The Role of C-erb B-2 in Predicting the Malignant Transformation of Complete Hydatidiform Moles: Final Report*

Satrina Rica A. Perez-Castillo, MD; Agnes D. Soriano-Estrella, MD, MHPEd and Margaret Joyce A. Cristi-Limson, MD

Department of Obstetrics and Gynecology, Philippine General Hospital, University of the Philippines Manila

Objective: This study aimed to determine the role of c-erb B-2 as an early predictor of gestational trophoblastic neoplasia after treatment for complete hydatidiform mole.
Study Design: A total of 64 patients who underwent suction curettage for complete hydatidiform mole were included in the study. Patients were divided into 2 groups: Group 1 included 32 cases who achieved and maintained normal βhCG titer after molar evacuation while group 2 consisted of 32 cases that progressed to gestational trophoblastic neoplasia. Immunocytochemical techniques were done to study the expression of the c-erb B-2 oncogene product in the paraffin blocks of the selected study sample. The intensity and percentage of c-erb B-2 staining were recorded. Z test for differences in two proportions was done to determine whether there was statistically significant difference in intensity between the c-erb B-2 immunoexpression of the two groups.
Results: A statistically significant difference between the two groups was seen only in those with weak immunoreactivity (1+) (P = 0.04). However, when data from patients with 0 and 1+ immunoreactivity as well as 2+ and 3+ immunoreactivity were combined, a statistically significant difference was shown between the two groups. There was no statistical difference noted between the two groups using the quantitative analysis.
Conclusion: C-erb B-2 immunostaining may have a role in the prediction of malignant transformation of complete hydatidiform moles. However, until further studies validate the reliability of the c-erb-B2 immunostaining as a predictor of PMGTN, clinicians should still be guided by other clinical parameters in deciding the proper post-evacuation treatment and follow-up of patients with CHM.

Key words: Gestational trophoblastic neoplasia, complete hydatidiform mole, c-erb B-2, malignant transformation

The major clinical concern about complete hydatidiform moles (CHM) is its risk for malignant degeneration or the development of post-molar gestational trophoblastic neoplasia (PMGTN).[1] The

* First place, 2012 SGOP Fellows' and Residents' Research Contest.

estimated risk for progression to PMGTN following a complete molar pregnancy is estimated at 18 to 28%.[2]

At present, monitoring of the level of serum beta-human chorionic gonadotropin (βhCG) is the only reliable way of detecting malignant transformation of CHM. In the Philippines, the protocol endorsed by the Philippine Society for the Study of Trophoblastic Diseases is as follows: βhCG determination is done a week after molar evacuation, then every 2 weeks until 3 consecutive normal βhCG titers are obtained, then monthly for 6 months, then every two months during the next 6 months.[3] So as not to confound the true trend of βhCG values, patients are advised not to get pregnant for at least 1 year after the first normal βhCG titer. Patients who present with any of the following conditions are considered to have PMGTN: 1) a serum level of 20,000 mIU/mL more than 4 weeks after evacuation, 2) a persistently elevated βhCG value 14 weeks post evacuation, 3) an increasing value of 10% or more, 4) elevation of a previously normal βhCG titer after evacuation provided pregnancy is excluded, 5) three weekly determinations of plateauing βhCG values, and 6) clinical or histologic evidence of metastasis at any site.[3] Following this protocol, a minimum of 1-2 months is needed to establish the course of the patient's disease process. Also, patients, most of whom are indigent, may find the frequency of serum βhCG determination too expensive and time consuming to have it done regularly, leading to poor follow-up. Thus, the search for a test that can predict the patient's risk for malignant transformation at the time of molar evacuation is of utmost importance. Early identification of patients who are at risk of developing PMGTN using immunostaining can facilitate precise identification of patients who will most benefit from chemoprophylaxis and intensive post-evacuation monitoring. Similarly, among patients identified as low risk for malignant transformation may be allowed decreased frequency or shortened βhCG monitoring.

The role of oncogenes in the development of various neoplasms in the body has been the focus of investigation in recent years. Oncogenes are set of genes that when altered are associated with the development of malignant cells.[4] It is postulated that a balance between oncogenes and tumor suppressor genes dictate the development of cancers.[5] Presence or absence of these oncogenes within specimens obtained from the actual tumor can now be determined using immunostains. In gestational trophoblastic diseases, studies have been conducted to investigate the possibility of predicting malignant transformation from a molar pregnancy based on immunostaining for oncogenes. So far immunostaining for c-ras, nm23, p53, c-myc, c-erb B-2, c-fms and bcl-2 have been investigated. Of these, c-erb B-2 has been the most studied, however with varying results.

The oncogene c-erb B-2 protein is a member of the epidermal growth factor (EGF) receptor products.[5] These proteins are transmembrane signaling molecules that share close structural homology and have been implicated in cell transformation and tumor pathogenesis.[5-10] Its presence is often associated with poor prognosis in several malignancies, particularly in breast cancer.[5,7,11] Immunostaining for c-erb B-2 is readily available in tertiary institutions, thus its role in predicting malignant transformation of complete hydatidiform mole is worth investigating.

In 2009, we reported the preliminary results of a study that was undertaken to evaluate the role of c-erb B-2 as a predictor of PMGTN.[12] Specifically, this study aimed to determine if there was a difference in the c-erb B-2 staining of specimens obtained from patients who remained to have normal βhCG titer and those who developed malignant degeneration. The degree of staining that was able to predict PMGTN was also obtained. Computed sample size for this study was 64. Of the 64, 32 had hydatidiform mole who attained normal βhCG titers and 32 were patients who developed PMGTN. However, only a total of 35 patients were obtained in this initial study, 15 of whom had PMGTN. The result of the initial study revealed that there was no statistically significant difference in the staining between those patients who remained to have normal βhCG and those who developed PMGTN. We then pursued the study aimed at completing the desired sample size and to

determine if there will be a difference in the results achieved.

Materials and Methods

Study Design and Population

This was a case control study approved by the research technical and ethical review board of the institution.

The study included patients admitted at the Department of Obstetrics and Gynecology of a tertiary hospital from January 2001 to June 2011 with histopathologically confirmed complete hydatidiform mole from specimens obtained during suction curettage. Patients who underwent suction curettage with at least 1 year of regular serial βhCG monitoring, as per treatment protocol, were included. For this study, all patients included in the study were those assessed to be at high-risk of developing PMGTN based on having at least one of the following clinical characteristics: Uterine size larger than age of gestation of more than 6 weeks, serum β-hCG titer more than or equal to 100,000 mIU / ml, theca lutein cysts more than or equal to 6 cm in size, maternal age greater than or equal to 35 years, gravidity of 4 or more, recurrent molar pregnancy, medical complications arising from trophoblastic proliferation (DIC, pre-eclampsia, thyrotoxicosis, pulmonary insufficiency).[3] All these patients received chemoprophylaxis. Those who underwent total hysterectomy, as well as patients who were lost to follow-up (did not reach at least 1 year follow-up or no adequate B-hCG monitoring for at least 3 months) were excluded from the study. Patients were then divided into two groups. Group 1 consisted of patients who remained to have normal βhCG titers after at least 1 year of follow-up as per treatment protocol, while group 2 consisted of patients who developed post-molar gestational trophoblastic neoplasia.

Sample Size Computation

Sample size calculations are based on a proportion of 0.920 (those with cancer) and a proportion of 0.594 among the controls (those without cancer). The test of equality of proportions will be carried out at the 0.05 level of significance. A sample size of 32 from each group gives a probability of 0.804 of rejecting the null hypothesis of equal proportions if the alternative holds.

Description of Study Procedure

The medical records of patients who underwent suction curettage for a complete hydatidiform mole at the Department of Obstetrics and Gynecology of a tertiary hospital from January 2008 to June 2011 were reviewed. A list of those who were able to complete their follow-up schedule as recommended by the Philippine Society for the Study of Trophoblastic Diseases was obtained. Among these patients, retrievable paraffin blocks of 32 patients who remained to have normal βhCG during the follow-up period and 32 patients who progressed to gestational trophoblastic neoplasia were collected. These were submitted to the Section of Surgical Pathology of the institution for immunostaining with c-erb B-2.

Paraffin sections were examined by a pathologist who had no access to the patients' clinical data. Expression of c-erb B-2 was analyzed semi-quantitatively and quantitatively. Semi-quantitative analysis was done by assessing the intensity of the staining. The results were classified according to the system used by previous studies.[7,16] This includes:

(0) negative, no immunoreactivity (Figure 1)
(1+) weak immunoreactivity slightly stronger than background staining (Figure 2)
(2+) moderate immunoreactivity (Figure 3)
(3+) strong immunoreactivity (Figure 4)

In the quantitative analysis, the percentage of cells with complete membranous delineation was determined. Similar to the study of Cameron, et al. and Fulop, et al., the following classification was used:[7,16]

0 - no cells staining (Figure 1)
Grade 1 - few cells staining (Figure 5)
Grade 2 - half of cells demonstrating staining (Figure 6)
Grade 3 - most of cells demonstrating staining (Figure 7)

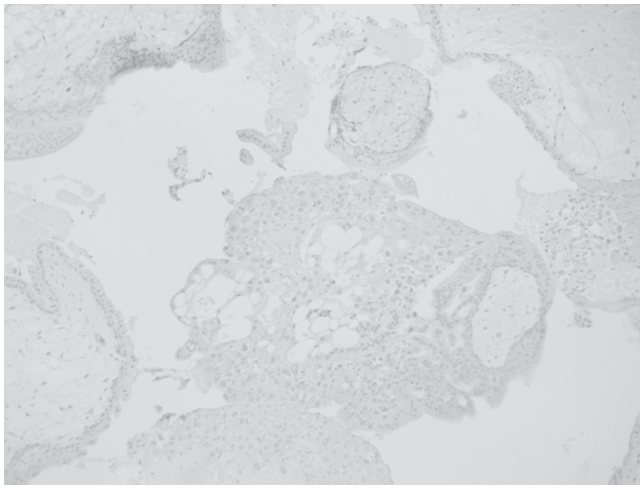


Figure 1. Photomicrograph showing negative or no immunoreactivity in the semi-quantitative and quantitative analysis.

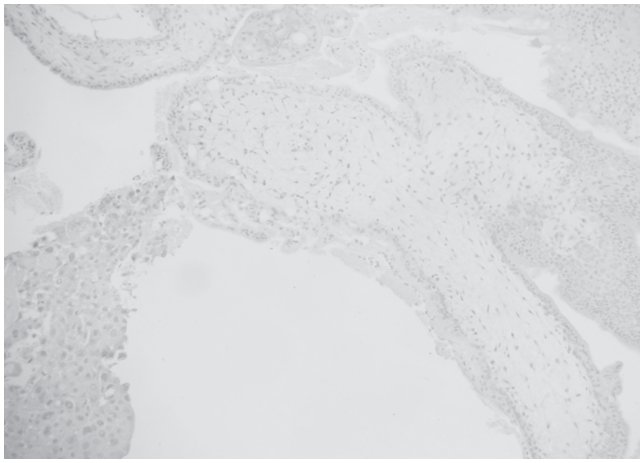


Figure 2. Photomicrograph showing weak immunoreactivity, slightly stronger than the background in the semi-quantitative analysis.

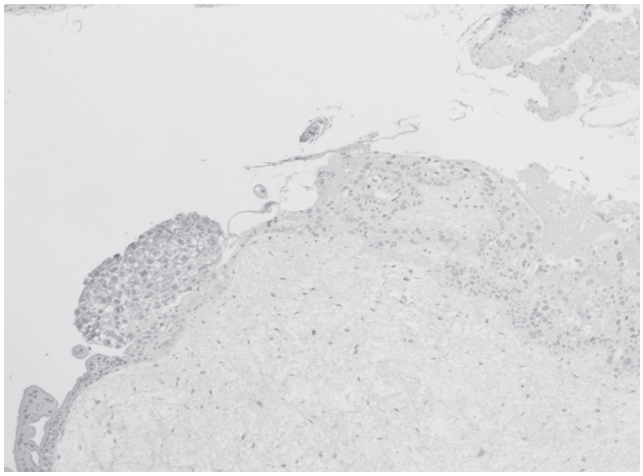


Figure 3. Photomicrograph showing moderate immunoreactivity in the semi-quantitative analysis.

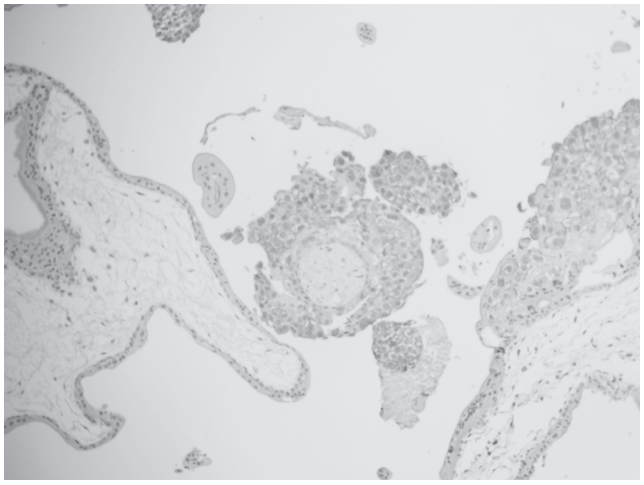


Figure 4. Photomicrograph showing strong immunoreactivity in the semi-quantitative analysis.

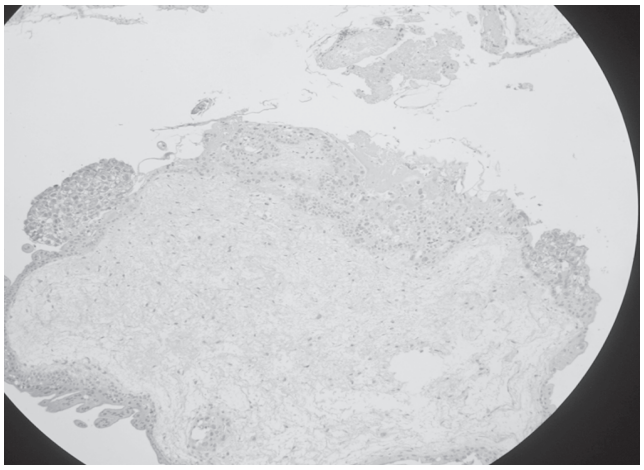


Figure 5. Photomicrograph showing few cells staining in the quantitative analysis.

