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ABOUT THE JOURNAL

Aims and Scope

Asian Archives of Pathology (AAP) is an open access, peer-reviewed journal. The journal was first published in 2002 under the Thai name "วารสารราชวิทยาลัยพยาธิแพทย์แห่งประเทศไทย" and English name "Journal of the Royal College of Pathologists of Thailand". The journal is a publication for workers in all disciplines of pathology and forensic medicine. In the first 3 years (volumes), the journal was published every 4 months. Until 2005, the journal has changed its name to be "Asian Archives of Pathology: The Official Journal of the Royal College of Pathologists of Thailand", published quarterly to expand the collaboration among people in the fields of pathology and forensic medicine in the Asia-Pacific regions and the Western countries.

The full articles of the journal are appeared in either Thai or English. However, the abstracts of all Thai articles are published in both Thai and English languages. The journal features letters to the editor, original articles, review articles, case reports, case illustrations, and technical notes. Diagnostic and research areas covered consist of (1) **Anatomical Pathology** (including cellular pathology, cytopathology, haematopathology, histopathology, immunopathology, and surgical pathology); (2) **Clinical Pathology (Laboratory Medicine)** [including blood banking and transfusion medicine, clinical chemistry (chemical pathology or clinical biochemistry), clinical immunology, clinical microbiology, clinical toxicology, cytogenetics, parasitology, and point-of-care testing]; (3) **Forensic Medicine (Legal Medicine or Medical Jurisprudence)** (including forensic science and forensic pathology); (4) **Molecular Medicine** (including molecular genetics, molecular oncology, and molecular pathology); (5) **Pathobiology**; and (6) **Pathophysiology**.

All issues of our journal have been printed in hard copy since the beginning. Around the late 2014, we developed our website (www.asianarchpath.com) in order to increase our visibility. We would like to acknowledge that our journal has been sponsored by the Royal College of Pathologists of Thailand. We have the policy to disseminate the verified scientific knowledge to the public on a non-profit basis. Hence, we have not charged the authors whose manuscripts have been submitted or accepted for publication in our journal.

On the other hand, if any authors request a printed copy of the journal issue containing the articles, each of the copied journals costs 450 bahts for Thai authors and 30 United States dollars (USD) for international authors.

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Disclaimer

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MANUSCRIPT REVIEWERS

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LETTER TO THE EDITOR

Novel Roles of Troponin Testing in Myocarditis Following COVID-19 Infection and Vaccination

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The novel acute respiratory syndrome caused by coronavirus appears to have emerged from Wuhan, China, at the end of the year 2019 (COVID-19) and has now developed to a rapid pandemic spread. Two years after the outbreak of the disease in December 2021, the infection has resulted in more than 280 million documented cases with more than 5.4 million deaths worldwide. In COVID-19 patients, a spectrum of cardiovascular diseases including myocardial infarction, stress cardiomyopathy, and myocarditis has been described. In all of these conditions, increases in serum concentrations of cardiac troponins indicative of myocardial injury are usually observed and have diagnostic and prognostic significance. In this context, it should be noted that elevations of serum troponins have also been reported in COVID-19 patients who did not have pre-existing cardiovascular co-morbidities. Myocardial injury, as defined by an increase in troponin serum concentrations above the 99th percentile reference limit, seems to be a common manifestation in COVID-19 infection, with the reported prevalence of troponin elevation in upto 30% of the patients. It has been reported that significantly higher serum levels of the troponins are associated with severe disease requiring ICU admission as well as with nonsurvivors as compared to those with milder symtoms and survivors. More importantly, high troponin elevations were observed in patients with severe forms of the disease who finally progressed to multiorgan dysfunction, failure, and death.

In the past year, the increasingly use of mRNA vaccines (Pfizer or Moderna) to prevent COVID-19 infection in children and adolescents has led to safety concern of the vaccination. A risk of myopericarditis has been observed particularly in male patients under 30 year of age after the second vaccine dose. Whatever the causes, in young and old people presenting with symptoms suggestive of myocarditis, troponin testings along with the use of 12-lead ECG and, wenn indicated, echocardiogram or cardiac MRI have been recommended in the diagnosis and risk assessment of the disease. Accumulating data also point to the usefulness of cardiac

troponins in the evaluation of COVID-19 patients with persistent cardiovascular symptoms ("Long COVID") several months after discharges from the hospital. Measurements of troponin concentrations early after hospital admission as well as longitudinal monitoring during and after hospital stay, therefore, may provide crucial diagnostic and prognostic tools in the management of COVID-19 patients with myocarditis.

