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Ki-67 Immunoexpression in Gestational Trophoblastic Diseases

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Gestational trophoblastic diseases comprise of hydatidiform mole, invasive mole, choriocarcinoma and placental site trophoblastic tumor having different biological behavior. Ki-67 is a proliferative marker used to evaluate the biological behavior of different tumors. The study was aimed to determine the Ki-67 immunoexpression in gestational trophoblastic diseases and its association in different types of gestational trophoblastic diseases. A cross-sectional, descriptive study was done on 50 cases of gestational trophoblastic diseases, histologically identified as 10 cases of partial hydatidiform mole, 23 cases of complete hydatidiform mole, 9 cases of invasive mole and 8 cases of choriocarcinoma. Immunoexpression of Ki-67 was determined by PAP immunohistochemistry. All cases showed Ki-67 immunopositivity. High Ki-67 labeling index (≥50% of Ki-67 positivity) was found in 10% of partial hydatidiform mole, 73.9% of complete hydatidiform mole, 33.3% of invasive mole and 100% of choriocarcinoma. Low Ki-67 labeling index (<50% of Ki-67 positivity) was found in 90% of partial hydatidiform mole, 26.1% of complete hydatidiform mole, 66.7% of invasive mole (p=0.000). These findings indicated that high Ki-67 labeling index was observed in gestational trophoblastic diseases having worse biological behavior such as complete hydatidiform mole and choriocarcinoma. So this study supports that Ki-67 immunoexpression might predict the biological behavior and prognosis of gestational trophoblastic diseases.

Key words: Gestational trophoblastic diseases, Histological types, Ki-67 immunoexpression, Ki-67 labeling index

INTRODUCTION

Gestational trophoblastic diseases consist of a group of neoplastic disorders arising from placental trophoblastic tissue after normal or abnormal fertilization.[1] They include hydatidiform mole (complete and partial), invasive mole, and frankly malignant choriocarcinoma and placental site trophoblastic tumor.[2] The incidence of gestational trophoblastic disease in the Asian population was 1.95 times higher than in the non-Asian population.[3] The incidence of hydatidiform mole in the UK is one in 714 pregnancies.[4] Recent results indicate that the previously documented higher rates in the Far East have fallen towards the stable levels found in Europe and North America, possibly because of dietary changes.[4] Hydatidiform mole is associated with increased risk of persistent trophoblastic disease or choriocarcinoma.[2] That is rapidly invasive and metastasizes widely, but once identified responds well to chemotherapy.[2]

The risk of choriocacinoma in complete hydatidiform mole is 10%-30% and in partial hydatidiform mole is 0.5%-5%.[5] Early diagnosis and biological behaviours of gestational trophoblastic diseases are important for follow up and prognosis of the patients. Ki-67 is a proliferative marker and high Ki-67 immunoexpression is also a marker of poor prognosis.[6] Ki-67 has been established as a valuable reflection of the

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tissue proliferative compartment and thus could be of value in studying the biologic behavior of the gestational trophoblastic diseases.[7] Ki-67 immunoexpression shows variable intensities in different subgroups of hydatidiform mole by determining the labeling index (number of positive nuclei/ total number of nuclei) in villous, cytotrophoblasts, syncytiotrophoblasts and stroma cells. Ki-67 labeling index of villous cells, especially cytotrophoblasts, is valuable in diagnosis and differentiation between different subgroups of molar pregnancy, being the highest in complete mole (more than 50%) followed by partial mole (more than 20%).[8]

In this study, the immunoreactivity of Ki-67 was assessed in gestational trophoblastic diseases and its association with different types of gestational trophoblastic diseases was studied. By determining the association of the Ki-67 immunoexpression and the gestational trophoblastic diseases, it was hoped that useful marker will be found for prediction of gestational trophoblastic diseases.

MATERIALS AND METHODS

A cross-sectional, descriptive study was done on 50 cases of gestational trophoblastic diseases attending Central Women's Hospital (CWH), Yangon. The biopsied samples sent to the histological section of Pathology Department, CWH were fixed with 10% buffered para-formaldehyde. After finishing the adequate fixation, tissue processing and proper paraffin wax embedding, all the tissue sections were stained with haematoxylin and eosin. Histological grading and types of gestational trophoblastic diseases were described by using WHO classification.[9]

