecological and ultrasound examination, serum β HCG levels and pathohistological tissue verification).

Analyzed parameters included age, blood type, parity and previous pregnancies (course and outcomes) and a gestational age of an ongoing pregnancy.

Conception tissue obtained after the suction curettage of the cavum uteri was fixed in buffered formaldehyde solution (pH 7.2-7.4), paraffin embedded, while 4μm thick histological sections were stained by haematoxylin and eosin method to examine the basic light-microscopic morphological characteristics.

**Statistical analysis**

Derived values were processed by standard statistical methods such as calculation of mean and standard deviation or median and interquartile range depending on data distribution. Standard level of significance p<0.05 was chosen as the statistical significance statistical tests the Mann Whitney test, $X^2$ test and Fisher test were also used for evaluation.

**RESULTS**

**Pregnant Women Age**

Information on a number and characteristics of patients in both groups according to their age are shown in Figure1.

The prevalent age was between 30 and 34 years in the study group, while the prevalent age was 35-39 years in the control group. There were 2.86% of pregnant woman aged over 40 years in the study group, which was not, however, recorded in the control group. The obtained results and the collected data show that the average age was 29.92 years in the study group with the average deviation of 7.96 years. The most frequent age in the study group was 33 years while the median age was 30 years. The youngest pregnant woman was 17 years old, and the eldest one was 46 years old, in the study group, while the age range was 29 years.
The average age was 28.63 years in the control group with the average deviation of 8.17 years. The most frequent age in the control group was 22 years while the median age was 27 years. The youngest and the eldest pregnant woman in the control group were 17 and 40 years old respectively, while the age range was 23 years.

Based on the results of the hypothesis test of difference of the median value in the groups, the empirical measure p>0.05 was determined at the significance level, which was used for testing a variable Age indicating that differences in average age between the groups were not statistically significant.

Parity characteristics
Table 2 displays the characteristics of pregnant women in the groups according to parity.

The p value in all parities was >0.05 at the significance level indicating that the statistically significant difference in the relative modality between the groups was not observed.

### TABLE 1. Average pregnant woman's age

| Observed characteristics | Hydatidiform Mole | Physiological Pregnancy | Total | df1 | df2 | F  | p   |
|--------------------------|-------------------|-------------------------|-------|-----|-----|----|-----|
| Age                      | 29.92 ± 7.96      | 28.63 ± 8.17            | 29.27 ± 8.03 | 1   | 68  | 0.445 | 0.507 |

### TABLE 2. Number and characteristics of patients according to parity and group

| Parity          | Molar Pregnancy | Physiological Pregnancy | Total | f | %  | F  | %  | f | %  | p   |
|-----------------|-----------------|-------------------------|-------|---|----|----|----|---|----|-----|
| Primipara       | 19              | 54.29                   | 17    | 48.57 | 36 | 51.43 | 0.632 |
| Multipara       | 14              | 40.00                   | 14    | 40.00 | 28 | 40.00 | 1.000 |
| Grand multipara | 2               | 5.71                    | 4     | 11.43 | 6  | 8.57  | 0.391 |
| Total           | 35              | 100.00                  | 35    | 100.00 | 70 | 100.00 |       |

### TABLE 3. Number and characteristics of pregnant women according to a type of miscarriage and hydatidiform mole prevalence in a previous pregnancy

| Previous Pregnancy | PATIENT GROUP              | f | %  | F  | %  | f | %  | p   |
|--------------------|---------------------------|---|----|----|----|---|----|-----|
|                    | Molar Pregnancy (35)      |   |     |   |     |   |     |     |
| Spontaneous abortion | 9                       | 25.71 |    | 10 | 28.57 | 19 | 27.14 | 0.250 |
| Induced abortion    | 6                        | 17.14 |    | 6  | 17.14 | 12 | 17.14 | 1.000 |
| Spontaneous and induced abortions | 3 | 8.57 |    | 0  | 0.00 | 3  | 4.29  | 0.070 |
| Total               | 18                       | 51.43 | 16   | 45.71 | 34 | 48.57 | 0.810 |
| Hydatidiform mole in a previous pregnancy | 4 | 11.43 |    | 0  | 0.00 | 4  | 5.71  | 0.034 |
Unfavourable Obstetrical History

The number and type of previous miscarriages and hydatidiform mole prevalence in previous pregnancies in the groups are shown in Table 3.