ORIGINAL ARTICLE

BCL-2 EXPRESSION IN DIFFERENT LUMINAL SUBTYPES OF BREAST CARCINOMA AND ITS PROGNOSTIC SIGNIFICANCE

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Abstract

Background: BCL-2 (B- cell Lymphoma 2) is expressed in breast cancer depending upon the ER expression.

Objective: The purpose of this study is to look for BCL-2 positivity and hence its prognostic value in different luminal types of breast carcinoma.

Materials and Methods: 30 cases of breast cancer were selected, 5 were excluded and finally 25 cases were selected for the study. ER, PR and HER-2 neu status was checked by immunohistochemistry and cases were divided in five molecular subtypes. Immuno-histochemical expression of BCL-2 was checked in correlation with ER, PR and HER-2 neu status.

Results: In this study BCL-2 was strongly positive in 6(24%) out of 25 cases. There was direct statistical correlation between BCL-2 expression and ER/PR status (p-value <0.05).

Conclusion: BCL-2 can be used as an independent prognostic marker for breast carcinoma and anti-BCL-2 therapy may reinforce the effectiveness of hormonal therapy in breast carcinoma.

Keywords: BCL-2, Breast Carcinoma, Luminal types, Prognostic markers.

Introduction

Breast carcinoma is the most common cancer among women with the range of biological subtypes, clinic-pathological and molecular features. The incidence of breast carcinoma is continuously increasing in Pakistan. Some of the recent studies show that every one in nine women in Pakistan carries the risk of developing breast cancer at some stage in her life. In Asia, Pakistan has the highest age standardized incidence rate of breast carcinoma. Pakistan is a developing country with a huge population living in the rural areas with very limited health facilities available. This makes the early diagnosis and hence the treatment of breast cancer a big challenge.

Incidence of breast cancer varies in different region of the world attributable to genetic differences, race, cultural differences and environmental exposure[1,2]. Breast cancer remains the most prevalent cancer in the world.

Different risk factors contributing to the development of breast carcinoma include genetic factors (breast carcinoma in first degree relatives, Li-Fraumeni Syndrome, BRCA1/BRCA2 mutations, heterozygosity for ataxia telangiectasia etc.), hormonal risk factors (early menarche, late menopause, nulliparity, obesity, late childbearing etc.) and environmental risk factors. Treatment of breast carcinoma still remains a challenge and is subjected to early diagnosis and different attributes of the tumor.

Breast can be involved a number of types of malignant tumors. Most common of these tumors is the invasive breast carcinoma. There are numerous subtypes of invasive breast carcinoma and the commonest is the invasive ductal carcinoma (NOS). All the cases chosen in this study are of invasive breast carcinoma NOS type. Other types of invasive breast carcinoma include oncocytic carcinoma, lipid rich carcinoma, glycogen rich carcinoma, sabaceous carcinoma, lobular carcinoma NOS, tubular carcinoma, cribriform carcinoma NOS, mucinous adenocarcinoma, mucinous cystadenocarcinoma NOS, invasive micro papillary carcinoma and metaplastic carcinoma NOS. In addition to these breast tissue can also be involved by neuroendocrine tumors, salivary gland tumors, epithelial-myoepithelial tumors, papillary neoplasms, mesenchymal tumors, fibroepithelial tumors, lymphomas and metastatic tumors.

Grossly invasive ductal carcinoma appears as a firm, poorly circumscribed mass. On sectioning the tumor has a hard cartilaginous surface and a gritty appearance. Chalky white crab like streaks of calcification can extend into the surrounding breast parenchyma. In cases

when the tumor has a very huge size there maybe signs of degeneration e.g. haemorrhage and cyst formation. The tumor may be fixed to the chest wall, can cause involvement of the skin (peau de orange skin or ulceration) and can also cause changes in appearance of the nipple (retraction).

Histologically the tumor maybe arranged in tubules, nests, sheets or cords (percentage of tubule formation in the tumor depends upon the histological grade of the tumor). The individual neoplastic cells reveal pleomorphism, high N/C ratio, irregular nuclear membranes and prominent nucleoli. The surrounding stroma may show pleomorphism. Calcification may also be noted. Myoepithelial lining is lost in neoplastic ducts. Abnormal mitotic figures are seen and number of mitosis varies with the histological grade of the tumor. Ductal Carcinoma In situ (DCIS) may also be seen. Involvement of the overlying skin, nipple and lympho-vascular invasion are checked during histological examination and they have a role in deciding the prognosis of the tumor.