Immunohistochemical staining method

The paraffin blocks were further processed for IHC staining with Ki-67 monoclonal antibody (Mouse Anti-human Ki-67 antigen, Clone: Ki88 Code: MU370-UC BioGenex, Emergo Europe) by using the Peroxidase-antiperoxidase method. Sections 4 µm thickness were placed on silanised slides and then dried at 37^{o}C overnight and incubated at 60^{o}C for half an hour. These slides were deparaffinized through xylene-ethanol series and washed with PBS (phosphate buffered saline) for 5 minutes, 3 times, followed by washing with distilled water for 5 minutes. Antigen retrieval with 10 mM citrate buffer (pH 6.0) was done by using heat method in microwave oven. Slides were immersed with 0.3% hydrogen peroxide in absolute methanol for 15 minutes to block endogenous peroxidase activity. Then, the slides were washed with PBS for 5 minutes, 3 times and incubated in a moist chamber overnight at 4°C with the primary antibodies: Ki-67 (dilution 1:100).

After incubation is completed, these slides were washed individually with PBS (pH 7.6) for 3 times (each time for 5 minutes and incubated with secondary antibody (Horse Radish peroxidase labeled Goat Anti-mouse IgG) for an hour at room temperature. Then these slides were washed with PBS for 5 minutes, three times. The diaminobenzidine substrate solution was freshly prepared and covered on the sections and was left at room temperature for 15-20 minutes. Then, the slides were washed with distilled water for 5 minutes, twice. Slides were counterstained with haematoxylin for 5-10 seconds and rinsed in water and proceeded in ethanol 70%, 80%, 90%, 100%, xylene III, II, I, 5 minutes each for dehydration and mounted with distene plasticizer xylene (DPX). Ki-67 immunopositivity was observed as brown nuclear staining. In this study, Ki-67 immunoexpression was determined by semi-quantitative scoring method and Ki-67 labeling index.[8]

Semi-quantitative scoring method was determined by the proportion of positive nuclear staining cells over total numbers of cytotrophoblasts and syncytiotrophoblasts. The distribution of Ki-67 immunopositivity in trophoblastic cells were assessed as negative (equal or less than 10% are

positive cells) and positive (more than 10% are positive cells). Positive immunoreactivity was graded as; weakly positive (1+) - (10-20%), moderately positive (2+) - (21-50%) and strongly positive (3+) - (more than 50%).[8] Two senior pathologists performed to count the immunopositivity of trophoblastic cells and immunohistochemical staining reaction of these cells by using specific grading.

Ki-67 labeling index was determined by counting positive brown nuclear staining cells among 1000 cells of cytotrophoblasts and syncytiotrophoblasts. The labeling index was expressed as percent positive.

$$\text{Ki-67 labeling Index} = \frac{\text{Total no. of positive}}{1000 \text{ cells}} = \text{LI\%}$$

Ki-67 labeling index and the immunoreactivity results were recorded and interpreted.

Data analysis

Statistical analysis of data was done by using the Statistical Package for Social Science Study (SPSS), version 20. The association between Ki-67 immunoexpression and different types of gestational trophoblastic diseases was tested using Chi-square test.

RESULTS

Age distribution of gestational trophoblastic diseases

Among 50 cases of gestational trophoblastic diseases, the youngest age of patient was 16 years and the oldest age was 52 years. The highest percentage belongs to the age group between 20-29 years, i.e. 19 cases (38%) of study population followed by over 40 years, 16 cases (32%).

Among 10 cases of partial mole, the youngest age of patient was 17 years and the oldest age was 44 years. The mean age of partial mole was 26 years. In complete hydatidiform mole, the youngest age was 16 years and the oldest was 52 years. The mean age was 34.08 years. Among 9 cases of invasive mole, the youngest age was 20 years and the oldest age was 47 years. The mean age was 35 years. In choriocarcinoma, the youngest age was 29 years and the oldest age was 50 years. The mean age was 38.5 years.

Distribution of gestational trophoblastic diseases by histological types

Among 50 cases of gestational trophoblastic diseases, 23 cases (46%) were complete hydatidiform mole, 10 cases (20%) were partial hydatidiform mole, 9 cases (18%) were invasive mole and 8 cases (16%) were choriocarcinoma.

Ki-67 immunoreactivity in gestational trophoblastic diseases

Ki-67 immunoreactivity was found in all 50 cases of gestational trophoblastic samples. The highest Ki-67 immunoreactivity was found in choriocarcinoma cases (77.50%) and the lowest Ki-67 immunoreactivity was seen in partial mole (31.25%) in this study (Table 1).