Analyzing the miscarriage type and number in previous pregnancies, the statistically significant difference in the relative prevalence between the groups was not observed. 4 (11.43%) cases of the same complications as in a previous pregnancy were reported in the study group.

Gestational Age of Previous Spontaneous Abortions

The statistically significant difference in the average gestational age of spontaneous abortions in the groups is shown in Table 5.

The statistically significant differences in the average gestational age of spontaneous abortions between the study and control group were not observed, based on the test results p>0.05 at the reliability level of 95% or the risk of 5%. Whereas at the reliability level of 90% or the risk of 10%, the statistically significant differences in the average gestational age of spontaneous abortions were observed.

Gestational Age of Pregnancy

The statistically significant differences in the average gestational age of pregnancy between the study and control group were observed, based on the test results p>0.05 at the reliability level of 95% or the risk of 5%.

Blood Type and Hydatidiform Mole

The pregnant women examined according to their

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**TABLE 4.** Number and characteristics of patients according to number and gestational age of spontaneous abortions

| Gestational age of spontaneous abortions | Molar Pregnancy (35) | Physiological Pregnancy (35) | Total (70) | p |
|------------------------------------------|----------------------|-----------------------------|------------|---|
|                                          | F        | %    | f     | %    | F       | %    |
| 6-7 weeks                                | 3        | 8.57 | 2     | 5.71 | 5       | 7.14 | 0.642 |
| 8-9 weeks                                | 4        | 11.43| 1     | 2.86 | 5       | 7.14 | 0.158 |
| 10-11 weeks                              | 0        | 0.00 | 1     | 2.86 | 1       | 1.43 | 0.310 |
| 12-13 weeks                              | 1        | 2.86 | 2     | 5.71 | 3       | 4.29 | 0.554 |
| 14-15 weeks                              | 1        | 2.86 | 3     | 8.57 | 4       | 5.71 | 0.299 |
| 17-18 weeks                              | 0        | 0.00 | 1     | 2.86 | 1       | 1.43 | 0.310 |
| Total                                    | 9        | 25.71| 10    | 28.57| 19      | 27.14| 0.788 |

**TABLE 5.** Difference in the average gestational age of spontaneous abortions in the groups

| Observed Characteristics | Hydatidiform Mole | Physiological Pregnancy | Total | df1 | df2 | F    | p   |
|--------------------------|-------------------|-------------------------|-------|-----|-----|------|-----|
| Gestational age of previous spontaneous abortions | 9.00 ± 2.55 | 11.70 ± 3.77 | 10.42 ± 3.45 | 1     | 17   | 3.26 | 0.089 |

**TABLE 6.** Difference in the average gestational age of pregnancy in the groups

| Observed Characteristics | Hydatidiform Mole | Physiological Pregnancy | Total | df1 | df2 | F    | p   |
|--------------------------|-------------------|-------------------------|-------|-----|-----|------|-----|
| Gestational age          | 9.63 ±1.83 | 8.25 ±2.03 | 8.94 ± 2.04 | 1     | 68   | 8.783| 0.004 |
blood type and their groups (study and control) are shown in Table 7.

A contingency table displays that the statistically significant difference in prevalence of individual modalities was not observed.

### Pathomorphological Characteristics of Hydatidiform Mole Villi

The typical microscopic characteristics were examined following the guidelines according to Genest (9).

Table 8 displays the pathomorphologic characteristics seen only in the study group. However, the variables presented below were not seen in the control group.

The prevalence of hydatidiform mole villi is statistically significant having the following characteristics: irregular borders of villi (71.43%); slightly enlarged villi (54.29%); moderate cisterns (65.71%); mild avascularisation of villi (57.14%).