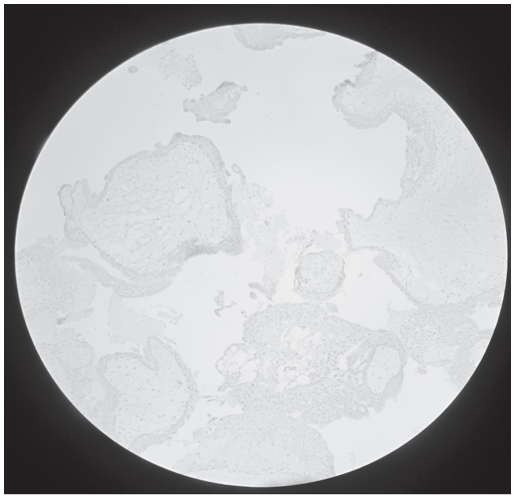


Figure 6. Photomicrograph showing half of cells staining in the quantitative analysis.

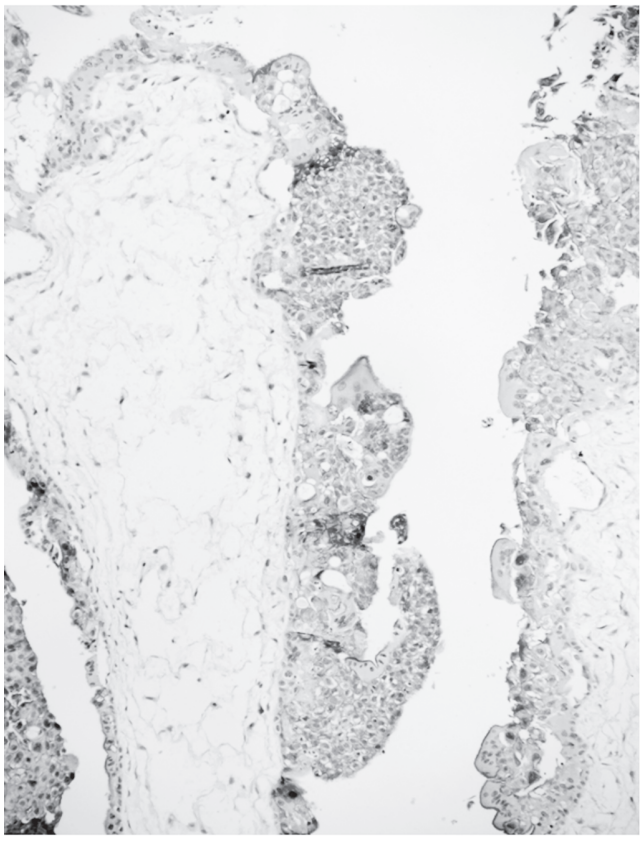


Figure 7. Photomicrograph showing most of cells staining in the quantitative analysis.

Data from the initial report were obtained and combined with the current study for statistical analysis.

Immunohistochemistry

Three-μm histological sections on polysine slides were used with the Dual Link System-HRP technique. The sections were deparaffinized with xylene and hydrated with decreasing graded ethanol concentrations. Antigenic recovery was performed using a microwave. Any endogenous peroxide activity was quenched by incubating the specimen for 10 minutes with dual endogenous enzyme block, after which the slides were washed in Dako wash buffer. The slides were incubated for 20 minutes with primary antibody (polyclonal rabbit anti-human c-erb B-2 oncoprotein). Slides were washed in Dako wash buffer, followed by incubation with the labelled polymer for 15 minutes. Slides were once again washed in Dako wash buffer. Staining was completed by a 10-minute incubation with 3,3'-diaminobenzidine (DAB) substrate chromogen, which results in brown-colored precipitate at the antigen site. Slides were washed in distilled water, and the process was finished with hematoxylin counterstaining.

Statistical Analysis

Z test for differences in two proportions was done to determine whether there was statistically significant difference in both the quantitative and semi-quantitative analysis of intensity of c-erb B-2 immunoexpression of the two groups.

Results

Patients' age ranged from 16-51 years old, with gravidity ranging from G1 to G8. None of the patients had recurrent molar pregnancies. Table 1 shows semi-quantitative analysis of c-erb B-2 expression in histological sections between the two studied populations. Among patients belonging to group 1, semi-quantitative analysis of c-erb B-2 immunoexpression revealed that most patients (35%) had weak immunoreactivity slightly stronger than background staining (1+). On the other hand, majority of patients with malignant degeneration (47%) had moderate immunoreactivity (2+). Among the different degrees of staining in the semi-quantitative analyisis, patients in group 1 generally showed less staining compared to those in group 2. However, a statistically significant difference between the two groups was only seen in those with weak immunoreactivity (1+) (P=0.04). When data from patients with 0 and 1+ immunoreactivity as well as 2+ and 3+ immunoreactivity were combined, a statistically significant difference was shown between two groups (Table 2).

Quantitative analysis of c-erb B-2 immunostaining on the other hand showed that most patients belonging to group 1 (41%) had few cells staining (Grade 1) while most of the patients belonging to group 2 (malignant degeneration) (47%) had most of the cells demonstrating staining (Grade 3) with c-erb B-2 (Table 3). Generally, patients who remained to have normal βhCG titers had less percentage of cells staining.

However. there was no statistical difference noted between the two groups when this form of immunostaining analysis was used, even if clustering of interpretation is done as shown in Table 4.

Table 1. Semi-quantitative analysis of c-erb β-2 expression in histological sections between the two studied populations.

Intensity of Staining	Group 1 (complete hydatidiform mole in remission) (n = 32)	Group 2 (complete hydatidiform mole with progression to GTN) (n = 32)	P-value*
0 (negative, no immunoreactivity)	1 (3%)	1 (3%)	.99
1+ (weak immunoreactivity, slightly stronger than background staining)	11 (35%)	4 (12%)	.04**
2+ (moderate immunoreactivity)	10 (31%)	15 (47%) .	20
3+ (strong immunoreactivity)	10 (31%)	12 (38%)	.60

* Computed using the Z-test for differences in two proportions
** Significant difference at P ≤ 0.05

Table 2. Semiquantitative analysis of c-erb β-2 expression after pooling of results.

Intensity of Staining	Group 1 (complete hydatidiform mole in remission) (n = 32)	Group 2 (complete hydatidiform mole with progression to GTN) (n = 32)	P-value*
0 to 1+	12 (38%)	5 (16%)	.047**
2+ - 3+	20 (62%)	27 (84%)	.047**

* Computed using the Z-test for differences in two proportions
** Significant difference at P ≤ 0.05

Table 3. Quantitative analysis of c-erb β-2 expression in histological sections between the two studied populations.

Percentage of Cells Staining	Group 1 (complete hydatidiform mole in remission) (n = 32)	Group 2 (complete hydatidiform mole with progression to GTN) (n = 32)	P-value
0 (no cells staining)	1 (3%)	1 (3%)	0.99
Grade 1 (few cells staining, more than 10% of cells)	13 (41%)	8 (25%)	0.18
Grade 2 (half of cells demonstrating staining)	7 (22%)	8 (25%)	0.77
Grade 3 (most of cells demonstrating staining)	11 (34%)	15 (47%)	0.31

* Computed using the Z-test for differences in two proportions

Table 4. Quantitative analysis of c-erb β-2 expression after pooling of results.

Percentage of cells with complete membrane staining	Group 1 (complete hydatidiform mole in remission) (n = 32)	Group 2 (complete hydatidiform mole with progression to GTN) (n = 32)	P-value
0 to Grade 1	14 (44%)	9 (28%)	.19
Grade 2 to Grade 3	18 (56%)	23 (72%)	.19

* Computed using the Z-test for differences in two proportions

Discussion

A single test that can accurately predict the risk for malignant transformation of a complete hydatidiform mole has been of great interest for the past years. Among patients predicted to be of low risk for PMGTN, it would mean decreased anxiety and the possibility of a less frequent and shortened follow-up period. This would be particularly important to patients older than 35 who are contemplating pregnancy but have a limited time frame for fertility. On the other hand, among patients who are predicted to be at high risk of developing PMGTN, it would mean closer and prolonged surveillance. In this subset of patients, administration of chemoprophylaxis would be of definite benefit.[13]

At present, a patient's demographic characteristics and clinical features are used to identify women at an increased risk for the development of postmolar gestational trophoblastic neoplasia, however, they still lack the ability to predict the course of disease for individual patients. Monitoring of βhCG remains to be the only acceptable tool of identifying hydatidiform mole patients who have progressed to trophoblastic neoplasia.[14,15] However, it has been a known fact that the use of serum βhCG surveillance can be time consuming and costly to patients.

The balance between cellular proliferation, maturation and cell apoptosis maintains a normal functional tissue.[7] The regulation of trophoblastic proliferation is dependent on the balance between proto-oncogenes and tumor suppressor genes, such that over-expression of the former or decreased function of the latter may lead to uncontrolled cell proliferation and eventually resulting to malignant transformation.[16]

The protein c-erb B-2, also known as HER2/neu is a member of the epidermal growth factor (EGF) receptor family of receptor products. Its expression has been associated with secretion of human chorionic gonadotropin.[8] Members of this EGF receptor family have intrinsic tyrosine kinase activity and are considered important mediators of cell growth, differentiation and survival. The protein product of c-erb B-2 is normally found in tissues such as epithelia of the breast, ovary, endometrium, lung, kidney and gastrointestinal tract, and in the central nervous system.[16] The use of c-erb B-2 staining has been widely used for prognostication in breast cancer.[6,7,11] Maruo, et al. in 1986, performed immunohistochemical staining for EGF receptor in developing human placenta.[11] Their findings suggested a role for EGF receptor in the induction of differentiation of trophoblasts. With this in consideration and because of the extensive homology of EGF to c-erb B-2 oncogene product, they initially proposed that there may lie a link between c-erb B-2 oncogenic stimulation and the trophoblasts' normal growth regulatory mechanism.[11]

In our preliminary report involving 35 patients, results showed no statistically significant difference in the immunoreactivity for c-erb B2 between patients who remained to have normal βhCG and those who developed PMGTN.[12] In this final report, with a total of 64 patients, results showed a statistically significant difference between the two groups, after combining the data from patients with 0 and +1 immunoreactivity as well as 2+ and 3+ immunoreactivity when semi-quantitative analysis of immunostaining is done.

Studies have likewise been done to evaluate the utility of the c-erb B-2 immunostaining as a predictor of postmolar tumor; however, results have varied. In 2002, Yang, et al. concluded that increased expression of c-erb B-2 protein and decreased expression of nm23 were strong predictors for the malignant transformation of complete mole.[16] However, results of other studies have suggested otherwise. In 1994, Cameron and associates found that out of 56 cases of trophoblastic tumors, only one stained positive for c-erb B-2. They concluded that the association between c-erb B-2 and persistent gestational trophoblastic disease was not significant.[17] Menczer, et al. found that although the rate of c-erb B-2 expression was somewhat higher in moles who subsequently developed gestational trophoblastic neoplasia (22.2%) than in moles who went in remission (16.6%), the difference was not statistically significant.[13]

In 2006, Yazaki-Sun and associates postulated that the c-erb B-2 protein likely promoted the growth of the inherently invasive extravillous trophoblasts, and that the over-expression resulted in the progression of molar pregnancies to gestational trophoblastic neoplasia.[18] Their study design was very similar to the present study. They compared the c-erb B-2 immunoreactivity of specimens obtained from patients who achieved normal βhCG titers to those who developed PMGTN. Quantitative and semi-quantitative analysis were also performed. Similar to the results of the present study, Yazaki-Sun, et al. found statistical difference when semi-quantitaive analysis is used and no statistical difference was noted when the quantitative analysis was used.[18] The previous study differed from the present study due to inclusion of partial hydatidiform moles (PHM) and patients who underwent hysterectomy as part of the management of molar pregnancy in the former study. In the present study, the authors deemed it necessary to exclude patients with partial moles in order to eliminate this as a confounding factor. Studies have shown that PHM carries a significantly lower risk of malignant transformation (2 - 4%) when compared to complete moles (18-28%).[2] Thus, inclusion of these patients in the study might affect the outcome. For the same reason, patients who underwent hysterectomy as a method of molar evacuation were excluded because this procedure decreases the risk of local invasion from 20%-33% to 3.5%-10% as compared to curettage.[19] The past study did not indicate whether patients were given methotrexate chemoprophylaxis. For the present study, all patients received chemoprophylaxis. Review of department records showed that majority of hydatidiform mole patients treated in the institution received chemoprophylaxis by virtue of having at least one risk factor for the development of PMGTN. Therefore, although this intervention has been shown to decrease the risk of PMGTN among high-risk molar patients, all patients included in the study received chemoprophylaxis in order to remove this as a confoundng factor.[20]

The findings of this study revealed that there was statistical difference between patients with complete hydatidiform mole with spontaneous remission and those with progression to gestational trophoblastic neoplasia when semi-quantitative analysis of staining is used. However this was only noted when negative or no immunoreactivity (0) and weak immunoreactivity slightly stronger than background staining (1+) are grouped together, and when moderate immunoreactivity (2+) and strong immunoreactivity (3+) were grouped together as well. The relative increase in the sample size when clustering of patients was done could have explained the finding of statistical significance. This means that semi-quantitative analysis of staining can probably be used to predict patients who are at risk in the future of developing gestational trophoblastic neoplasia. On the other hand, this study showed that quantitative analysis probably may not be a good tool to predict malignant tendencies of complete hydatidiform mole.

Given these findings, it can be suggested that immunostaining with c-erb B-2 may be done in patients with complete hydatidiform mole. Results can guide clinicians in determining the appropriate frequency and duration of serum βhCG monitoring as well as identify patients who would benefit most from methods that will decrease the risk for PMGTN such as chemoprophylaxis and hysterectomy.