Breast carcinoma is stratified into three histological grades and this grading system serves as a strong indicator of prognosis of the cancer. The Nottingham scoring system is used for the histological grading of breast carcinoma worldwide. The final score is a cumulative of three individual scores that are given to tubule formation (based on how much the tubules in the cancer resemble the normal breast tubules), nuclear pleomorphism and mitotic activity. Each one of these attributes of the tumor are scored from 1 to 3. 1 score given for being closest to normal and 3 given to the most abnormal. The minimum Nottingham score would be 3 and maximum 9. Based on the score the tumor is stratified into three grades. Grade I (well differentiated) is assigned if the score is 3-5. Grade II (moderately differentiated) for a score 6-7 and Grade III (poorly differentiated) for a score 8-9.

Histological grade correlates with the aggressiveness, prognosis and hormone receptor status of the tumor e.g. Grade I tumors are usually least aggressive, have a good prognosis and are ER positive. Whereas, Grade III tumors show a more aggressive behaviour, have a poorer prognosis and are usually TNBC. Histological grade also affects the treatment decisions e.g. radiation therapy is used in addition to surgery for grade III tumors to reduce the chances of recurrence of the cancer.

There has been much research and advancement in the treatment of breast carcinoma over the years. Different treatment options that are available include surgical procedures, chemotherapy, radiation therapy, hormonal therapy and biological therapy.

Recently there has been felt a need for tailoring the treatment for each patient. Every patient is different and so is the cancer. Breast carcinoma is not a fixed entity but rather a very diverse disease with several different histological and molecular types. The treatment for every patient should ideally be personalized on the basis of the genetic makeup of the tumor. Studying each and every molecular change can be an exhaustive task yet some markers that have a vital role in promoting the proliferation of cancer cells should be considered while

deciding the treatment options. This way instead of targeting every dividing cell the treatment could be focused on the proliferating cancer cells.

One of these tumor attributes that is most important for stratification of tumor prognosis and deciding treatment options is the molecular status of the tumor. Gene expression profiling is a microarray analysis technique that is used to view the transcriptional variations and expression of different genes in the breast cancer.

Gene expression profiling of breast carcinoma has resulted in classification of breast cancer into molecular subtypes luminal A (Hormone Receptor positive, Her 2 negative, low ki67), luminal B (Hormone Receptor positive, Her 2 positive/negative, high Ki 67), TNBC /Basal like (Hormone Receptor negative, Her 2 positive), Her 2 enriched (Hormone Receptor negative and Her 2 positive).

Immunohistochemistry is a well-established routine method that can be used for analysis of protein expression and can help to identify the molecular subtypes of breast carcinoma that are similar to the ones derived by gene expression arrays.

Currently, the decision regarding the treatment of a breast cancer patient depends upon several factors including tumor size, morphology, histological grade, lymph-node status and luminal type. The luminal subtypes have prognostic implications and also influence the treatment options which can be personalized for every patient according to the gene expression profiling of the tumor. Luminal A have the best prognosis. Luminal B have slightly worse prognosis than Luminal A. Triple Negative Breast Carcinoma (TNBC) is more common in women with BRCA 1 mutation. Her-2 enriched tumors have worse prognosis[(3)].

Hormones are the chemicals which act as messengers. They are released in the bloodstream and act on the tissue which carries their specific target receptor. Ovaries of premenopausal women produce Estrogen and Progesterone. Estrogen promotes the development of secondary sex characters while progesterone has an important role in the menstrual cycle regulation and maintenance of pregnancy. Some types of breast cancers (i.e. the HR positive breast cancers) carry the estrogen and progesterone receptors and are sensitive to the estrogen and progesterone. These hormones upregulate the proliferation of the cancer cells in the HR positive type breast cancers. When the hormone circulating in the blood reaches the breast tissue, it binds to the receptor on the cancer cell and upregulates the expression of certain genes. These transcriptional changes and increased expression of certain proteins increases the cell division of the cancer cells and thus promotes the growth of the cancer. Testing the breast cancer for the status of these hormone receptors is vital for risk stratification and treatment decisions. If a tumor is positive for estrogen receptor, it is very much likely that it will also be positive for progesterone receptor.