### DISCUSSION

#### Pregnant Women Age

Age is a likely risk factor for hydatidiform mole in addition to the geographical variations. The lowest hydatidiform mole incidence was reported in women between age 25 and 29 years (10). Risk of hydatidiform mole was tenfold increased in women conceiving at maternal age under 16 or after 40 years (11). Sebire (7) reported ten to twenty fold increased risk of molar pregnancy in women conceiving under age 15; for women aged over 45 and 50 the risk was 20 times higher; for women over 50 the risk was even 200 times higher. Failure in fertilization might be one of the causes. The younger...
women more frequently have triploid conceptions, while the maternal triploid occurs more frequently in elderly patients.

In this study, maternal age ranged from 17 to 46 years and 17 to 40 in hydatidiform mole and in physiological pregnancy respectively. The most frequent incidence was seen at age 30 to 34 years (28.57%) in the study group, and at age 35 to 39 years (31.43%) in the control group.

The average age of the study group was 29.92±7.96 years, and of the control group was 28.63±8.17 years thus, the difference between the groups was not significant. In the study conducted by Osamur, the average age was 28 years (12), and in the Khabouse study it was 28 years (13). The molar pregnancy prevalence was seen to be higher in elderly women (14). In his study Ben Temime (15) reported 30% of patients aged over 35.

In our study we found 22.85% and 2.86% of patients with hydatidiform mole aged 35 to 39 years and over 40 respectively. The eldest pregnant woman was 46 years old. However, the difference in relation to a miscarriage caused by the cervical insufficiency was not statistically significant.

The molar pregnancy risk was increased in elderly women and progressively increased with age (8). The abnormal ovum fertilization is more likely to occur in elderly women than it is seen in younger women. The increased risk was particularly seen in pregnant women aged over 35 and 40 years (1,16).

According to literature date on age as a likely risk factor, we found that 11.43% and 14.29% of pregnant women were under 20 years in the study and control group respectively, thus with no significant difference.

In women under 20 years of age, who are at the beginning of their reproductive years, the increased incidence of hydatidiform mole was observed (8). Other authors described the higher risk of molar pregnancy in younger women as well (17,4). It was reported that a relative risk was six time higher for women under 15 years of age (10). Audu (17) reported 17.5 years of age as the greatest risk for molar pregnancy, while Hancock (18) reported the two-fold higher incidence in teenage women than in elderly women. Parazzini (19), however, stated that advanced age increased only the complete mole risk, but not the partial mole.

Difference in age among the patients in this study was not observed complying with the study conducted by Slim (20) in which the clear correlation between the incidence of hydatidiform mole and maternal age was not observed as well. It might have been caused by marriage age and pregnancy across countries, environmental factors or by relatively few patients.

**Parity Characteristics**

It is well known that maternal age, parity and difference between births significantly affect both mother and child’s health. Number of births is biologically, medically and socially significant. Most authors emphasized that the first pregnancy was at the highest risk (21).

Ben Temime (15) reported the molar pregnancy in nullipara (28.88%). The similar results were reported by Audu (17) and Altiere (22) as well. In contrast, Nowak (23) reported the higher incidence of hydatidiform mole among women who had given more births. Our results indicate that women with hydatidiform mole who gave birth once prevailed (54.29%). Women who gave birth once also prevailed in the control group (48.57%). Statistically, there was no significant difference between the numbers of births in the groups. The risk of molar pregnancy is present whether a woman has already given a birth or not.

**Unfavorable Obstetrical History**

According to Kuvačić (24), the analysis of previous reproductive health is important for assessment of reproductive health as well as differences in an ongoing pregnancy. It was noticed in epidemiological studies that previous spontaneous abortions increased the risk of gestational trophoblastic disease whereas one or more previous pregnancies decreased it (25).