Conclusion and Recommendation

C-erb B-2 immunostaining may have a role in the prediction of malignant transformation of complete

hydatidiform moles. Results on the semi-quantitative analysis are particularly encouraging. However, until further studies validate the reliability of the c-erb-B2 immunistaining as a predictor of PMGTN, the authors of this study still advise clinicians to be guided by other clinical parameters in deciding the proper post-evacuation treatment and follow-up of patients with CHM.

This study was limited to patients diagnosed with complete hydatidiform moles. The investigators recommends future research on the role of c-erb B-2 in prediciting malignant transformation in cases of partial hydatidiform moles. The involvement of other tumor markers in the development of gestational trophoblastic disease should also be further explored.

References

1. Khoo SK, Baartz D, Sidhu M, Yip WL, Tripcony L. Analysis of risk factors for persistent trophoblastic disease. Aust N Z J Obstet Gynecol 2009; 49: 657-9.
2. Garner EI, Goldestein DP, Feltmate CM and Berkowit RS. Gestational trophoblastic disease. Clin Obstet Gynecol 2007; 50(1): 112-22.
3. Clinical Practice Guideline for the Diagnosis and Management of Gestational Trophoblastic Diseases, Philippine Society for the Study of Trophoblastic Diseases, Inc. Nov 2006.
4. Lentz GM, Lobo RA, Gershenson DM and Katz VL. Molecular Oncology in Gynecologic Cancer. Comprehensive Gynecology, Mosby, an imprint of Elsevier Inc. 2012: 627.
5. William GT, Smith A. Molecular regulation of apoptosis: Genetic controls on cell death. Cell 1993; 74: 777-9.
6. Downward J, Yarden Y, Mayes E, Scrace G, Totty N, Stockwell P, Watefield MD. Close similarity of epidermal growth factor receptor and c-erb B oncogene protein sequences. Nature 1994; 307: 521-7.
7. Fulop V, Mok S, Genest D, Szigetvari I, Cseh I, Berkowitz R. C-myc, c-erb B-2, c-fms and bcl-2 oncoproteins expression in normal placenta, partial and complete mole and choriocarcinoma. J Reprod Med 1998; (43)2: 101-10.
8. Tuncer ZS, Vegh GL, Fulop V, Genest DR, Mok SC, Berkowitz RS. Expression of epidermal growth factor receptor-related family products in gestational trophoblastic diseases and normal placenta and its relationship with development of postmolar tumor. Gynecol Oncol 2000; 77(3): 389-93.
9. Meric F, Hung MC, Hortobagyi GN, Hunt KK. HER2/neu in the management of invasive breast cancer. J Am Coll Surg 2002; 194(4): 488-501.
10. Lee WS. Analysis of prognostic factors and treatment modality changes in breast cancer: a single institution study in Korea. Yonsei Med J 2007; 48(3): 465-73.
11. Maruo T, Mochizuki M. Immunocytochemical localization of epidermal growth factor receptor and myc oncogene product in human placenta: implication for trophoblast proliferation and differentiation. Am J Obstet Gynecol 1987; 156: 721-7.
12. Cristi M, Soriano-Estrella A. The role of c-erb B2 in the malignant transformation of complete hydatidiform mole. Phil J Obstet Gynecol 2009; 33(3): 89-93.
13. Menczer J, Schreiber L, Berger E, Golan A, Levy T. Assessment of Her-2/neu expression in hydatidiform moles for prediction of subsequent gestational trophoblastic neoplasia. Gynecol Oncol 2007; 104: 675-9.
14. Trommel N, Sweep F, Schijf C, Massuger L, Thomas C. Diagnosis of hydatidiform mole and persistent trophoblastic disease: diagnostic accuracy of total human chorionic gonadotropin (hCG), free hCG a- and b-subunits, and their ratios. Eur J Endocrinol 2005; 153: 565-75.
15. Kerkmeijer L. Human chorionic gonadotropin in the prediction of persistent trophoblastic disease. Obstet Gynecol 2009; 326-31.
16. Yang X, Zhang Z, Jia C, Li J, Yin L, Jiang S. The relationship between expression of c-ras, c-erb B-2, nm23, and p53 gene products and development of trophoblastic tumor and their predictive significance for the malignant transformation of complete hydatidiform mole. Gynecol Oncol 2002; 85: 438-44.
17. Cameron B, Gown AM, Tamimi HK. Expression of c-erb B-2 oncogene product in persistent gestational trophoblastic disease. Am J Obstet Gynecol 1994; 170: 1616-21.
18. Yazaki-Sun S, Daher S, de Souza. Correlation of c-erbB-2 oooncogene and p53 tumor suppressor gene with malignant transformation of hydatidiform mole. J Obstet Gynecol Res 2006; 32(3): 265-72.
19. Berkowitz RS, Goldstein DP. Current management of gestational trophoblastic diseases. Gynecol Oncol 2009; 112(3): 654-62.
20. Kim DS, Moon H, Kim KT, et al. Effects of prophylactic chemotherapy for persistent trophoblastic disease in patients with complete hydatidiform mole. Obstet Gynecol 1986; 67(5): 690-4.

Long-Term Prognosis and Risk Factors for Recurrence Among Patients with Borderline Epithelial Ovarian Tumors in a Tertiary Hospital - A Local Experience*

Angelito D. Magno, MD and Jean Anne B. Toral, MD, MSc

Section of Gynecologic Oncology, Department of Obstetrics and Gynecology, Philippine General Hospital, University of the Philippines Manila

This retrospective study investigated the long-term prognosis of borderline ovarian tumors and determined risk factors for recurrence. Ninety-nine patients treated between 2002-2011 were investigated for clinical stage, histopathologic subtype, surgical techniques, intraoperative findings, adjuvant therapy, presence and absence of recurrence and prognosis. Follow-up ranged from 8 months to 144 months (median= 17months). The average age was 42 years old. Eighty-six (87%) of patients had stage 1 disease. Histopathologic subtypes included mucinous (80%), serous (17%), endometrioid (1%) and mixed epithelial type (1%). Conservative surgery was used in 39% of cases and radical surgery in 61% and staging procedure in 60% and post-operative adjuvant therapy in 23%. Recurrence was found in 13 patients, and one of which died of the disease. The average disease-free interval was 46 months (6-148months). The 5 and 10-year overall survival for stage 1 disease was 98 % and 93% respectively. Age (≥50), stage (II-IV), unstaged procedure, tumor rupture, presence of peritoneal implants, and residual tumors were significantly associated with tumor recurrence ($P < 0.05$). However, surgical procedure and histologic type were not significantly correlated with recurrence.

Key words: ovarian tumors

Long before Howard Taylor, in 1929, introduced the term 'semi-malignant' tumor in the literature, many physicians described papillary ovarian cystadenomas that clinically behave between benign and malignant tumors. It has been four decades since the International Federation of Gynecology and Obstetrics (FIGO) and World Health Organization (WHO) in the 1970s formally introduced the term "carcinoma of low malignant potential" or "borderline tumor."[1-3,5-6,8-10] The term "borderline tumor" is synonymous with "carcinoma/tumor of low malignant potential" or "atypical ovarian tumor".

According to the WHO definition, a borderline epithelial tumor lacks obvious invasion of the stroma and has mitotic activity and nuclear abnormalities intermediate between clearly benign and unquestionably malignant tumors of a similar cell. Histologic criteria for making the diagnosis include nuclear atypia, stratification of the epithelium, formation of microscopic papillary projections and the absence of stromal invasion. [1-2,4,6-8,11-13]

Some authors labeled serous type as the most common histologic type followed by mucinous.

* Second place, 2012 SGOP Fellows' and Residents' Research Contest.

(60.3% vs 33%).[1,5,6,9] In others, mucinous tumor is more common (75.2%) than serous (22.3%). Least common are endometrioid and Brenner types and mixed type.[15]

Surgery is the primary treatment of patients with ovarian tumors.[1,17] According to the FIGO classification, the surgical treatment includes a detailed exploration of the entire abdomen, bilateral salpingo-oophorectomy (BSO), hysterectomy, omentectomy, peritoneal lavage (or ascites sample), peritoneal biopsies, resection of all suspected lesions and retroperitoneal lymphadenectomy. For mucinous tumors, an appendectomy should be performed to exclude any ovarian metastasis of possible mucinous tumor of the appendix.[11,17] However, many authors suggested that since borderline ovarian tumors are less aggressive than the ovarian carcinoma counterpart, conservative surgical approach is more appropriate, even for more advanced stage disease. Some do not even recommend routine retroperitoneal lymphadenectomy.[1,4,8,12]

Similarly, the use of chemotherapy in borderline ovarian tumors is still controversial. Many studies advocate the use of adjuvant chemotherapy for advanced stage borderline tumors. However, some authors also questioned its routine use citing the lack of large significant study, and that chemotherapy does not affect the over-all survival.[1,4,8] In addition, complications from adjuvant treatment contribute to the total morbidity and mortality rates and not necessarily from the disease itself.[9,12] Due to the lack of large randomized trial on the use of chemotherapy in borderline ovarian tumors, the Society of Gynecologic Oncologists of the Philippines, in their 2008 Clinical Practice Guidelines, came up with a decision, based on consensus among its members for the use of chemotherapy in advanced borderline ovarian tumor (stage IC-IV).[17]

Early stage borderline ovarian tumor has very good prognosis.[3,6,1] Recurrence rate of borderline ovarian tumor is very low, ranging from 2-7%.[3] Recurrent tumor may develop after latent intervals as long as 20-50 years.[6] In the literature, there are different risk factors associated with tumor recurrence or progression of borderline tumor to frankly malignant tumors. However, most studies have conflicting data, reporting statistical insignificance. These factors include: stage[3,9,15,18], histologic type (serous versus non-serous)[1,9,15], type of surgery (conservative versus radical or if staging procedure was done)[1,8,9,11,15], administration of adjuvant treatment, either in the form of radiation or chemotherapy[15], residual tumor[3,9], presence and type of implants (either non-invasive, invasive and micropapillary)[1,3,4,8,11,13,18], and tumor ploidy.[9]

The aim of this study was to investigate the clinical outcome of patients with borderline ovarian tumors, and to determine the risk factors for its recurrence. To date, there is no local study on the long-term prognosis and risk factors for recurrence of patients diagnosed with borderline ovarian tumor.

Objectives

The general objective of this study was to determine the long-term prognosis of patients with borderline epithelial ovarian tumors and to determine the risk factors for its recurrence.

The specific objectives were as follows: 1) to determine the long term prognosis, 5-year and 10-year over-all survival (OS) stage per stage of patients diagnosed with borderline epithelial ovarian tumor, in the local setting; 2) to determine the local experience in the management of borderline epithelial ovarian tumor, particularly surgical and adjuvant treatment; 3) to determine the factors associated with tumor recurrence.

Materials and Methods

Study Design

This is a retrospective study, which evaluated the long-term prognosis of patients with borderline epithelial ovarian tumors and the risk factors for its recurrence. This study underwent technical and ethical review and was subsequently approved by the Institutional Review Board.

Scope and Limitations

The study involved chart review of all patients with borderline ovarian tumors referred and managed in

the Cancer Institute of UP-PGH from 2002-2011. The study has limited its subjects to patients seen in the Cancer Institute. Not all patients operated for borderline ovarian tumor were seen and managed by the Gynecologic Oncology Service. Another limitation was since this study was purely based on chart review, many charts of patients could not be retrieved anymore in the medical records, especially those who were diagnosed in 2002-2004.

Patient Population

All borderline ovarian tumor patients managed by the Gynecologic Oncology Service in the Cancer Institute form 2002-2011 were included in the study. Patients, who were diagnosed with any cancers, concomitant with the borderline tumors that may affect the prognosis of the patient were excluded in the study.

Methodology

Prior to data collection, the list of all patients, who met the inclusion and exclusion criteria, were obtained from the Weekly OPD Census. An information sheet was developed (Appendix 1), which included patient's profile and other demographic data, surgical procedure done, histologic type of ovarian tumor, date of operation, final stage based on the histopathologic result, tumor ploidy if available, presence or absence and size of residual tumor, presence or absence and type of peritoneal implants, adjuvant treatment, if any, patient's present status, site of recurrence, timing of diagnosis of recurrence. Charts were borrowed from the Medical Records of the Cancer Institute. Chart review was done in all patients included in the study. Patients who were lost to follow-up were contacted, and asked for signs and symptoms of recurrence like abdominal bloatedness, early satiety, pelvic heaviness or painful abdominal enlargement or any limitation on personal activity. Also, these patients were asked to consult if possible to check their present status. If not possible, survival was computed from the time no evidence of disease was first noted up to the last consult. The primary outcomes of time study include 5-year and 10-year overall survival and the correlation of risk factors to recurrence.

Statistical Analysis

Data were encoded in the Excel Worksheet, which percentage, average and median tools were used. Pearson product moment correlation and risk ratio were used to analyze the risk factors for recurrence. Kaplan-Meier was used to determine overall survival.

Results

A total of 99 patients were included in the study. Seventy nine patients are alive with no evidence of disease. Thirteen patients had recurrence, one of whom died of the disease. Seven patients, who were seen in the Cancer Institute once and lost to follow, were excluded from data analysis for recurrence, since patients status cannot be determined. The median follow-up was 17 months (range 8-144 months). (Table 1).

The 5-year and 10-year survival of all borderline ovarian tumor patients are in table 2. The 5-year and 10-year overall survival for borderline ovarian tumor were 89% and 83.9%, respectively (Figure 1). For patients with stage I disease, the 5 and 10 year overall survival are 98.6% and 92.5%, respectively (Figure 2). For advanced stage disease, the 5 and 10-year overall survival is only 22.5% (Figure 3). This is far lower than in the report of Chambers, et al. with a 5-year survival rate of 60 - 80%.

The average age of patients with borderline ovarian tumor was 42 years old (range 14-91 years old). Majority of patients with borderline ovarian tumor are 41 years old and above accounting for 52% followed by women 40 years old and younger accounting for 47.5%.