Hormonal/Endocrine therapy is a treatment option for cancers showing hormone receptor positivity. This therapy works either by downregulating the production of hormones or decreasing the effect of hormones on the cancer cells by blocking the hormone receptors.

In case of breast cancer, the patients with HR positive cancers the patient can benefit by a hormone therapy that blocks the production of estrogen and progesterone by the ovaries (ovarian ablation). This is done by either surgically removing the ovaries (oophorectomy) or by radiation. Another option is to do functional ablation of the ovaries by drugs comprising gonadotrophin releasing hormone GnRH agonists. These drugs interfere with the pituitary and ovarian axis and decrease the production of hormones by the ovaries. Drugs that contain aromatase inhibitors can block the enzyme aromatase which is required for estrogen formation.

Another treatment option is to block the hormone receptors on the cells. SERMs (selective estrogen receptor modulators) attach themselves to the estrogen receptors on the cells and block the estrogen from binding to the receptors. They have a selective action depending upon the tissue i.e. they have an estrogen blocking effect in the breast cancer cells whereas they can mimic estrogen and act as agonists on the estrogen receptors of the endometrium and thus pose a risk of developing endometrial carcinoma. There are some pure anti-estrogenic drugs available which do not have an estrogenic action on any tissue in the body e.g. fluvestrant.

There are several ways to include the hormonal therapy in the treatment of breast cancer. In cases that present at an early stage and are positive for estrogen receptor, hormonal therapy can be used as an adjuvant therapy after having the surgical removal of the cancer. This reduces the chances of recurrence. Hormonal therapy can be used as a neoadjuvant therapy to decrease the size of the tumor before surgical removal. It can also be used as a treatment option for patients with advanced or metastatic disease in which surgery is not a treatment option.

The ER positive tumor patients benefit from endocrine therapy against estrogen receptors e.g. tamoxifen. However they don't respond well to cytotoxic chemotherapy. PR positive tumors show good response to endocrine chemotherapy even if the PR expression is weak.

HER2 gene overexpression has been associated with worse prognosis but still it is a strong predictive marker of response to anti- HER2 neu therapy e.g. Herceptin.

Therapies in breast cancer are influenced by hormonal receptors status and customised treatment options can be devised for patients to ensure maximum benefit. Therefore, it is invaluable to search for new prognostic markers for risk stratification and to devise new treatment modalities.

The product of the B cell leukemia / lymphoma (BCL-2) proto-oncogene had been predominantly studied in Haematological malignancies where it had been associated with a 14;18 chromosomal translocation in follicular lymphoma. This translocation juxtaposes the BCL 2 gene located at 18q21 with the immunoglobulin heavy chain locus located at 14q32. As a result of this, there is an increased production of the BCL-2 gene product. BCL-2 provides

survival advantage by blocking apoptosis. BCL-2 overexpression increases the lifespan of B cells; may maintain memory B cells, plasma cells and neurons by increasing their lifespan. It is also involved in the formation of ion channels and hence changing the membrane permeability which is required for apoptosis. BCL-2 exists in two forms, non-phosphorylated (which inhibits apoptosis) and Bax homodimers (which can promote apoptosis by binding to and inhibiting the phosphorylated form). BCL-2 gene expression is not specific for 14;18 translocation.[4,5]

Recent studies have identified BCL-2 expression in non – haematological malignancies including malignancies of Lungs, prostate, nasopharynx and neuroblastoma. In our study, we analysed BCL-2 immunoreactivity in breast cancer & correlated it with ER, PR, Her 2 status of tumor as well as with histological grade[6].

Via transcriptional induction, Estrogen upregulates BCL-2 in breast cancer. Therefore, if BCL-2 is positive then it could indirectly show the estrogen receptor (ER) functional activity and hence it could affect the clinical outcomes of the breast cancer[7].

Few studies have been done worldwide regarding correlation of BCL-2 positivity in breast cancer with different histological grades of tumor and molecular subtypes of the tumor and have shown BCL-2 to be a good prognostic marker of breast carcinoma. But no such study has been conducted in our country to the best of my knowledge. This study will be conducted in our population to correlate BCL-2 expression with different molecular subtypes of the tumor and to determine BCL-2 as a subtype specific prognostic marker

Materials and Methods

This is a descriptive, cross-sectional study held in Sheikh Zayed Hospital Lahore over a duration of six months. The calculated sample size (collected by Non-probability consecutive sampling) taking confidence level of 95% and margin of error of 5% and average prevalence of 9.25% is 25. All incisional biopsies and mastectomy specimens from female patients with ages 35-55 years and greater. Only Invasive Ductal Carcinoma of Breast are included. Block for review and immunohistochemistry from outside laboratories were also included. All autolysed/unfixed samples (samples in weak formalin) were excluded from the study.