Although the statistically significant difference in relative incidence (number and types of spontaneous abortions) was not observed in this study, it was noticed that spontaneous and induced abortions prevailed in the study group (8.57%), while spontaneous abortions prevailed in the control group (19-27.14%). The cervical insufficiency which causes a spontaneous abortion apparently had been present before an ongoing pregnancy causing its negative outcome.
Hydatidiform mole was recognized as the risk factor for spontaneous abortion (8). The majority of molar pregnancies were discovered by warning signs indicated a threatened or started spontaneous abortion (11). In his study Kashanian (26) reported the increased risk of molar pregnancies in women who had history of two or more miscarriages. Rezavanet (27) reported 14.5% of patients with hydatidiform mole who had previous spontaneous miscarriages in contrast to 9.5% of non-molar pregnancies. However, the difference was not statistically significant.

Investigating the gestational age of previous spontaneous abortions, we found that the majority of pregnant women with molar pregnancy had had spontaneous abortions in the 8th and 9th (11.43%), whereas the majority of pregnant women with physiological pregnancy had had previously spontaneous abortion in the 14th and 15th week (8.57%).

The prevalence of spontaneous abortions at advanced gestational age in the control group proved the theory that the cervical insufficiency is a factor which causes termination of pregnancy but not insufficiency of yellow body or hormonal abnormality. History of hydatidiform mole in previous pregnancy was recognized as the risk factor in literature – it is a predisposition to another molar pregnancy. Women who had molar pregnancy were at risk of recurrence in a subsequent pregnancy (27). Moreover, the recurrence of molar pregnancies was likely to occur at random after one or more normal pregnancies regardless of a partner (1).

According to Grgurević (11) women who suffered from mole had 20 to 40 times greater risk of a recurrent molar pregnancy, and the similar results were reported by Audu (17).

We found that 4 (11.4%) patients had a history of previous molar pregnancy, and 1 (2.85%) patient had two previous molar pregnancies. These findings are in accordance with the study conducted by Sebire (28) suggesting that the risk of recurrence in patients who had two previous molar pregnancies increased 10% to 20%. Recurrent molar pregnancy increased the risk of choriocarcinoma as well (28). Rezavanet (27) in his study reported history of previous molar pregnancy in 2.5% of cases, while Ben Temime (15) in 3.3% of cases. In Garret study (29) in the US, hydatidiform mole recurrence was likely in 1.2% of cases, and in Vakili study (30) 1.2%. Lorigan study (31) emphasized that women with history of molar pregnancy had greater risk of proliferation. Potential reasons might have been the presence and continuation of previous causes of molar pregnancy (genetic, environmental and diet).

**Blood Type and Hydatidiform Mole**

Bagshawe (5) and Berkowitz (4) suggested the blood type A as a risk factor for molar pregnancy. In the study group, 40% of pregnant women had the blood type A, regarded as a risk factor in literature. However, differences in blood types were not statistically significant. In Rezavanet study (27) the blood type 0 prevailed due to its predominance in population. He further suggested that discrepant study results were indicated by various blood types in regions, which might be applied to an interpretation of our results.

**Gestational Age of Pregnancy**

Hydatidiform mole, a pathological condition, affects the course of a normal pregnancy (32). Our results on gestational age of pregnancy in partial mole and physiological pregnancy terminated by spontaneous abortion indicate to the prevalence of advanced gestational age in molar pregnancy. According to Ben Temime (15) in 81.24% of patients molar pregnancy was diagnosed between the 8th and 16th weeks. Our results suggest that spontaneous abortions, caused by the cervical insufficiency, may have some typical symptoms rather than signs indicated the molar disorder. Gestational age of pregnancy is an important parameter for establishing diagnosis of molar pregnancy since the reliability of diagnostic methods significantly increases with gestational age (8).

