Immunohistochemistry Study of P53 and C-erbB-2 Expression in Trophoblastic Tissue and Their Predictive Values in Diagnosing Malignant Progression of Simple Molar Pregnancy

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Received 2015 September 22; Revised 2015 December 09; Accepted 2016 June 05.

Abstract

Background: Finding a tumor marker to predict the aggressive behavior of molar pregnancy in early stages has yet been a topic for studies.

Objectives: In this survey we planned to study patients with molar pregnancy to 1) assess the p53 and c-erbB-2 expression in trophoblastic tissue, 2) to study the relationship between their expression intensity and progression of a molar pregnancy to gestational trophoblastic neoplasia, and 3) to determine a cut off value for the amount of p53 and c-erbB-2 expression which might correlate with aggressive behavior of molar pregnancy.

Patients and Methods: In a prospective cross sectional study by using a high accuracy technique EnVision Tm system for immunohistochemistry staining of molar pregnancy samples, we evaluated p53 and c-erbB-2 expression in cytotrophoblast and syncytiotrophoblast and the correlation of their expression with progression of molar pregnancy to gestational trophoblastic neoplasia (GTN). Normal prostatic tissue and Breast cancer tissue were used as positive controls.

Results: We studied 28 patients with simple molar pregnancy (SMP) and 30 with GTN. Cytotrophoblast had significantly higher expression of p53 and c-erbB-2 and syncytiotrophoblast had greater expression of p53 in GTN group as compared to SMP group. The cut off values for percentage of p53 positive immunostained cytotrophoblast and syncytiotrophoblast were 5.5% and 2.5%. In c-erbB-2 positive membranous stained cytotrophoblast the cut off was 12.5%.

Conclusions: Our data suggests that over expression of p53 and c-erbB-2 is associated with malignant progression of molar pregnancy. We encountered that high expression of p53 and c-erbB-2 in trophoblastic cells could predict gestational trophoblastic neoplasia during the early stages.

Keywords: Complete Hydatiform Mole, P53, C-erbB-2, Prognosis

1. Background

Gestational trophoblastic disease is a group of heterogeneous conditions; ranging from simple molar pregnancy to gestational trophoblastic neoplasia with aggressive behavior and metastasis (1-3). It has an incidence rate of 1 per 1000 pregnancies in Western countries (4) and is more common among Eastern populations (5, 6). Although the metastatic form could be potentially fatal, early diagnosis and chemotherapy makes it as one of the most curable solid tumors (7-9).

Current guidelines suggest measuring weekly serum βhCG (human chorionic gonadotropin) following the evacuation of pregnancy products and in case of plateau or rising pattern; the persistent gestational trophoblastic disease would be suspected and chemotherapy should be started (10, 11) it is a great advantage if one could predict aggressive behavior of the disease before an increase in serum βhCG. Researchers have studied molecular pathogenesis of gestational trophoblastic neoplasia and some found p53 and c-erbB-2 to have a role in malignant behaviors of these tumors (12-16). However, there were controversies whether p53 and c-erbB-2 expression could act as tumor markers.

2. Objectives

In this survey we planned to study patients with molar pregnancy using immunohistochemistry staining to 1) as-
cess $p53$ and $c$-$erbB$-$2$ expression in trophoblastic tissue, 2) to study the relationship between their expression intensity and progression of a molar pregnancy to gestational trophoblastic neoplasia, and 3) to determine a cut off value for $p53$ and $c$-$erbB$-$2$ expression intensity in case of correlation with aggressive behavior of molar pregnancy.

3. Patients and Methods

3.1. Population

In a prospective cross sectional study, we included patients with primary diagnosis of molar pregnancy referring to oncology clinic of Qaem hospital, affiliated to MUMS. All patients underwent evacuation and curettage, followed by weekly βhCG measurements. Patients were divided into two groups: (1) gestational trophoblastic neoplasia (GTN) group if serum βhCG level rose or did not change during study; (2) simple molar pregnancy group whose serum βhCG underwent gradual decrease. Serum βhCG level < 5 mIU/mL was considered as normal. Patients’ specimen of curettage were referred to pathology laboratory of hospital for histological and immunohistochemistry studies.