Table 1. Ki-67 Immunoreactivity in gestational trophoblastic diseases

Histological types	No. of cases	Mean (%)	Standard deviation	Min	Max
Partial mole	10	31.25	10.09	20	50
Complete mole	23	50.78	12.01	25	80
Invasive mole	9	48.33	16.91	30	70
Choriocarcinoma	8	77.50	10.00	55	90
Total	50	50.71	18.44	20	90

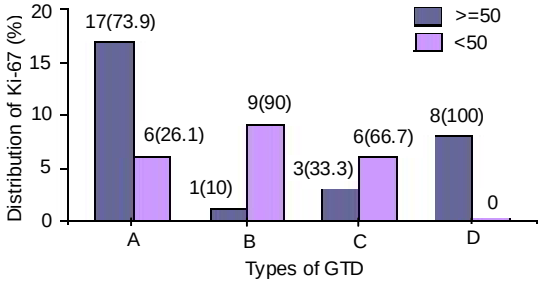
Max=Maximum, Min=Minimum

The mean immunoreactivity of 50 cases of gestational trophoblastic diseases was 50.710±18.438 (%). The median value was 50.00. The minimum expression of Ki-67 immunoreactivity was 20 (%). The maximum immunoreactivity was 90 (%) (Table1).

According to the mean and median value of Ki-67 immunoexpression in gestational trophoblastic diseases in this study, the best cut-off point for gestational trophoblastic diseases in labeling index score was 50 (%). The score less than 50 (%) was considered as low and more than or equal to 50 (%) as high labeling index score.

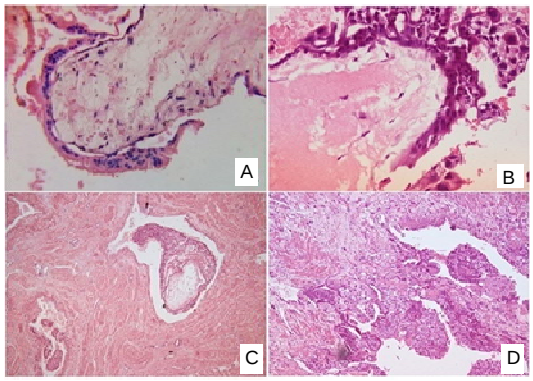
Distribution of Ki-67 labeling index in gestational trophoblastic diseases

Out of 50 cases of gestational trophoblastic diseases, 29 cases (58%) showed presence of high Ki-67 index scores and 21 cases (42%) showed low index scores. Among complete hydatidiform mole, 17 cases (73.9%) showed high Ki-67 index scores and 6 cases (26.1%) showed low index scores. Out of 10 cases of partial hydatidiform mole, only 1 case (10%) showed high Ki-67 index scores and 9 cases (90%) showed low index scores. Among invasive mole, 3 cases (33.3%) showed high Ki-67 index scores and 6 cases (66.7%) showed low index scores. All 8 cases (100%) of choriocarcinoma showed high Ki-67 index scores (Fig. 1).



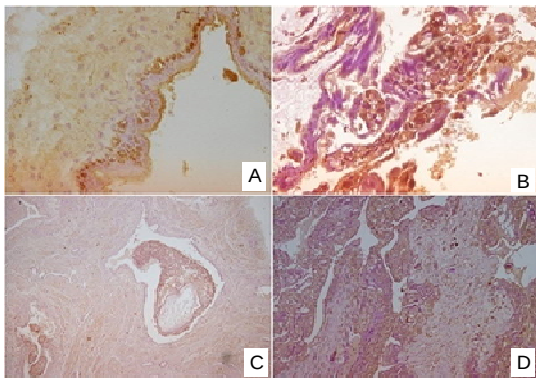
A=Complete H mole C=Invasive H mole
B=Partial H mole D=Chorio CA

Fig. 1. Distribution of Ki-67 labeling index in different types of gestational trophoblastic diseases



A=Partial H mole (H&E x 400)
B=Complete H mole (H&E x 400)
C=Invasive mole (H&E x 100)
D=Choriocarcinoma (H&E x 100)

Fig. 2. H and E staining of different types of GTD



A=Partial H mole (Ki-67 LI=37.5%)
B=Complete H mole (Ki-67 LI=80%)
C=Invasive mole (Ki-67 LI=70%)
D=Choriocarcinoma (Ki-67 LI=90%)

Fig. 3. IHC staining of Ki-67 (Ki-67 labeling index) in different histological types of GTD

Association of Ki-67 immunoexpression with different histological types of gestational trophoblastic diseases

Among 10 case of partial hydatidiform mole, 2 cases (20%) showed the intensity of Ki-67 immunoexpression 1+ and 8 cases (80%) showed 2+ intensity. Among complete hydatidiform mole, the 2+ intensity of Ki-67 immunoexpression was found to be (52.2%) of cases and the remaining (47.8%) showed 3+ intensity. Among 9 cases of invasive mole, the intensity 2+ was seen in (66.7%) of cases and 3+ in (33.3%). All 8 cases of choriocarcinoma (100%) showed 3+ intensity of Ki-67 immunoexpression (Fig. 2 & 3).