Eighty-one patients (81.8%) with borderline ovarian tumors had non-serous type histology, 80% of which were mucinous type and only 1 case of endometrioid borderline type was noted. The serous type, including the serous papillary, accounted for 17 percent of patients. There was one case of mixed epithelial borderline tumor noted.

Majority of women with borderline tumor were in the early stage of the disease. Stage 1 disease

Table 1. Clinical and histopathologic characteristics of patients with borderline tumors.

Number of Patients	Alive NED (n=79)	Recurrence (n=12)	Died of Recurrence (n=1)	Lost (n=7)	TOTAL (n=99)
Age:					
<20	3	0	0	0	3 (3.0%)
20-30	25	0	0	2	27 (27.3%)
31-40	11	3	0	3	17 (17.2%)
41-50	17	1	0	0	18 (18.2%)
51-60	19	6	1	1	27 (27.3%)
>60	4	2	0	1	7 (7.1%)
Histologic type:					
Mucinous/Non-serous	67	9	1	4	81 (81.8%)
Serous	12	3	0	2	17 (17.2%)
Mixed	0	0	0	1	1 (1%)
Stage:					
I	76	3	1	6	86 (86.9%)
IA	63	0	0	0	63
IB-IC	13	3	1	6	23
II-IV	3	9	0	1	13 (13.1%)
Surgical Procedure:					
Radical	45	7	1	7	60 (60.6%)
Conservative	34	5	0	0	39 (39.4%)
Cystectomy	2	3	0	0	5
USO/adnexectomy	32	2	0	0	34
Staging Laparotomy:					
Staged	51	3	0	5	59 (59.6%)
Unstaged	28	9	1	2	40 (40.4%)
Adjuvant Therapy:					
Yes	10	11	1	4	26 (26.3%)
No	69	1	0	3	73 (73.7%)
Other risk factors:					
Tumor rupture:					
Yes	11	9	1	4	25 (25.3%)
No	68	3	0	3	74 (74.7%)
Implants:					
None	76	6	1	5	88 (88.9%)
Yes	3	6	0	2	11 (11.1%)
Not biopsied	1	3		0	4
Non-invasive	1	2		2	5
Invasive	1	1		0	2
Residual:					
Yes	0	2	0	0	2 (2%)
No	79	10	1	7	97 (98%)
Tumor ploidy: n=38					
Diploid	34	2	0	2	38
Low risk (low proliferative rate)	6	0		1	7
High risk (high proliferative rate)	28	2		1	31
Aneuploid	0	0	0	0	

Terminology:

Radical surgery: Hysterectomy with bilateral salpingo-oophorectomy
Conservative surgery: surgery that preserves the uterus, either ovarian cystectomy or adnexectomy or salpingo-oophorectomy only.
Staged: if peritoneal fluid cytology, pelvic with or without paraaortic lymphadenectomy, partial omentectomy and random peritoneal biopsy were done.
Unstaged: if peritoneal fluid cytology, pelvic with or without paraaortic lymphadenectomy, with partial omentectomy and random peritoneal biopsy were not done.
Adjuvant treatment: any treatment given post-operative, either radiation, chemotherapy, surgery or combination.
Over-all survival: time from diagnosis of the disease to present or latest status of the patient.
Progression-free survival: time from diagnosis of the disease up to diagnosis of recurrence.
Recurrence: occurrence of new lesion by any imaging modalities and/or increase in tumor markers beyond normal values and/or biopsy proven tumor on re-exploration. These factors constitute the term "recurrence", which make the physician give adjuvant treatment.

Table 2. Percentage survival of long term prognosis of patients diagnosed with borderline epithelial ovarian tumor.

Survival	5 years (60 months)	10 years (120 months)
Overall	89.0 (82.6 - 95.4)	83.8 (76.2 - 91.4)
Stage (IA - IC)	98.6 (96.2 - 99.8)	92.5 (87.1 - 97.9)
Stage (II - IV)	22.5 (13.9 - 31.1)	22.5 (13.9 - 31.1)

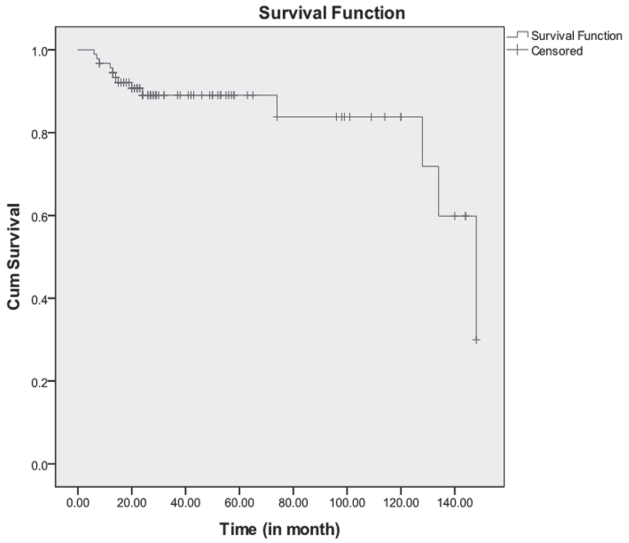


Figure 1. Overall survival of patients with borderline ovarian tumor (Kaplan Meier).

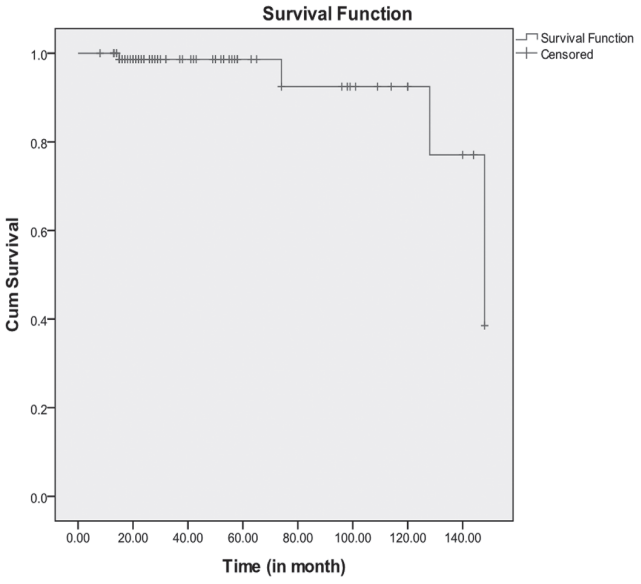


Figure 2. Survival curve of patients with stage I disease (Kaplan Meier).

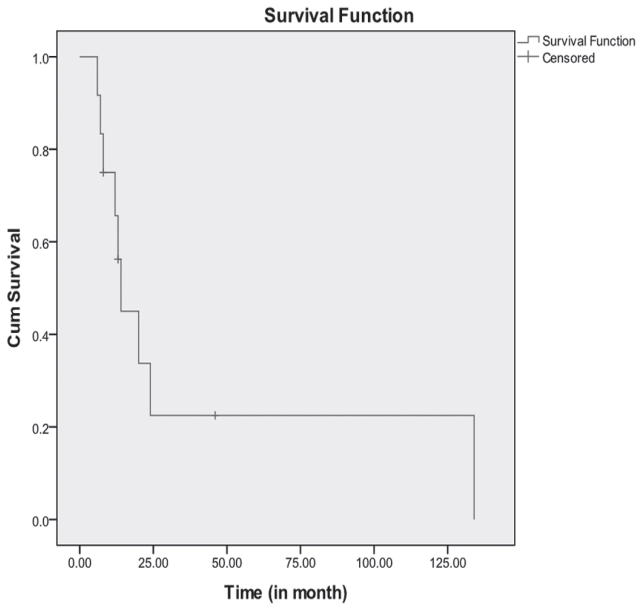


Figure 3. Survival curve of patients with stage II-IV (Kaplan Meier).

accounted for 87% of cases. Of these, 63 patients had stage 1A and 23 patients had stage 1C. Thirteen patients had advanced stage II-IV diseases.

Sixty patients (60.6%) underwent radical surgery. There were 39 patients who underwent conservative surgery, 87% of which had salpingo-oophorectomy and 13% had cystectomy or ovarian biopsy only. Among the 99 patients, 60% underwent staging procedure and 40% were either inadequately staged or not staged at all.

There were only 25 cases of tumor rupture, either preoperative or intraoperative. There were 11 patients for which tumor implants were noted. Five were non-invasive type of implants, 2 had invasive implants and 4 patients had implants but were not biopsied. There were only 2 patients with residual tumors, both had >10mm in residual size.

Of the 36 patients who required adjuvant chemotherapy (stage 1C=23, stage II-IV=13), only 25 patients (69%) received adjuvant therapy. Thirty one percent did not receive adjuvant therapy. Adjuvant therapy was either in the form of chemotherapy alone or surgery plus chemotherapy. Interestingly, there were 2 stage 1A patients who received adjuvant chemotherapy. The first patient was a 14-year old,

diagnosed with borderline mucinous tumor, which on tumor ploidy study showed diploid DNA but with higher proliferative rate (S-phase). The other was a 57-year-old patient, diagnosed with borderline serous tumor, who underwent total hysterectomy but no surgical staging procedure was done. Both patients are alive and have no evidence of disease.

Tumor Recurrence

Risk Factors

Data analysis was done by comparing the presence of risk factors in patients with tumor recurrence versus with no recurrence. The seven patients who were lost to follow-up were excluded from the analysis since presence of recurrence could not be determined. Only 92 patients were analyzed (79 patients with no recurrence and 13 patients with recurrence). The average disease-free interval was 46 months (6-148 months). Table 3 shows the patients' risk factors and its correlation with recurrence. Table 4 shows the risk ratio of each factor for tumor recurrence. Table 5 shows the characteristics of patients with tumor recurrence.

Age

Among patients 40 years old and younger, 93% (n=39) had no recurrence and 7 % had recurrence. Among patients older than 40 years old, 20% had tumor recurrence. Age is a significant factor for recurrence ($P<0.05$). Patients with age 50 years and above were at higher risk of 4.2 times.

Histologic Type

Among patients with mucinous tumor, only 13% had tumor recurrence as compared to patients with serous histology (25%). Tumor histologic type is not significantly correlated with tumor recurrence ($P=0.48$).

Stage

Among patients with stage 1 borderline tumors, there were only 4 cases of tumor recurrence; all 4 had stage 1C disease. There was no recurrence in patients with stage 1A disease. However, in patients with advanced disease (stage II-IV, n=12), 9 had tumor recurrence (75%). There is correlation between stage and tumor recurrence ($P< 0.05$). Patients with stage II-IV had 15 times risk of developing recurrence as compared to stage I patients.

Surgical and Staging Procedure

Among patients who underwent radical surgery (n=53), only 15% had tumor recurrence. Patients who underwent conservative surgery, tumor recurrence rate was 12.8%. The correlation between surgical procedure (conservative versus radical) and recurrence is not significant ($P=0.76$).

Among patients who underwent staging procedure, only 6% had recurrence as compared with patients who had inadequate or no staging procedure (24.3%). This factor is significantly correlated with tumor recurrence ($P<0.05$). Patients who were unstaged or inadequately staged had risk ratio of 4.74.

Intraoperative Findings

Forty eight percent of patients (n=10) who had tumor rupture had tumor recurrence, as compared to patients with no rupture (n=3, 4%). Among patients with tumor implants (n=9), 67% had recurrence, whereas, in patients with no implants, only 8% had recurrence. The type of implants was not significant due to small number of patients. There were no cases of recurrence in patients with no residual tumor. However, in patients with tumor recurrence, only 18% of patients had residual tumor.

Adjuvant Therapy

Among patients who are alive and no evidence of disease, 16 were required to undergo adjuvant therapy. Fifty percent received adjuvant therapy and 50% did not receive any form of therapy. However, among the 13 patients with recurrence, 12 had previous adjuvant therapy. Only one patient, who was required to have chemotherapy but did not receive any, had recurrence.

Table 3. Patient's risk factors distribution and its correlation to tumor recurrence.

Risk Factors		No Recurrence (n = 79)	Recurrence (n = 13)	TOTAL	Correlation Coefficient	*P-value*
Age: (42.2 ± 15.7)					.28*	.01
	<20	3 (3.3)	0	3 (3.3)		
	20-30	25 (27.2)	0	25 (27.2)		
	31-40	11 (12.0)	3 (3.3)	14 (15.2)		
	41-50	17 (18.5)	1 (1.1)	18 (19.6)		
	51-60	19 (20.7)	6 (6.5)	25 (27.2)		
	>60	4 (4.3)	3 (3.3)	7 (7.6)		
HistologicType:					.07	.48
	Mucinous/non serous	67 (72.8)	10 (10.9)	77 (83.7)		
	Serous	12 (13)	3 (3.3)	15 (16.3)		
Stage:					.68*	.00
	I	76 (82.6)	4 (4.3)	80 (87)		
	IA	63 (78.8)	0	63 (78.8)		
	IB-IC	13 (16.3)	4 (5.0)	17 (21.3)		
	II-IV	3 (3.3)	9 (9.8)	12 (13)		
Surgical Procedure:					-.03	.76
	Radical	45 (48.9)	8 (8.7)	53 (57.6)		
	Conservative	34 (37.0)	5 (5.4)	39 (42.4)		
	Cystectomy	2 (5.1)	3 (7.7)	5 (12.8)		
	USO/adnexectomy	32 (82.1)	2 (5.1)	34 (87.2)		
Staging Laparotomy:					.29*	.00
	Staged	51 (55.4)	3 (3.3)	54 (58.7)		
	Unstaged	28 (30.4)	10 (10.9)	38 (41.3)		
Adjuvant Therapy:					.65*	.00
	Yes	10 (10.9)	12 (13)	22 (23.9)		
	No	69 (75.0)	1 (1.1)	70 (76.1)		
Other Risk Factors						
Tumor rupture:					.52*	.00
	Yes	11 (12.0)	10 (10.9)	21 (22.8)		
	No	68 (73.9)	3 (3.3)	71 (77.2)		
Implants:					.50*	.00
	Yes	3 (3.3)	6 (6.5)	9 (9.8)		
	None	76 (82.6)	7 (7.6)	83 (90.2)		
Residual:					.37*	.00
	Yes	0	2 (2.2)	2 (2.2)		
	No	79 (85.9)	11 (12.0)	90 (97.8)		

- Significant correlation at .05 level

Tumor Ploidy

Among the 99 patients included in this study, only 38 patients were able to do tumor ploidy. All were cases of diploid borderline tumor. Among patients with no recurrence, 82% had intermediate-high risk diploid DNA and only 18% had diploid low risk DNA. Among patients with recurrence, only two were able to do tumor ploidy, both had intermediate-high risk diploid DNA.