3.2. Histological and Immunohistochemistry Studies

Immunohistochemistry staining was performed on multiple 4 µm sections of paraffin blocks provided from formalin fixed trophoblastic tissues. In order to evaluate the immunoreactivity of $c$-$erbB$-$2$ oncogene and $p53$ tumor suppressor gene, we applied a polymer based Dako Envision Tm system technique; (Do-7, Dakocytomation, N1581, DAKO Corporation, Carpiteria, CA 93013 USA) for $p53$ antigen and (Clone PN2A, Dakocytomation, Denmark A/S, DK-2600 Glostrup, Denmark) for $c$-$erbB$-$2$. Normal prostatic tissue and breast cancer slides were used as positive controls for $p53$ and $c$-$erbB$-$2$ respectively due to company protocols. As negative controls, phosphate buffered saline (PBS) was substituted with antibodies. All slides were observed by a single pathologist under a light microscope (Olympus BX 50; Olympus optical Co, Ltd, Tokyo Japan). The rate of $p53$ expression was reported as percentage of cytoto phoblastic and syncytiotrophoblastic cells with positive nuclear immunoreactivity. The $c$-$erbB$-$2$ oncogene expression rate was calculated as percentage of cells with positive membranous staining. To grade $p53$ staining intensity semi quantitatively, we applied 0 for no stained cells, + for staining of less than 10% of cells, ++ for 10 to 50% of cells, +++ for staining in more than 50% of cells. To score $c$-$erbB$-$2$ staining intensity we used negative as no or less than 10% of cells’ membranes stained, 1+ for faint membranous staining in more than 10% of cells, 2+ for weak to moderate complete membranous staining in more than 10% of cells and evaluate 3+ as strong for complete membranous staining in more than 30% of cells (17). All tissue preparation stages were performed based on Dako Envision Tm company protocols (18).

3.3. Statistical Analysis

Data were entered on SPSS for windows software version 21. Categorical data were analyzed by chi-square or exact Fischer test. Mann-Whitney test and independent sample t-test were applied to compare continuous variables. To estimate a cut off for percentage of positive immunostained cells ROC (receiver operating characteristic) curve analysis was applied to evaluate the risk of transformation of molar pregnancy to gestational trophoblastic neoplasia. The P value < 0.05 was considered statistically significant.

3.4. Ethics

Informed consents were signed by all patients. All diagnostic and therapeutic interventions including evacuation of pregnancy products, serial weekly measurement of serum βhCG, and histological evaluation for their primary diagnosis (complete or partial molar pregnancy) were performed according to indications for patients with molar pregnancy diagnosis. Immunohistochemistry expenses were covered by the research budget.

4. Results

We included 58 patients: 30 with final diagnosis of Gestational Trophoblastic Neoplasia (GTN) and 28 with simple molar pregnancy. Although Patients with GTN diagnosis had a higher age average, gravidity and parity number in comparison with simple molar pregnancy group, only age had a significant correlation with GTN. The primary diagnosis of 28 patients with (GTN) was complete molar pregnancy (P value < 0.05). Table 1 displays patients’ demographics.

The immunohistochemistry staining results in GTN group showed a significantly higher average percentage of cytotrophoblast and syncytiotrophoblast with positive nuclear immunoreactivity for $p53$ in comparison with simple molar patients. The membranous immunostaining of cytotrophoblast for $c$-$erbB$-$2$ was also significantly greater in GTN group. Patients with primary diagnosis of complete mole had significantly higher percentage of $p53$ positive cytotrophoblast and syncytiotrophoblast in comparison with partial mole group (Table 2 and Figure 1).

Patients in GTN group displayed a significantly higher immunoreactivity score for $p53$ among both cytotrophoblast and syncytiotrophoblast as compared to patients...
with simple molar pregnancy. Membranous immunoreactivity score of cytotrophoblast for c-erbB-2 marker were also higher among GTN group. Syncytiotrophoblast showed fairly similar immunoreactivity for c-erbB-2 marker in both GTN and simple molar pregnancy groups with no statistical meaningful difference (Table 3).

The receiver operating characteristic (ROC) curve analysis displayed the 5.5% as a cutoff percentage for cytotrophoblast with p53 nuclear immunostaining (93.3% sensitivity, 88% specificity). For syncytiotrophoblast with sensitivity and specificity of 90% and 88% respectively, the cutoff value of 2.5% was determined. We found the cut off value of 12% for the percentage of cytotrophoblast with c-erbB-2 membranous staining (sensitivity of 90% and a specificity of 92%) which might increase the risk of progression of a molar pregnancy to GTN (Figure 2). The positive predictive values were as 90%, 88.8% 88.4% for calculated cut off of p53-positive cytotrophoblast, p53 positive syncytiotrophoblast and c-erbB-2 positive cytotrophoblast respectively (Table 4).