**Pathomorphologic Analysis**

Hystopathologically, the partial hydatidiform mole was diagnosed when four microscopic features co-exist: 1) two populations of villi; 2) enlarged villi (more than 3 to 4 mm with central presence); 3) irregular villi with geographical divided borders with trophoblastic inclusions; 4) trophoblastic hyperplasia – usually focal and with syncytiotrophoblast (9). Partial hydatidiform mole or “transitional mole” occurs when a normal egg is fertilized by two sperms or by one diploid sperm. The developed tissue might be larger than expected for a gestational age (33).
Partial mole appears as a mosaic of normal and pathologically changed villi; it is characterized by the existence of a mixture of various villi population consisting of morphologically normal villi and edematous ones of irregular shape that have cisterns and trophoblastic hyperplasia (7). In this study, we found in all examined partial hydatidiform moles the enlarged villi, statistically slightly enlarged (54.29%). The results comply with the results reported by other authors, thus Fowler (34) reported diameter of villi to be statistically significantly greater in partial mole, while Howat (35), in the study of histological examination, reported 50 cases of molar pregnancy.

We also found the evidence that only particular villi were changed in partial mole. In all examined moles, the presence of normal villi was seen in this study as well, which was also reported by other authors (36). Sebire (37) is his study emphasized the importance of villous dysmorphism, and Szulman (33) reported dual populations of villi. The enlarged villi were not seen in physiological pregnancies reported by Van Lijnschoten as well (38). However, Salafia (39) found mild/moderate enlarged villi in 35% of cases and markedly enlarged in 20% of cases in spontaneously aborted pregnancies with no chromosome abnormalities. According to Sebire (7) the enlarged hydropic villi in molar pregnancy usually had irregular outline, while in spontaneously aborted pregnancies the enlarged villi were of a round outline with hypocellular villous stroma on cross section.

Examining villous borders, we found statistically great number of irregular ones (71.43%) in contrast to 100% of smooth and regular ones in physiological pregnancies. Sebire (7) reported that in partial mole the enlarged hydorphic villi can have abnormal jagged-like appearance with cleft formation, and round or oval pseudo inclusions are often present in some cross sections of villi.

Microscopically, the one of main characteristics of molar pregnancy is the hydropic swelling of chorionic villi. The villi consist of loose mucous edematous stroma (40).

The great number of moderate cisterns (65.71%) were seen in this study. Cisterns, however, were not noticed in physiological pregnancies. Mild cisterns in partial mole were also reported by Salafia (39). Examining pathomorphological changes in partial mole, Jaffar (14) found cisterns present in 80% of cases.

In earlier studies, the hydropic swelling of villi (cisterns) were suggested to be the most significant characteristic of conception affected by the molar disease. The degree of hydropic changes was less pronounced and incomplete during the first trimester (7). Therefore, this explains the greater number of moderate cisterns in contrast to pronounced once seen in this study. There was not enough time for complete hydropic degeneration of villi in an earlier gestation. Nonexistence of cisterns in physiological pregnancies in our study complies with the results reported by Funkunaga (41). He found hydropic degeneration of villi and focal hyperplasia only in pregnancies spontaneously aborted with abnormal karyotype.

The presence of villous avascularity was seen in all examined molas especially statistically the great number of slightly avascular ones (57.14%), in contrast to moderate ones (34.29%). The similar results were reported by other authors (7,39). Normal villi in physiological pregnancies were vascularised in 100% of cases. Salafia (39) reported a normal villous circulation in 46% and 59% of euploid and aneuploid spontaneous abortions respectively.

Amnion and fetus elements might be present in partial mole, and chorionic villi usually have blood vessels frequently containing fetal nucleated red blood cells. In some cases angiomatoid change might be seen focally with advancing gestational age appearing as dilated vessels with thick walls within swollen villi (42).

CONCLUSION

Risk factors were the most likely present in patients who had mole in previous pregnancies, symptoms were more typical for advanced gestational age; however, differences in age, parity and blood type were not observed. Chorionic villi of hydatidiform mole show typical pathomorphologic changes that are an important factor for establishing a proper diagnosis. The proper diagnosis is important in the successful management and prevention of complications of hydatidiform mole. Further education of women with molar pregnancies and the follow up after the treatment are required in order to maintain and improve reproductive health.
CONFLICT OF INTEREST
The authors declare no conflict of interest.

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