Discussion

Borderline ovarian tumors (BTO) represent 5-20% of all ovarian epithelial tumors.[3, 8, 11-14] Some authors labeled serous type as the most common histologic

Table 4. Patient's risk ratio to tumor recurrence.

Risk Factors	Risk Ratio	Confidence Interval (95%)	P-value
Age (> 50)	4.22*	1.41 - 12.63	0.01
Histologic Type (Serous)	1.54	.48 - 4.94	0.49
Stage (II - IV)	15.00*	5.47 - 41.16	0.00
Surgical Procedure (Conservative)	0.85	.30 - 2.40	0.78
Staging Laparotomy (Unstage)	4.74*	1.40 - 16.07	0.01
Adjuvant Therapy (Yes)	38.18*	5.26 - 277.3	0.00
Tumor Rupture (Yes)	11.27*	3.41 - 37.22	0.00
Implants (Yes)	7.91*	3.39 - 18.42	0.00
Residual (Yes)	8.18*	24.70 - 14.23	0.02

* Significant ratio at .05 level.

type followed by mucinous. (60.3% vs 33%).[1,5,6,9] However, in this report, the most common histology was mucinous type, followed by serous type borderline tumor. There was one case of endometrioid and one case of mixed epithelial type of tumor.

In general, patients with borderline ovarian tumors are younger than those with invasive ovarian carcinoma, with more than half of patients are premenopausal.[3] The average age of patients in this study was 42 years, which is similar to the report of Yokoyama, et al.[15] Chambers reported the mean age of patients with borderline tumors as 44 years and a range of 15 to 85 years, and 47 percent were younger than age 40.[1] Levi reported high prevalence of borderline ovarian tumor in patients in the fourth to fifth decade of life.[16]

Staging of borderline ovarian tumor is similar to frankly malignant cancers, which is based on International Federation of Gynecology and Obstetrics (FIGO) 2009 Staging. The FIGO 1998 Ovarian Cancer staging was only updated to FIGO 2009 Staging of Ovarian Cancer, but there was no changing in the staging. Most of the patients with borderline ovarian tumors are diagnosed at an early stage I.[10,11] Up to 90% of ovarian borderline tumors (range 60-90%) present as stage I disease, similar to this study. The approximate incidence of stage II disease has been reported to be 15% to 20% in most studies, followed by stage III (6-10%) and stage IV disease is rarely encountered (0.4-1%).[1,3,6,10,15]

Recurrence rate of borderline ovarian tumor is very low, ranging from 2-7%.[3] However, in this report, the recurrence rate was 13%. High recurrence rate is due to inclusion of patients who were diagnosed with Pseudomyxoma peritonei as the recurrence. In the study of Yokoyama, et al.[15], they excluded patients with pseudomyxoma peritonei in the study on the basis that the disease changes the clinical course of patients with borderline mucinous tumor. However, patients with pseudomyxoma peritonei were included in this study since the disease was considered a recurrence in the local setting. Six (46%) patients with recurrence were diagnosed with Pseudomyxoma peritonei and underwent peritonectomy and confirmed to have diffuse peritoneal adenomucinosis (DPAM), which is a benign long-term complication of mucinous tumor. Recurrent tumor may develop after latent intervals as long as 20-50 years.[6] In the literature, there are different risk factors associated with tumor recurrence or progression of borderline tumors. However most studies have conflicting data, reporting statistical insignificance.

Age, advanced stage, inadequate or no performance of surgical staging, tumor rupture,

presence of peritoneal implants and residual tumor were the significant risk factors seen in this study.

In the study of Shih, et al.[19], advanced age was a risk factor for recurrence of borderline ovarian tumor. Our study noted that age 50 years and older was associated with tumor recurrence. Seidman, et al. concluded in their study of 4,129 patients with borderline serous tumors that surgical stage was one of the most important prognostic factors of borderline ovarian tumor. In addition, the recurrence rate per year for stage 1 tumors was 0.27%, but the recurrence rate per year for advanced stage tumors was 2.4%.[18,20] In this study, patients with advanced disease stage II-IV had 15 times higher risk for recurrence. Patients who were unstaged or inadequately staged were 5 times of higher risk for recurrence. However, Rao, et al.[12] reported that in borderline ovarian tumor, staging procedure specifically pelvic and para-aortic lymphadenectomy were not associated with tumor recurrence, and suggested that surgical staging procedure can be omitted in the management of borderline ovarian tumor. Presence of peritoneal implants, particularly the invasive type, and residual tumors had been considered as poor prognostic factors in many studies and were associated with tumor recurrence.[1,3,4,8,11,13,18] This study reported an eight times higher risk for recurrence if tumor implants or residual tumor was noted.

Histologic type and surgical procedure (either radical or conservative) were not seen to have significant effect on tumor recurrence. Chambers[1] reported that conservative surgery in the form of unilateral salpingo-oophorectomy has a role in the management of patients with borderline tumor especially in younger patients. This can be attributed to the fact that majority of patients with borderline ovarian tumors are young and at the early stage of the disease, thus conservative surgery is appropriate. This was contrary to the report of Yokoyama, et al.[15], who reported a difference in 10-year disease-free survival rate between radical surgery (89%) and conservative surgery (57.4%).

Adjuvant chemotherapy, after surgery, has been the mainstay in the management of ovarian cancers. However, in borderline ovarian tumor, chemotherapy has no role according to Society of Gynecology and Obstetrics of Canada[9], even in advanced stages, unless there are invasive or microinvasive implants. In this study, adjuvant therapy has no effect the risk of recurrence of borderline tumor. All but one patient who had recurrence had previous adjuvant therapy, which means that even if chemotherapy was given to a patient, it is not a guarantee that recurrence will not recur.

Tumor ploidy was not included in statistical analysis due to limited number of patients who had performed this test. Diploid DNA, regardless of risk category, was not associated with recurrence. In addition, Libalova reported that DNA aneuploidy is also not associated with tumor recurrence.[21] Thus, tumor ploidy study has no role in the management of borderline ovarian tumor.

In the 3rd edition of SGOP Clinical Practice Guideline 22 (Table 6), the society suggested radical surgery for patients with stage IC disease, regardless of age and patient's desire for fertility, which is similar to the management of frankly malignant ovarian tumor. As noted in this study, stage I disease including stage IC disease has high overall survival and low recurrence rate. Moreover, surgical procedure was not associated with tumor recurrence.

Suggestions in the management of borderline tumors based on the findings in this study can be seen in table 7.

It is therefore suggested that for patients with early stage disease, stage IA-IC, conservative surgery is appropriate especially in patients who desire future fertility. In addition, decision to give adjuvant chemotherapy should include the presence of risk factors, which were noted have good correlation with tumor recurrence in this study.

Conclusion

Management of borderline ovarian tumor requires more conservative approach as compared to frank malignancy. Presence of risk factors associated with tumor recurrence would be a guide as to whether chemotherapy is warranted in a particular patients. It is therefore recommended that a prospective study with the same objective would better determine the

Table 6. Management of epithelial ovarian tumors of low malignant potential SGOP clinical practice guidelines 2008.

Stage	Status	Intervention[4,5] (Level 2b)	Adjuvant Therapy
	Young/desirous of pregnancy	USO/BSO, IO, PFC, complete surgical staging	None*
IA - IB	Reproductive function not desired	THBSO, IO, PFC, complete surgical staging	None*
IC - IV	Optimally debulked (residual tumor <2.0 cm) or Sub-optimally debulked (residual tumor ≥ 2.0cm)	THBSO, IO, PFC ± tumor debulking	Chemotherapy (Consensus-based) GPP

Table 7. Suggested revisions in the management of ovarian tumors of low malignant potential.

Stage	Status	Intervention	Adjuvant Therapy
IA - IC	Young/desirous of pregnancy	USO/BSO, frozen section, IO, PFC, complete surgical staging	None*
	Reproductive function not desired	THBSO, frozen section of ovarian cyst IO, PFC, complete surgical staging	None*
II - IV	Young/desirous of pregnancy	USO/BSO, frozen section, IO, biopsy of all implants/adhesions, complete surgical staging + tumor debulking	Chemotherapy if with risk factors for recurrence*
	Reproductive function not desired	THBSO, IO, biopsy of all implants/adhesions, complete surgical staging, ± tumor debulking	

* Risk factors for recurrence
  Age (> 50)
  Stage (II - IV)
  Inadequately staged or unstaged
  Presence of tumor rupture
  Presence of implants
  Presence of residual tumors

- Tumor ploidy study may be done to check aneuploidy.
- Appendectomy should be done in all borderline mucinous tumor
- Post-treatment monitoring should be less frequent than the frankly malignant counterpart (every 6-12 months) for the 1st 2 years then yearly thereafter

long term prognosis of borderline ovarian tumor and its risk factors for recurrence.

References

1. Chambers JT. Borderline ovarian tumors: A review of treatment. The Yale J Biol Med 1989; 62: 351-5.
2. Gardner GJ. Ovarian tumors of low malignant potential: Can molecular biology solve this enigma? J National Cancer Institute 2001; 93(15).
3. Buttin BM. Epithelial ovarian tumors of low malignant potential: The role of microinvasion. Obstet Gynecol 2002; 99(1).
4. Morice P. Prognostic factors for patients with advanced stage serous borderline tumors of the ovary. Ann Oncol 2003; 14: 592-8.
5. Acs G. Serous and mucinous borderline (low malignant potential) tumors of the ovary. Am J Clin Pathol 2005; 123(Suppl 1): S13-S57.
6. Hart WR. Borderline epithelial tumors of the ovary. Modern Pathology 2005; 18: S33-S50.
7. Kane A. Prognostic factors in patients with ovarian serous low malignant potential (Borderline) tumors with peritoneal implants. The Oncologist 2009;14: 591-600.

8. Simo-Es NR. Parameters of blood count and tumor markers in patients with borderline ovarian tumors: A retrospective analysis and relation to staging. International Scholarly Research Network In Oncology Volume 2012, Article Id 947831, Doi:10.5402/2012/947831
9. Ehlen TG. Management of low malignant potential tumor of the ovary. J Soc Obstet Gynaecol Can 2000; 22(1): 19-21.
10. Leitao MM Jr. Micropapillary pattern in newly diagnosed borderline tumors of the ovary: What's in a name? The Oncologist 2011; 16: 133-5.
11. Coumbos A, et al. Clinical management of borderline tumors of the ovary: Results of a multicenter survey of 323 clinics in Germany. Br J Cancer 2009; 100: 1731-8.
12. Rao GG. Surgical staging of ovarian low malignant potential tumors. Obstet Gynecol 2004; 104(2).
13. Jian Gu, et al. Molecular evidence for the independent origin of extra-ovarian papillary serous tumors of low malignant potential. J National Cancer Institute 2001; 93(15).
14. Jin Hwi Kim, et al. Clinical analysis of intra-operative frozen section proven borderline tumors of the ovary. J Gynecol Oncol 2009; 20(3): 176-80.
15. Yokoyama Y, et al. Clinical outcome in borderline ovarian tumors. Br J Cancer 2006; 94(11): 1586-91.
16. Levi F, et al. Borderline ovarian tumors in Vaud, Switzerland: Incidence, survival and second neoplasms. Br J Cancer 1999; 79: 1, 4-6.
17. Domingo EJ. Society of Gynecologic Oncologists of the Philippines, Clinical Practice Guidelines 2nd Ed. Aug 2008.
18. Seidman JD, Kurman RJ. Ovarian serous borderline tumors: A critical review of the literature with emphasis on prognostic indicators. Hum Pathol 2000; 31(5): 539-57.
19. Shih KK, et al. Risk factors for recurrence of ovarian borderline tumors. Gynecol Oncol 2011; 120(3): 480-4.
20. Kim K, et al. Risk factors and pattern of treatment failures in patients with borderline ovarian tumors. J Women's Med 2010; 3(2).
21. Líbalová P. Risk factors for recurrent disease in borderline ovarian tumors. Ceska Gynekol 2012; 77(1): 31-5.
22. Domingo EJ. Society Of Gynecologic Oncologists of The Philippines, Clinical Practice Guidelines 3rd Ed. Aug 2012, (Unpublised).

Treatment Outcome After Cold Knife Conization for Pre-Malignant Cervical Lesions: A Five Year Review in a Tertiary Care Government Hospital*

Rommel A. Garcia, MD and Maria Lilibeth L. Sia Su, MD

Section of Gynecologic Oncology, Department of Obstetrics and Gynecology, Philippine General Hospital, University of the Philippines Manila

Cervical cancer is the leading female genital tract malignancy in the Philippines. In 2008, the estimated age-standardized national incidence rate was 11.7 per 100,000.
In countries with organized Paps smear screening programs, there is a consistent and dramatic decline of up to 70% in both cervical cancer incidence and mortality. The detection and appropriate treatment of the cervical pre-malignant lesion, the cervical intraepithelial neoplasia (CIN), also contribute to the decline in cervical cancer incidence.
This study was conducted to determine the incidence of persistence, recurrence and progression of the CIN, the incidence of underlying invasive carcinoma, the incidence of stenosis and obstetrical complications after cold knife conization.
The study was a retrospective descriptive study which included 35 patients who underwent cold knife conization for pre-malignant cervical lesion from January 2007 to December 2011. The data obtained from the study were analysed using descriptive statistics.
In this review series, the CIN recurrence rate was 3.25%, the incidence of underlying carcinoma was 11.43% and the incidence of cervical stenosis was 6.45%. No case of persistence was noted, no case of true progression was documented and, as none of the patients had a pregnancy after the conization, the incidence of post-conization obstetrical complications could not be determined.