5. Discussion

In patients with simple molar pregnancy, gynecologists are always concerned about their progression toward the gestational trophoblastic neoplasia. To date the only way to evaluate patients following the evacuation and curettage, is serial measurement of serum βhCG (11). Regarding the invasive and metastatic behavior of malignant transformations of molar pregnancy, finding a marker with high predictive value to diagnose the malignant forms early after evacuation is of great importance. In this survey, we found that p53 and c-erbB-2 genes had higher expressions in both cytotrophoblast and syncy-
Table 2. Percentage of Cytotrophoblasts and Syncytiotrophoblasts with Positive Immunoreactivity for c-erbB-2 and P53 of Patients Regarding Their Final Diagnosis, Primary Diagnosis and Mann-Whitney Test Results\(^a,b\)

|                      | GTN          | Simple Mole | P Value | Complete Mole | Partial Mole | P Value |
|----------------------|--------------|-------------|---------|---------------|--------------|---------|
| P53 Cytotrophoblasts | 41.6 ± 25.4  | 5.3 ± 9.3   | 0.000   | 27.9 ± 26.7   | 9.0 ± 21.1   | 0.007   |
| P53 Syncytiotrophoblasts | 19.3 ± 18.8 | 1.2 ± 1.1   | 0.000   | 12.1 ± 17.1   | 4.0 ± 9.8    | 0.027   |
| C-erbB-2 Cytotrophoblasts | 18.4 ± 26.4 | 2.5 ± 8.6   | 0.000   | 11.7 ± 21.6   | 6.2 ± 20.1   | 0.053   |
| C-erbB-2 Syncytiotrophoblasts | 5.4 ± 14.5 | 1.6 ± 5.4   | 0.308   | 4.0 ± 12.1    | 1.6 ± 5.7    | 0.437   |

Abbreviation: GTN, Gestational Trophoblastic Neoplasia.
\(^a\)P value < 0.05 considered significant.
\(^b\)Values are expressed as mean (SD).

Table 3. Semi Quantitative Immunoreactivity Score of Cytotrophoblasts and Syncytiotrophoblasts for P53 and C-erbB-2 Markers\(^a,b,c\)

|                      | P53 Cytotrophoblast | P53 Syncytiotrophoblast | C-erbB-2 Cytotrophoblast | C-erbB-2 Syncytiotrophoblast |
|----------------------|----------------------|-------------------------|--------------------------|------------------------------|
| SMP GTN SMP GTN SMP GTN SMP GTN | SMP GTN SMP GTN SMP GTN | SMP GTN SMP GTN SMP GTN | SMP GTN SMP GTN SMP GTN | SMP GTN SMP GTN SMP GTN |
| Negative             | 1 (3.8)              | 1 (3.3)                 | 6 (23.1)                 | 2 (6.9)                      |
|                      | 23 (88.5)            | 7 (23.3)                | 23 (88.5)                | 25 (83.3)                    |
| +                    | 22 (84.6)            | 3 (10)                  | 20 (76.9)                | 11 (37.9)                    |
|                      | 1 (3.8)              | 12 (40)                 | 1 (3.8)                  | 1 (3.3)                      |
| ++                   | 3 (11.5)             | 17 (56.7)               | 0 (0)                    | 15 (51.7)                    |
|                      | 2 (7.7)              | 7 (23.3)                | 2 (7.7)                  | 3 (10)                       |
| +++                  | 0 (0)                | 9 (30)                  | 0 (0)                    | 1 (3.4)                      |
|                      | 4 (13.3)             | 0 (0)                   | 4 (13.3)                 | 1 (3.3)                      |
| P Value              | 0.000                | 0.000                   | 0.000                    | 1.000                        |

Abbreviations: GTN, Gestational Trophoblastic Neoplasia; SMP, Simple Molar Pregnancy.
\(^a\)For p53 marker, we applied 0 for no stained cells, + for staining of less than 10% of cells, ++ for 10 to 50% of cells, +++ for staining in more than 50% of cells. To score c-erbB-2 staining intensity we used negative as of no or less than 10% of cells’ membranes stained, + for faint membranous staining in more than 10% of cells, ++ for weak to moderate complete membranous staining in more than 10% of cells and evaluate +++ as strong, for complete membranous staining in more than 50% of cells, results of chi-square test.
\(^b\)P value < 0.05 considered significant.
\(^c\)Values are expressed as No. (%).

Table 4. The Positive and Negative Predictive Values for Calculated Cut Offs for Percentage of Cells with Positive Immunostaining to Diagnose Malignant Progression of Molar Pregnancy

|                      | P53 Cytotrophoblasts, (%) | P53 Syncytiotrophoblast, (%) | C-erbB-2 Cytotrophoblasts, (%) |
|----------------------|----------------------------|-------------------------------|--------------------------------|
| Cut off for percentage of cells with positive immunostaining | 5.5                        | 2.5                          | 12.5                           |
| Positive predictive value | 90                        | 88.8                         | 88.4                           |
| Negative predictive value | 92                        | 82.1                         | 76.6                           |

tiotrophoblast of GTN patients in comparison with simple molar patients with significant difference.