DISCUSSION

Maternal age has an influence on the incidence of gestational trophoblastic disease. There was an excess of molar pregnancies in the extremes of reproductive age.[3] In this study, the age distribution of H mole was 16-52 years. Most of the patients were found to be 20-29 years age group (38%) and the second commonest age group was 40 years and above (32%). Lurain found that advanced or very young maternal age had consistently correlation with higher rates of complete hydatidiform mole.[9] There was a small difference of age incidence in this study compared with the previous studies.

In comparison with Ki-67 immuno-expression of partial hydatidiform mole, all cases of complete hydatidiform mole showed a higher Ki-67 intensity with mean Ki-67 immunoreactivity 50.783±12.014 (%). The study done by Ali, *et al.*[8] showed Ki-67 immunoexpression of all villous components especially of cytotrophoblasts; being the highest in complete hydatidiform mole (68.542±11.275) followed by partial hydatidiform mole (22.5±16.611).

In this study, all 9 cases of invasive mole showed positive Ki-67 immunoexpression with the mean immunoreactivity 48.33 ±16.91 (%). In the study carried out by Makovitzky, *et al.*[10] the invasive mole labeled strongly positive for Ki-67 antigen. All cases of chorioarcinoma showed strongly positive Ki-67 immunoexpression with the mean Ki-67 immunoreactivity 77.5±10.0 (%). The study done by Erfanian, *et al.*[7] showed strongly positive Ki-67 immunoexpression in cytotrophoblasts 80.41±9.40 (%) and in syncytiotrophoblasts 58.75± 18.10 (%).

In the current study, highest proliferative activity of Ki-67 (≥50% of Ki-67 labeling index) was found in partial mole (10%), complete hydatidiform mole (73.9%), invasive mole (33.3%) and all cases of choriocarcinoma. Therefore, it might be evident that complete hydatidiform mole was more increasing risk of chorio-carcinoma than partial hydatidiform mole.

On the other hand, among the histological subtypes of trophoblastic diseases, partial hydatidiform mole showed the lowest Ki-67 labeling index and choriocarcinoma showed the highest. In comparison with choriocarcinoma, invasive mole showed low Ki-67 labeling index score. There was significantly associated between Ki-67 labeling index and histological subtypes of gestational trophoblastic diseases (p=0.000). In the present study, Ki-67 immunoexpression was detected in all cases of partial hydatidiform mole (10 cases), complete hydatidiform mole (23 cases), invasive mole (9 cases) and chorio-carcinoma (8 cases). According to the findings, it showed that the higher grade of histological subtypes of gestational trophoblastic diseases, the stronger the intensity of Ki-67 immunoexpression. In complete hydatidiform mole and invasive mole, there were no cases of weakly positive (1+) Ki-67 immunoexpression.

In comparison of partial hydatidiform mole with complete hydatidiform mole, there were no cases of partial hydatidiform mole showing strongly positive (3+) intensity of Ki-67 immunoexpression, whereas 47.8% of complete hydatidiform moles showed strongly positive (3+) intensity. All cases of choriocarcinoma showed strongly positive (3+) Ki-67 immunoexpression. There was a significantly association between Ki-67 immunoexpression and different histological types of gestational trophoblastic diseases (p=0.000).

Conclusion

This study was determined Ki-67 immuno-expression and Ki-67 labeling index in different types of gestational trophoblastic cases from Central Women's Hospital, Yangon. Ki-67 is a proliferative marker and high Ki-67 immunoexpression showed a marker of bad biological behavior and poor prognosis. Early diagnosis and accurate biological behavior is important for gestational trophoblastic diseases because early effective treatment can prevent the progression of the diseases. This is the preliminary study done on association of Ki-67 immunoexpression in different histological types of gestational tropho-blastic diseases in Myanmar.

The findings of this study showed that the higher grade of histological types of the gestational trophoblastic diseases, the stronger the intensity of Ki-67 immuno-expression. The findings support that Ki-67 proliferative marker might be useful in determining the prognosis of gestational trophoblastic diseases and early treatment.

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