Key words: cervical intraepithelial neoplasia, cold knife conization

Background, Rationale and Significance

Cervical cancer is the leading female genital tract malignancy in the Philippines. In 2008, the estimated age-standardized national incidence rate was 11.7 per 100,000.[1] In countries with organized Paps smear screening programs, there is a consistent and dramatic decline of up to 70% in both cervical cancer incidence and mortality.[2,3] The detection and appropriate treatment of the cervical pre-malignant lesion, the cervical intraepithelial neoplasia (CIN), also contribute to the decline in cervical cancer incidence.[4] There is however a lack of international consensus on the optimal surveillance strategies after treatment of CIN and follow-up is usually individualized.[5,6] Lack of data

* Third place, 2012 SGOP Fellows' and Residents' Research Contest.

on long-term outcomes, including risk of recurrence and progression to cancer among women treated for CIN, is a critical barrier to the formulation of evidence-based recommendations for follow-up surveillance strategies.[7]

Treatment of CIN is divided into ablative and excisional techniques. Ablative therapy destroys the abnormal cells and the surface of the transformation zone. Excisional therapy provides a surgical specimen that gives histologic proof of the severity of the disease and the completeness of the procedure. With proper patient selection, both techniques have cure rates of 80-90% with single treatment and 95% with repeated treatment.[8] Excisional therapy is usually the preferred procedure for CIN II-III on biopsy and persistent CIN III.

In our institution, excisional therapy includes cold knife conization and loop electrosurgical excision procedure (LEEP), the former an in-patient procedure and the latter done on an out-patient basis. The indications for both procedures are almost similar and the decision is made by the preferences of the clinician. However, for larger high grade lesions (carcinoma-in-situ and adenocarcinoma-in-situ suspects), those with suspected microinvasion and those with possible involvement of the endocervix, cold knife conization is the preferred excisional therapy.

Risk of cold knife conization include costs (including hospitalization), intraoperative and post-operative bleeding, infection, cervical stenosis, infertility and cervical incompetence.[8]

Studies on the long-term outcome of the different techniques of conization had been done abroad. Cold knife conization, LEEP and laser conization were compared. Coagulation of cone margins were present in more than half of the LEEP and laser cones. On long-term follow-up, cold knife conization is associated with the lowest recurrence rate of high-grade CIN and is also associated with virtually no case of invasive cervical cancer if the margins of the specimen was clear of the lesion. However, cold knife conization is more closely associated with cervical stenosis and adverse obstetrical outcome.[7,9]

A local study was conducted in 2007 to determine the incidence of dysplasia at the cervical cone margins and the presence or absence of residual disease in the post-cone hysterectomy specimens. There is however no local data on long-term treatment outcome after cold knife conization for pre-malignant cervical disease. This new study was thus conducted to determine the incidence of persistence, recurrence and progression of the CIN, the incidence of underlying invasive carcinoma, the incidence of stenosis and obstetrical complications.

Review of Related Literature

An online internet search for relevant abstracts and full-text journals was done using the terms "treatment outcome", "long-term outcome", "conization", "cold knife" and "cervical intraepithelial neoplasia (CIN)". Articles taken for review were those that took into consideration the rates of persistence, recurrence and progression of CIN after treatment and post-treatment complications.

A randomized trial in Hopital Edouard Herriot in France compared three techniques of conization (cold knife, laser and LEEP) which involved one hundred ten patients. Eighty six of these patients were followed up for more than three years. Of these, 28 had been treated with cold knife, 29 with LEEP and 29 by laser. Five recurrences were observed, one in the cold knife group, two in the LEEP group and two in the laser conization group. The main factor identified was the margin of resection. The only observed sequela was cervical stenosis: one case in the LEEP group, four cases in the cold knife group and none in the laser group. Thirty nine patients got pregnant (total number of 50 pregnancies). Two cervical cerclage were performed for shortened cervix both on a patient treated with LEEP. A total of nine cesarean sections were performed with two cases for failure of cervical dilatation secondary to the excisional procedure, which the study termed as *cervical dystocia* (one treated with LEEP and another one treated with laser conization).[9]

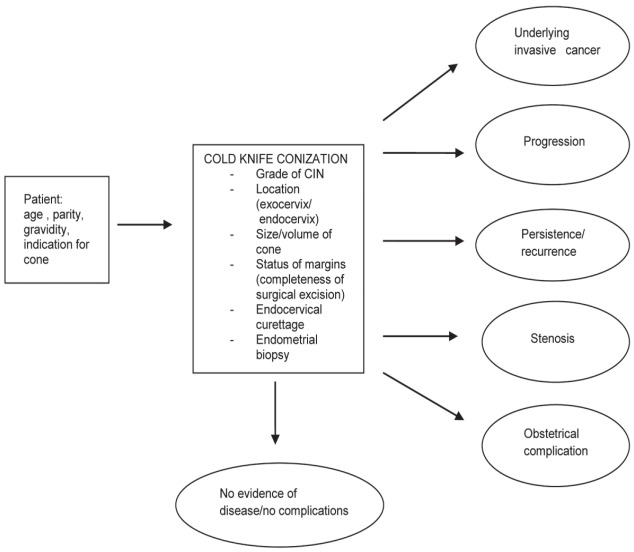
A total of 4417 women aged 18-72 years (mean age: 36 years) had cold knife conization with clear margins for histologically confirmed CIN III at the University of Graz, Austria between the years 1970 and 1994. All patients were followed up with colposcopy, cytology, and pelvic examination for a mean of 18 years. New high-grade CIN developed in

15 patients (0.35%) after a median of 107 months (8.9 years). A total of 4402 patients (99.65%) were free of high-grade CIN after a mean follow-up of 18 years. The reappearance of high-grade CIN years after cold knife conization with clear margins appeared to be new disease rather than recurrence. High-grade glandular intraepithelial lesions developed in two patients (0.05%): 14 and 17 years after conization. Twelve patients (0.3%) had metachronous vulvar intraepithelial neoplasia (VIN) grade III or vaginal intraepithelial neoplasia (VAIN) grade III, and one patient (0.02%) had invasive vaginal carcinoma 10 years after conization. The study concluded that cold knife conization with clear margins was an adequate method to definitively treat CIN III with advise for post treatment protocol of yearly colposcopic and cytologic follow-up.[10]

A retrospective study involved 37,142 women treated for CIN I, II, III from January 1986 to December 2000 from the British Columbia Cancer Agency. Treatment included cryotherapy, loop electrosurgical excision procedure (LEEP), cone biopsy and laser vaporization or excision. Overall observed cumulative rates of CIN II/III in the first 6 years after treatment were 14% for women treated for CIN III, 9.3% for CIN II and 5.6% for CIN I. Initial diagnosis, age and treatment type were associated with a diagnosis of CIN II/III after treatment, with 6-year adjusted rates for women aged 40-49 years ranging from 2.6% for treatment of CIN I with LEEP, to 34% for treatment of CIN III with cryotherapy. The CIN II/III recurrence rates after treatment were lowest for cone biopsy. Cryotherapy, compared with other treatments, was associated with the highest rate of recurrent disease.[7]

A local retrospective study was conducted from January 1, 1994 to October 31, 2003 which included 50 patients who underwent cold knife conization. The objective of the study was to determine the incidence of dysplasia at the cervical cone margins and the presence or absence of residual disease in the post-cone hysterectomy specimens. Thirty three patients or 66% had pathology ranging from CIN to invasive cancer, with 10 having positive resection margins. Out of the 33 patients who had pathologic findings in the conization specimen, 27 underwent subsequent hysterectomy, 5 opted to have conservative management (follow-up with cytology and colposcopy) and one got lost to follow-up. Fifteen out of the 27 patients (56%) who had hysterectomy had no residual disease. The incidence of residual disease was 44% in the 12 patients with positive hysterectomy specimens. For the women who had CIN on the cone specimen, 75% had no residual disease in the hysterectomy specimen. For the women who had invasive cancer on cone specimen, 72.7% had residual disease in the hysterectomy specimen. The study concluded that the presence or absence of residual disease in the post-cone hysterectomy specimen correlated with the degree of dysplasia at the cervical cone margin. Age, gravidity and parity were not associated with positive residual disease.[11]

Based on the review of related literature, the conceptual framework for the study focused on the factors that has a potential to affect the treatment outcome after cold knife conization for pre-malignant cervical lesion.



Flowchart 1. Conceptual framework of the study.

Research Project Objectives

General Objectives

This study is conducted to determine the incidence of persistence, recurrence and progression of the CIN,

the incidence of underlying invasive carcinoma, the incidence of stenosis and obstetrical complications after cold knife conization for pre-malignant cervical disease.

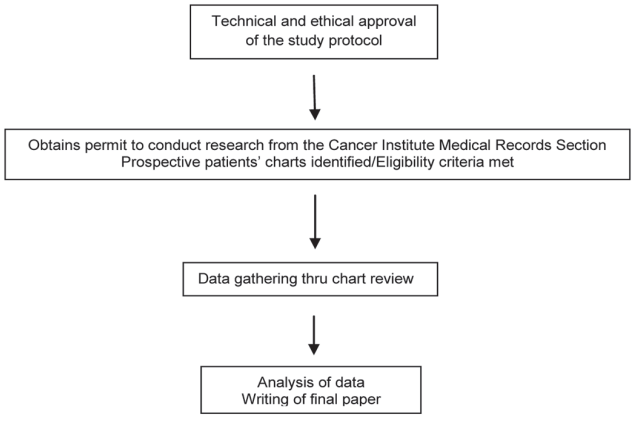
Specific Objectives

1. To describe the demographic characteristics of the cold knife conization patients in terms of age, gravidity, parity and the indication for the conization.
2. To describe the surgico-pathologic features in terms of the grade of neoplasia, location of the lesion, size or volume of the cone specimen, status of surgical margins, results of the endocervical curettage (if done) and results of the endometrial curettage (if done).
3. To determine the incidence of persistence, recurrence and progression of the CIN.
4. To determine the incidence of underlying invasive cervical cancer.
5. To determine the incidence of post-conization complications, e.g. stenosis and obstetrical complications.

Materials and Methods

The Study Design

The study is a retrospective descriptive study. A flowchart of the study is seen below.



Flowchart 2. Flowchart of study activities.

Operational Definition of Terms

Cervical intraepithelial neoplasia - an interval of epithelial dysplastic changes in the transformation zone of the cervix which has the potential to progress to invasive cancer. Low-grade dysplasia (CIN I) is confined to the basal one third of the epithelium and is usually described in terms of koilocytic atypia, whereas high-grade dysplasia (CIN II-III) involves two thirds or greater of the epithelial thickness. Full thickness involvement is known as carcinoma in situ (CIS).[12]

Cold knife conization - (also *conisation*) a surgical procedure to remove the entire cervical transformation zone including the cervical lesion by a knife or scalpel. It involves cutting out the area around the cervical os, removing the entire transformation zone and much of the endocervical canal. It results in the removal of a conical area from the surface of the cervix into the cervical canal. It is performed in an operating room and requires general or regional anesthesia.[2,8]

Persistence - the continuance of cervical intraepithelial neoplasia despite treatment by cold knife conization as evidenced by abnormal cytology and/or colposcopy with or without biopsy.

Recurrence - the diagnosis of cervical intraepithelial neoplasia after cold knife conization followed by a documented disease free interval of at least 3 months. The recurrent CIN and the disease free interval are established by cytology and/or colposcopy with or without biopsy.

Progression - the advancement from a low grade CIN to a higher grade CIN, or from CIN to invasive cancer.

Cervical stenosis - obliteration of the cervical canal as demonstrated by failure to visualize the endocervical canal (even with an endocervical retractor), or failed calibration with a 3mm Hegar dilator or no demonstrable endocervical canal in the hysterectomy specimen.

Obstetrical complications - pregnancy-related complications attributed to cold knife conization namely late abortion, cervical incompetence, premature labor, premature rupture of membrane and cervical dystocia.

Study Population

Target population

The target population consisted of patients at the Cancer Institute of UP-PGH who underwent cold knife conization for pre-malignant cervical lesion from January 2007 to December 2011.

Inclusion and Exclusion Criteria

Inclusion criteria

- All patients at the Cancer Institute who had cold knife conization for cervical intraepithelial neoplasia from January 2007 to December 2011.
- Patients who had cold knife conization in other institutions with follow-up or treatment for sequelae or complications at the Cancer Institute from January 2007 to December 2011.
- Must have had good follow-up and/or complete medical records for the purpose of this study.

Exclusion criteria

- Patients who had other methods of excisional procedure for the treatment of cervical pre-malignant lesion.
- Patients who had cold knife conization for indications other than cervical intraepithelial neoplasia or cervical pre-malignant lesion.
- Those with incomplete medical records.

Sampling Design and Sample Size

The study is a retrospective descriptive study of all patients who had cold knife conization from the year 2007 to 2011.

Procedure

The study was conducted at the Cancer Institute of UP-PGH. This paper was approved by the technical and review board of the institution.

After study protocol approval, the principal investigator secured permit to conduct the research and retrieve the medical records from the Cancer Institute Medical Records Section. The medical records of patients who underwent cold knife conization from the years 2007 to 2011 were identified through the logbooks and record books of the Section of Gynecologic Oncology, Department of Obstetrics and Gynecology. The medical records of patients who satisfied the inclusion criteria were included in the study. Data gathering was done thru chart review.

Data Collection Tool

A case registry form for each patient was filled up by the principal investigator. The case registry form recorded the patients' clinical and demographic characteristics. The cold knife conization surgico-pathologic features were gathered from the Surgical Pathology Reports. The treatment outcomes were likewise recorded.

Data Analysis

Descriptive analysis was used for the patients' clinical and demographic characteristics, surgico-pathologic features and treatment outcomes.

The data obtained from the study were analysed using descriptive statistics (mean, range and percentage).