P53 is known as a tumor suppressor gene which encodes a nuclear phosphoprotein and its mutation seems to involve in many human cancers’ pathogenesis (13, 19, 20). Several studies have been performed on the role of p53 in gestational trophoblastic neoplasia. Petignat et al. reported the over expression of mutant p53 in complete moles and malignant forms (21). Although many studies reported an increase in p53 expression in GTN and complete mole in comparison with simple molar pregnancy and partial mole (12, 14 - 16, 22-24), some found that the increased type is rather the wild type to the mutant type of p53 (12, 23, 25, 26). Yang et al. also reported an increased expression of p53 in GTN and complete mole although he did not find a significant predictive value for such increase to diagnose the malignant forms in early stages (16). Whether the mutant type or wild type are over expressed, in this survey we found that p53 expression increased significantly in cytotrophoblast and syncytiotrophoblast of GTN and complete moles. We found the positive predictive values of 90% and 88.8% when 5.5% and 2.5% of cytotrophoblast and syncytiotrophoblast with positive nuclear immunoreactivity were used as the cut off respectively. Using the same
method of immunostaining, Chen Y et al. results support our data and find it useful to evaluate p53 expression as adjuncts to conventional methods of diagnosis (24).

Epidermal growth factor receptors (EGFRs) are a big family of transmembrane signaling proteins which are involved in many human neoplasms pathogenesis (27-29). C-erbB-2 is a member of (EGFRs) family and is involved in pathogenesis of different malignancies including melanomas, breast cancer, and colorectal cancer (30, 31) as well as complete mole and choriocarcinoma (16, 27, 32, 33). Yang et al. reported a prognostic value of 84% for percentage of cytotrophoblasts with positive cytoplasmic immunostaining to predict the malignant progression of simple molar pregnancy (16). However, there are conflicting reports: Cameron et al. reported the expression of c-erbB-2 in only one case out of 20 patients with persistent gestational trophoblastic disease (34); Dehaghani AS et al. found that the mean serum c-erbB-2 does not differ significantly between GTD patients and normal pregnant controls (35). In contrast to these reports, we found an over expression of c-erbB-2 in cytotrophoblast of patients with GTN and complete mole with significant difference to sim-
ple molar pregnancy and partial mole. We determined a cut off value of 12.5% for the percentage of cytotrophoblast with c-erbB-2 membranous staining (sensitivity of 90% and a specificity of 92%) which might increase the risk of progression of a molar pregnancy to GTN. Many researchers have supported our data: Yang et al. has found c-erbB-2 as a strong predictor for malignant behavior of molar pregnancies; Yazaki et al. proposed to use c-erbB-2 expression as well as hCG in therapeutic protocols (16, 27, 32, 33, 36).

Our data is noteworthy because of the more accurate method of immunohistochemistry we have used; unlike the most studies on this issue using avidin-biotin methods (16, 23, 32, 33, 37, 38), our study was performed on EnVision™ system. Avidin-biotin methods are widely used since their introduction in 1981 but because of the background staining due to tissues endogenous biotin and decreasing the expression of biotin in formalin fixation and paraffin blocking of tissues, the new method of polymer based methods have been established (39, 40). EnVision™ system is a new polymer based technique which has higher sensitivity than routine avidin-biotin method without its limitations (41).

Although our study was performed only by one pathologist and was not a blind study, using a more accurate technique of immunostaining and counting immunostained cells separately on each cell population (cytotrophoblast and syncytiotrophoblast) might make our results more practical to be a base for future studies. Finally, further collaboration of pathologists and gynecologists would be suggested to establish comprehensive guidelines for early diagnosis of malignant progression of molar pregnancies.

Our data suggests that over expression of p53 and c-erbB-2 is associated with malignant progression of molar pregnancy. We encountered that high expression of p53 and c-erbB-2 in trophoblastic cells could predict gestational trophoblastic neoplasia in early stages. Supposed our data could be supported with more studies on this issue, it might be useful to evaluate the immuno-expression of p53 and c-erbB-2 genes on primary samples of pregnancy products of patients with molar pregnancy to estimate their risk of progression toward the malignant forms.

Acknowledgments

None declared.

Footnotes

Authors’ Contribution: Malihe Hasanzadeh designed the study, performed the operations and biopsy procedures and supervised the entire project. Norrie Sharifi performed the staining step and studied the samples and supervised the pathological aspect of discussion. Marjaneh Farazestanlan contributed to data collection and entry; literature review and writing process. Saman Nazemian and Faezeh Madani sani contributed to data analysis; literature review and writing up process.

Conflict of Interest: All authors have declared no conflict of interests and the whole project has paid from Dr. Hasanzade grant from research budget of Mashhad University of Medical Sciences.

Financial Disclosure: None declared.

Funding/Support: This survey was financially supported by Mashhad University of Medical Sciences (MUMS) as Malihe Hasanzade research grant.

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