Results

The objectives of the study required a descriptive profiling of patients and a review of the treatment outcomes after cold knife conization for pre-malignant cervical lesions. Descriptive statistics such as means (± S.D.), median and range of quantitative variables were computed. For qualitative variables such as final pathologic diagnosis of cone specimen and presence of obstetrical complications after the procedure,

proportions were computed. Data were then presented in tables and graphs. Although not stated as an objective, the mean length of follow-up was also described.

Patient Characteristics

A total of 35 patients were included in this analysis. The mean age of the patients was 46.37 years (SD±10.28). The ages of the patients ranged from 23 to 65 years. The median age of the patients was 46 years. Seventy four percent of the patients were aged 40 years and above.

The mean number of pregnancies experienced by the patients (gravidity) prior to conization was 4.63 (SD±2.2) with a corresponding range of 0 to 10 pregnancies. Sixty percent (21 out of 35) of these patients had been pregnant for 3 to 5 times at the time of the conization. One patient had not experienced pregnancy at the time of conization.

Table 1. Patients' characteristics and surgico-pathologic profile.

ID #	Age (years)	Gravidity/ Parity	Pre-cone lesion	Histopath diagnosis of cone specimen	Location of lesion	Surgical margin	ECC	EM biopsy	Size of Cone	Outcome
1	52	G7P6	CIN III	Cervicitis					2.5x2x1.5	NED
2	46	G4P3	CIN II	MICA	Exocervix	(-)			No report	MICA
3	52	G4P2	CIN III	Cervicitis					2.5x2x1.3	NED
4	41	G5P4	CIS	CIS	Exocervix	(-)		Simple hyperplasia	4.2x1.6x0.7	NED
5	46	G10P8	CIN III	Cervicitis					4x3x2.5	NED
6	43	G3P3	CIN III	CIN III	Exocervix	(-)	(-)		3.5x2x3	NED
7	36	G3P3	MICA	Cervicitis					4.5x4.5x3	Progression
8	64	G7P7	CIN II	No residual lesion					2.5x3x2.5	NED
9	63	G6P6	CIN III	CIN I	Exocervix	(-)	(-)		2x2x1.8	NED
10	36	G4P4	CIN III	CIN III	Exocervix	(-)	(-)		3x3x3	NED
11	52	G3P3	CIN III	Cervicitis					3x2x3	NED
12	56	G0	CIN II	Adenocarcinoma	Exocervix	(-)	(-)		3x2x0.5	Adeno CA
13	38	G4P3	CIN III	CIN I	Exocervix	(-)			2.5x2.5x2.5	NED
14	54	G3P3	CIN III	CIN I	Exocervix	(-)	(-)		3.5x2x3	NED
15	30	G5P3	CIN III	CIN III	Exocervix	(-)			3x3x2	NED
16	44	G5P5	CIN III	Cervicitis					3x2.5x2	NED
17	44	G4P4	CIN III	SCCA	Exocervix	(+)			2.7x4.5x3.5	SCAA
18	36	G4P4	CIN III	CIN III	Exocervix	(-)			3x3x3	NED
19	34	G6P6	CIN III	CIN III	Exocervix	(-)			3x3.5x2.5	Stenosis
20	65	G9P7	CIN III	CIN II	Exocervix	(-)			2x2x2	NED
21	63	G7P6	CIN III	CIN III	Exocervix	(+)	(-)		2.5x2.5x2.5	NED
22	50	G8P4	CIN III	CIN III	Exocervix	(-)	(-)		3.5x3.5x2	NED
23	23	G1P1	CIN III	CIN I	Exocervix	(-)			3x2x2	NED
24	46	G5P4	CIN III	CIS	Exocervix	(-)			3.2x3x3	NED
25	53	G3P3	CIN III	CIS	Exocervix	(-)			3.8x3x3	NED
26	34	G4P2	CIN III	CIS	Excoervix	(-)			3x2.5x2.7	NED
27	50	G4P3	CIN III	CIN I	Exocervix	(-)			2.5x2.5x3	NED
28	45	G5P4	CIN III	CIN I	Exocervix	(-)	(-)		2.5x2.5x3	NED
29	60	G3P3	CIN II	Adenocarcinoma	Endocervix	(-)		Chronic endometritis	2.5x2.5x2.7	Adeno CA
30	57	G2P2	CIN II	CIN II	Exocervix	(-)			2.5x2.5x2.5	NED
31	42	G5P5	CIN III	CIN III	Exocervix	(-)	(-)		2.8x2.5x2	NED
32	41	G2P2	CIN III	CIN I	Exocervix	(-)			4x3x2.5	Recurrence, Stenosis
33	46	G8P7	CIN III	CIN III	Exocervix, Endocervix	(-)	(+)		3x3x2	NED
34	47	G3P3	CIN III	CIS	Exocervix	(-)		Proliferative endometrium	4x3x3	NED
35	34	G4P2	CIN III	CIN I	Exocervix	(-)			4x3x3	NED

MICA= microinvasive carcinoma SCCA= squamous cell CA NED= no evidence of disease
ECC=endocervical curettage EM biopsy= endometrial biopsy

The mean number of children born by the patients (parity) prior to conization was 3.86 (SD±1.85) with a corresponding range of 0 to 8 children. The mean number of gravidity was consistently higher than the mean number of parity in all age groups, implying that some of the patients' pregnancies were not successful.

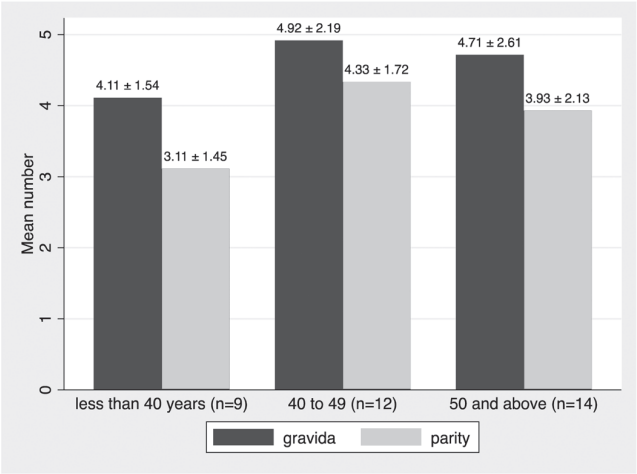


Figure 1. Mean gravidity and mean parity of the patients by age group

Eighty percent (28 out of 35) of the patients were diagnosed with CIN III prior to conization. Fourteen percent (5 out of 35) of the patients were diagnosed with CIN II prior to the procedure. One patient was diagnosed with carcinoma-in-situ (CIS) and another patient was diagnosed with microinvasive carcinoma (MICA).

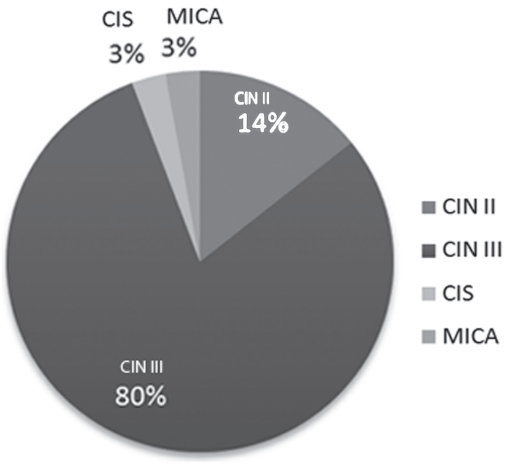


Figure 2. Pre-conization diagnosis (n=35)

Surgico-Pathologic Features

Histologic Diagnosis of the Cone Specimen

Twenty per cent (7 out of 35) had no evidence of neoplasia on the cone specimen.

Eighty per cent (28 out of 35) had evidence of disease on the cone specimen. Nine had CIN III, 8 had CIN I, 5 had CIS, 4 had carcinoma and 2 had CIN II.

Table 2. Histologic diagnosis of the cone specimen.

Histologic Diagnosis of the Cone Specimens	Number	Percent
Negative for Neoplasia		
Chronic cervicitis	6	17.14
Negative for tumor	1	2.86
Positive for Neoplasia		
CIN I	8	22.86
CIN II	2	5.71
CIN III	9	25.71
MICA	1	2.86
Carcinoma in situ (CIS)	5	14.29
Adenocarcinoma	2	5.71
Squamous cell carcinoma	1	2.86
Total	35	100.00

Location of the Lesion

Ninety three percent (26 out of 28) of patients had lesions located in the exocervix. One patient had an endocervical lesion, while one patient had both an exocervical and endocervical lesion.

Size of the Cone Specimens

.

Among patients with neoplasia, the largest cone specimens removed had a dimension of 4cm x 3cm x 3cm. These cones came from two patients with carcinoma-in-situ and CIN I. The smallest cone specimen removed among the patients with neoplasia had a dimension of 2cm x 2cm x 1.8cm. This came from a patient aged 63 years, with a pre-conization diagnosis of CIN III and had a cone biopsy diagnosis of CIN I.

Among patients without neoplasia, the largest cone specimen removed had a dimension of 4.5cm x 4.5cm x 3cm. The said cone came from a 36 year old patient with a pre-conization diagnosis of microinvasive carcinoma (MICA) and a cone biopsy diagnosis of chronic cervicitis. The smallest cone specimen removed among the patients without neoplasia had a dimension of 2.5mm x 2mm x 1.5mm. The said cone came from a 52 year old patient with CIN II on pre-conization diagnosis and chronic cervicitis on cone biopsy diagnosis.

Status of the Surgical Margins

Ninety three percent (26 out of 28) of the cone specimens had surgical margins negative for tumor.

Two cone specimens had resection margins positive for tumor. First was a cone from a 44 year old with an exocervical lesion and a cone size of 2.7cm x 4.5cm x 3.5cm. The pre-conization diagnosis was CIN III and the cone biopsy diagnosis was squamous cell carcinoma. This patient eventually underwent radical hysterectomy with bilateral salpingo-oophorectomy. The second was a cone specimen from a 63 year old with an exocervical lesion and a cone size of 2.5cm x 2.5cm x 2.5cm. The pre-conization diagnosis was CIN III and the cone biopsy diagnosis was also CIN III. This patient was lost to follow-up.

Result of the Endocervical Curettage

Endocervical curettage was performed in only 10 patients. Only one had positive endocervical curettage while the other 9 had negative results.

Result of the Endometrial Curettage

Endometrial curettage was performed in only three patients. One had simple cystic hyperplasia, one had acute on chronic endometritis and the third had a proliferative endometrium.

The first patient was a 41 year old with a pre-conization diagnosis of CIS and a cone biopsy diagnosis of focal CIS. Her endometrial biopsy result was simple cystic hyperplasia. This patient eventually underwent modified radical mastectomy and total hysterectomy with bilateral salpingo-oophorectomy for Invasive Ductal Carcinoma Stage II. The

Table 3. Patients with positive cone margins.

ID	Age	Gravidity/ Parity	Pre-cone Lesion	Histopath Diagnosis of Cone Specimen	Location of Lesion	Surgical Margin	Size of Cone	Post cone Intervention
17	44	G4P4	CIN III	SCCA	Exocervix	(+)	2.7x4.5x3.5	RHBSO
21	63	G7P6	CIN III	CIN III	Exocervix	(+)	2.5x2.5x2.5	Lost to ff-up

Table 4. Result of the endocervical curettage.

ID	Age	Gravidity/ Parity	Pre-cone Lesion	Histopath Diagnosis of Cone Specimen	Location of Lesion	Surgical Margin	ECC
6	43	G3P3	CIN III	CIN III	Exocervix	(-)	(-)
9	63	G6P6	CIN III	CIN I	Exocervix	(-)	(-)
10	36	G4P4	CIN III	CIN III	Exocervix	(-)	(-)
12	56	G0	CIN II	Adenocarcinoma	Exocervix	(-)	(-)
14	54	G3P3	CIN III	CIN I	Excoervix	(-)	(-)
21	63	G7P6	CIN III	CIN III	Exocervix	(+)	(-)
22	50	G8P4	CIN III	CIN III	Exocervix	(-)	(-)
28	45	G5P4	CIN III	CIN I	Exocervix	(-)	(-)
31	42	G5P5	CIN III	CIN III	Exocervix	(-)	(-)
33	46	G8P7	CIN III	CIN III	Exocervix Endocervix	(-)	(+)

hysterectomy specimen showed secretory endometrium. The second patient was a 60 year old with a pre-conization diagnosis of CIN II and a cone biopsy diagnosis of mucinous adenocarcinoma. Her endometrial biopsy result was acute on chronic endometritis. This patient eventually underwent total hysterectomy with bilateral salpingo-oophorectomy and bilateral pelvic lymph node dissection. The hysterectomy specimen showed atrophic endometrium. The third patient was a 47 year old with a pre-conization diagnosis of CIN III and a cone biopsy diagnosis of carcinoma-in-situ. Her endometrial biopsy result was proliferative endometrium. This patient was lost to follow-up.

Persistence, Recurrence and Progression of the CIN

Four of the 35 patients had an underlying carcinoma. Ninety three percent of the remaining 31 patients (29 out of 31) remained free of disease on follow-up.

One patient had tumor recurrence post-conization. This was a 41 year old patient who had a pre-conization diagnosis of CIN III and a cone specimen diagnosis of CIN I. The surgical margin was free of tumor. Recurrence was detected six months post-conization on colposcopy with a biopsy which showed CIN II. Cervical stenosis was also diagnosed on recurrence. The patient underwent hysterectomy with a diagnosis of chronic cervicitis on the hysterectomy specimen.

One patient had disease progression. This was a 36 year old who had a pre-conization diagnosis of microinvasive carcinoma and a cone biopsy diagnosis of chronic cervicitis. Six years after conization, this patient was diagnosed to have progression with a diagnosis of cervical squamous cell carcinoma stage IA1 based on clinical grounds. She underwent extrafascial hysterectomy with bilateral salpingo-oophorectomy with a diagnosis of cervical adenomyosis with chronic cervicitis on the hysterectomy specimen.

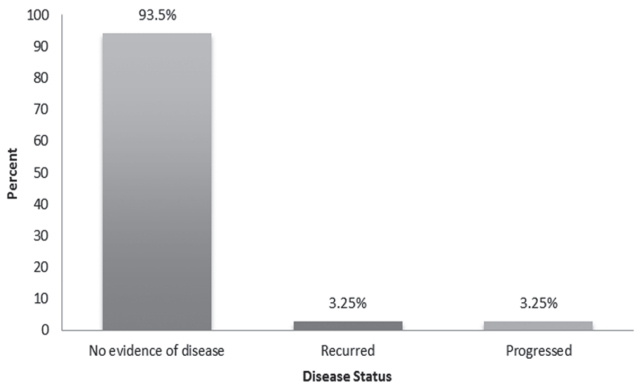


Figure 3. Incidence of recurrence and progression.

Underlying Invasive Cervical Carcinoma

The incidence of underlying cervical cancer in this series was 11.43% (4 out of 35). Two of these patients

Table 5. Result of endometrial curettage.

ID	Age	Gravidity/ Parity	Pre-cone Lesion	Histopath Diagnosis of Cone Specimen	Location of Lesion	Surgical Margin	EM Biopsy	Post cone Procedure	Hysterectomy Histopath
4	41	G5P4	CIS	CIS	Exocervix	(-)	Simple cystic hyperplasia	MRM THBSO	Secretory Endometrium Chronic cervicitis
29	60	G3P3	CIN II	Adenocarcinoma	Endocervix	(-)	Acute or Chronic endometritis	THBSO BLND	Atrophic Endometrium No residual tumor, cervix
34	47	G3P3	CIN III	CIS	Exocervix	(-)	Proliferative endometrium	Lost to ff-up	

were diagnosed with adenocarcinoma, while one patient had microinvasive carcinoma (MICA) and one had squamous cell carcinoma.

The first patient was a 46 year old who had pre-conization diagnosis of CIN II and a cone biopsy diagnosis of MICA. She underwent modified radical hysterectomy with bilateral salpingo-oophorectomy. Pathologic diagnosis on the hysterectomy specimen was carcinoma-in-situ (CIS). The second patient was a 44 year old with a pre-conization diagnosis of CIN III and a cone biopsy diagnosis of squamous cell carcinoma (SCCA). She underwent radical hysterectomy with bilateral salpingo-oophorectomy. The hysterectomy specimen was negative for residual tumor.

The third patient was a 56 year old with a pre-conization diagnosis of CIN II and a cone biopsy diagnosis of adenocarcinoma. She underwent radical hysterectomy with bilateral salpingo-oophorectomy. The hysterectomy specimen was negative for residual tumor.

The fourth patient was a 60 year old with a pre-conization diagnosis of CIN II and a cone biopsy diagnosis adenocarcinoma. She underwent total hysterectomy with bilateral hysterectomy. The hysterectomy specimen was also negative for residual tumor.

Table 6. Patients with recurrence and progression.

ID	Age	Gravidity/ Parity	Pre-cone Lesion	Histopath Diagnosis of Cone Specimen	Post cone Disease	Post cone Intervention	Hysterectomy Histopath
7	36	G3P3	MICA	Cervicitis	Progression, Cervical CA (Clinical diagnosis)	EL EHBSO	Negative for tumor, cervix Adenomyosis, Cervix
32	41	G2P2	CIN III	CIN I	Recurrence, CIN II Stenosis	EL TH	Chronic Cervicitis with Koilocytic Change Secretory Endometrium Negative for Tumor

Table 7. Patients with underlying carcinoma.

ID	Age	Gravidity/ Parity	Pre-cone Lesion	Histopath Diagnosis of Cone Specimen	Post cone Intervention	Hysterectomy Histopath
2	46	G4P3	CIN II	MICA	EL, MRHBSO BLND	CIS
12	56	G0	CIN II	Adenocarcinoma	EL, RHBSO BLND, PALS	Chronic cervicitis No malignancy seen
17	44	G4P4	CIN III	SCCA	EL, RHBSO BLND	Negative for residual Tumor
29	60	G3P3	CIN II	Adenocarcinoma	EL, THBSO BLND	No residual tumor seen

RH= radical hysterectomy MRH= modified radical hysterectomy

Incidence of Post-conization Complications

Two patients developed stenosis after conization with an incidence rate of 6.45% (2 out of 31, excluding the 4 cases of cancer who underwent hysterectomy). The first one was a 34 year old with a pre-conization diagnosis of CIN III and a cone biopsy diagnosis of CIN III also. The cone size was 3cm x 3.5cm x 2.5cm. Stenosis was diagnosed 14 months after the conization and she eventually had total hysterectomy for severe dysmenorrhea secondary to cervical stenosis. The hysterectomy specimen showed chronic cervicitis. The second patient was a 41 year old with a pre-conization diagnosis of CIN III and a cone biopsy diagnosis of CIN I. The cone size was 4cm x 3cm x 2.5cm. Colposcopy-guided biopsy showed recurrence of CIN II six months post-conization and was diagnosed with stenosis at the same time. She underwent total hysterectomy. The hysterectomy specimen also showed chronic cervicitis.

None of the patients on follow-up got pregnant. There were no data on obstetrical complications post-conization.

Other Relevant Statistics: Length of Follow-up

The mean length of follow-up of post-conization patients was 11 months (SD±14.58). The length of patient follow-up ranged from 0 to 50 months. Forty five percent (16 out of 35) had follow-up of 2 months or less. Nine patients (25.71%) followed up for more than a year while 4 patients (11.43%) did not follow-up at all.

Discussion

There has been no local long-term study on the treatment outcome of patients who had cold knife conization for CIN. The result of this 5-year review on the treatment outcome of CIN treated by cold knife conization was comparable to international literature.

In the presentation of the results of histologic diagnosis of the cone biopsy, there could be a basis for the argument against classifying CIN I under the group of those with evidence of disease, considering that this was a low-grade lesion with a very minimal chance of progression to carcinoma. This may distort the statistics considering that CIN I comprised twenty nine per cent (8 out of 28) of those with evidence of disease.

In this review series, there were two cone specimens with margins positive for tumor, with an incidence rate of seven percent. Both had CIN III as pre-conization diagnosis. One had CIN III on the cone biopsy while the other had squamous cell

Table 8. Patients who developed cervical stenosis.

ID	Age	Gravidity/ Parity	Pre-cone Lesion	Histopath Diagnosis of Cone Specimen	Surgical Margin	Size of Cone	Outcome	Post cone Intervention	Hysterectomy Histopath
19	34	G6P6	CIN III	CIN III	(-)	3x3.5x2.5	Stenosis	EL, TH	Chronic cervicitis
32	41	G2P2	CIN III	CIN I	(-)	4x3x2.5	Recurrence, Stenosis	EL, TH	Chronic cervicitis

Table 9. Length of follow-up of patients.

Length of Follow-up	Number	Percent
Zero	4	11.43
1 to 3 months	14	40
4 to 6 months	3	8.57
7 months to 1 year	5	14.29
More than 1 year	9	25.71
Total	35	100

carcinoma. In this series, the presence of positive margins was associated with high grade lesions on the cone specimen.

The recurrence rate of CIN after cold knife conization is at three per cent (1 out of 31) which is similar to foreign data.[9] Recurrence is usually associated with a positive surgical margin, however for this particular case, the surgical margin of resection of the cone specimen was negative for tumor.

With a mean follow-up time of eleven months, the incidence of progression to cancer after cold knife conization is zero. There was one case in the review that was clinically diagnosed as progression to cancer but was later found to have cervical adenomyosis on the final hysterectomy specimen. This was also similar to foreign data which stated that " if conization has ruled out invasive cancer, those with free surgical margins have almost a 100% disease-free follow-up".[13]

The incidence of underlying invasive cancer in the cone specimen was 11 percent as seen in 4 cases in the series. There was no invasive cancer in the hysterectomy specimen of these four patients, although carcinoma-in-situ was seen in one case. Cold knife conization thus can be surmised to be curative in all but one case, with a cure rate of 97 percent (34 out of 35). This cure rate was higher than European data wherein cold knife conization appeared to be curative in 87 percent of cases.[13]

Foreign data showed that the incidence of cervical stenosis after cold knife conization is 14 percent (14.3%).[9] In this review series, the incidence of cervical stenosis was 6.45% (2 out of 31, excluding the 4 cases of cancer who had hysterectomy). The main predictor for cervical stenosis was the size or volume of the cone. The two cases of cervical stenosis in this series came from the patients with large cone sizes, one of whom was associated with recurrence of CIN II.

The incidence of obstetrical complications in post-conization patients could not be determined as none of these patients had a pregnancy after the conization procedure.

Although not stated as an objective, the length of follow-up post conization was also described. Only half of the total number of patients followed-up for about two months after the cold knife conization. These patients may have had a false sense of security when their pre-malignant lesions were removed by the cold knife conization. Or it could be that the clinicians failed to impart to their patients the importance of close and long-term follow-up after the cold knife conization. Emphasis on long-term follow-up of post-conization patients is important to establish the diagnosis of persistence and recurrence of CIN and to prevent further progression to invasive cervical cancer if it may happen.

Summary and Conclusion

This 5-year retrospective descriptive study included 35 patients who had cold knife conization for pre-malignant cervical lesion from January 2007 to December 2011 in the Cancer Institute of UP-PGH. Data collection was done by medical chart review and data obtained were analysed using descriptive statistics. This paper was approved by the technical and review board of the institution.

This study was conducted to determine the incidence of persistence, recurrence and progression of the CIN, the incidence of underlying invasive carcinoma, the incidence of stenosis and obstetrical complications after cold knife conization.

Based on this review series, the CIN recurrence rate was 3.25%, the incidence of underlying carcinoma was 11.43% and the incidence of cervical stenosis was 6.45%. No case of persistence of the CIN was noted. No case of true progression was documented as the one case diagnosed as progression to invasive cervical cancer based on clinical examination turned out to be benign on the final hysterectomy specimen. None of the patients had pregnancy after the conization hence the incidence of post-conization obstetrical complications could not be determined.

Based on this series, cold knife conization appears to have a cure rate of 97% (34 out of 35).

Limitations and Recommendations

The 5-year treatment outcome in this review series seems reassuring but caution should be observed mainly because of the relatively small number of patients in the study and the short follow-up of the majority of these post-conization patients. The results

of this review should be confirmed in a larger group of patients with a longer post-conization follow-up period. A study with a larger population involving multiple centers is recommended.

Ethical Considerations

The research proposal and protocol entitled "'Treatment outcome after cold knife conization for cervical pre-malignant lesion: a five year review in a tertiary care government hospital" was submitted to the Research Ethics Board for ethics review and approval. The study was conducted only after approval from the board.

This is a retrospective descriptive study of patients who underwent cold knife conization at UP-PGH using review of medical records for data collection. Medical records were reviewed for the treatment outcome of cold knife conization. Only patient charts that fulfil the inclusion criteria were included.

All patients' information are strictly confidential.

The results of this study may be published in order that other interested parties may learn from this research. However, confidential information will not be shared. Patients' information will remain confidential and anonymized.

This study was undertaken as a partial fulfilment of the requirements for completion of the fellowship training in Gynecologic Oncology, as such all expenses for the conduct of the study was shouldered by the principal investigator.

There was no conflict of interest with other agencies/sections/department arising from this study.

References

1. Laudico AV, Medina V, Lumague MRM, Mapua CA, Redaniel MTM, Valenzuela FG, Pukkala E. 2010 Philippine Cancer Facts and Estimates, Philippine Cancer Society 2010: 34-7.
2. Werner C. Preinvasive lesions of the lower genital tract. Williams Gynecology 2008; 1224-84.
3. World Health Organization: Screening and early detection of cancer. Cervical cancer screening. Cytology screening. http://www.who.int/cancer/detection/cytologyscreen/en/index.html.
4. Surveillance, Epidemiology, and End Results (SEER) Program . SEER*Stat Database: Incidence - SEER 17 Regs Limited-Use, Nov 2006 Sub. National Cancer Institute, DCCPS, Surveillance Research Program, Cancer Statistics Branch . Released April 2007, based on the November 2006 submission. Available at: www.seer.cancer.gov .
5. Kyrgiou M, Tsoumpou I, Vrekoussis T, et al. The up-to-date evidence on colposcopy practice and treatment of cervical intraepithelial neoplasia: The cochrane colposcopy and cervical cytopathology collaborative group (C5 group) approach. Cancer Treatment Reviews. 2006; 32(7): 516-23.
6. Ortac F, Gungor M, Taskin S. Management of margin positive cases after loop electrosurgical excision procedure. Textbook of Gyneacological Oncology. 2010; 10: 40-3.
7. Melnikow J, McGahan C, Sawaya GF, Ehlen T, Coldman A. Cervical intraepithelial neoplasia outcomes after treatment: Long-term follow-up from the British Columbia Cohort Study. J Natl Cancer Inst 2009; 101: 721-28.
8. Tulunay G, Ozgul N. Treatment modalities for cervical preinvasive lesions. Textbook of Gyneacological Oncology 2010; 9: 36-9.
9. Mathevet P, Chemali E, Roy M, Dargent D. Long-term outcome of a randomized study comparing three techniques of conisation: cold knife, laser and LEEP. Eur J Obstet Gynecol Reprod Biol 2003; 106: 214-8.
10. Reich O, Pickel H, Lahousen M, Tamussimo K, Winter R. Cervical intraepithelial neoplasia III: Long-term outcome after cold-knife conization with clear margins. Obstet Gynecol 2001; 97: 428-30.
11. Heuvos AB, Luna JTP. Cervical cold knife conization at the University of the Philippines-Philippine General Hospital: A 10-year retrospective study. 2007 (Unpublished).
12. Randall M, Michael H, Long III H, Tedjarati S. Uterine cervix. Principles and Practice of Gynecologic Oncology 5th Ed. 2009; 14:623-81.
13. Creasman WT. Preinvasive disease of the cervix. Clinical Gynecologic Oncology 8th Ed. 2012; 1:1-30.