The biopsy specimens fulfilling the inclusion criteria were enrolled in the study. All data was collected by using a proforma. The requisition forms sent from surgery department were retrieved along with other relevant investigations. The clinical parameters like age were recorded. The histological preparation of the slides for this study was performed by using the standard method for inclusion in paraffin followed by the standard hematoxylin–eosin staining. The immune-histochemical analysis of all the cases was performed on serial sections obtained after processing and cutting. Immune-enzymatic soluble complex method was used. The antibody used was Bcl-2 polyclonal antibodies from DAKO.

Strong nuclear and cytoplasmic staining in almost all tumor cells was taken as positive for ER and PR (Allred score >3). Immunohistochemistry was used to check the HER 2 neu status of all cases. Only cases with an IHC score of 3+ were considered as positive. To check for BCL-2 status 10% was taken as the cut-off (only cases with >10% tumor cells showing strong BCL-2 expression were considered as positive). One consultant histopathologist (minimum experience of 5 years) examined each case and his/her diagnosis and interpretation of IHC was taken as confirmatory. Outcome variables were recorded.

SPSS version 23 was used for data entry and analysis. Effect modifiers like age and duration of disease were controlled through stratification. Post stratification chi-square test were applied by taking P value of 0.05 as significant.

Results

The table no.1 shows the age stratification of breast carcinoma in the local population. BCL-2 status was checked in all cases of breast carcinoma that were included in this study. The BCL-2 status of all cases is shown in Table no.2. ER, PR and HER 2 neu status of all cases were also checked by immunohistochemistry. BCL-2 status and ER status is compiled in Table no.3. BCL-2 and PR status of all breast carcinoma cases is shown in Table no.4. HER 2 neu and BCL-2 status of the cases is represented in Table no.5.

BCL-2 positivity in correlation with the ER, PR and HER 2 neu status can be seen in these tables.

Table 1: Incidence of breast carcinoma in different ages

AGE	Number of cases	Percentage
<35 years	5	20%
35-45 years	4	16%
45-55 years	9	36%
>55 years	7	28%
Total	25	100%

The incidence of breast carcinoma was highest in age group 45-55 years.

Table 2: BCL-2 status in breast carcinoma

Positive	6	24%

Negative	19	76%
Total	25	100%

6(24%) out of total 25 cases were strongly positive for BCL-2.

Table 3: BCL-2 and ER status in breast carcinoma

ER	BCL-2 Negative	BCL-2 Positive	p- value
Negative	15 (100%)	0 (0%)	0.000
Positive	4 (42%)	6 (58%)	

Table 3: BCL-2 and PR status in breast carcinoma

PR	BCL-2 Negative	BCL-2 Positive	p- value
Negative	15 (100%)	0 (0%)	0.000
Positive	4 (42%)	6 (58%)	

BCL-2 expression was noted in 6(58%) out of 10 ER/PR positive cases.

Table 3: BCL-2 and HER-2 neu status in breast carcinoma

Her-2 neu	BCL-2 Negative	BCL-2 Positive	p- value
Negative	16 (72%)	6 (28%)	0.237
Positive	3 (100%)	0 (0%)	

Discussion

The results of this study reveal that BCL-2 positivity varies in different molecular subtypes of breast cancer. BCL-2 expression is more common in luminal A type breast carcinoma and luminal B type breast carcinoma. In our study BCL-2 was strongly positive (+++) in 6 (24%) out of total 25 cases. A study was conducted in St. Mary's hospital by Yeng Hwa Eom 53.8% of breast cancer cases were BCL-2 +ve & 46.2% were -ve. BCL-2 positivity was more common in luminal A and Luminal B breast CA than in Basal like and Her 2 neu enriched types. BCL-2 positivity is related with younger age, low histological grade, low ki-67 index, Hormone receptor positivity, negative HER 2 neu status, EGFR negativity, p53 and CK5/6 negativity.

In this study ER/PR positivity was strongly related with BCL-2 positivity. 10 out of 25 cases were strongly ER/PR positive. Among these 10 cases 6 (58%) cases showed strong BCL-2 expression as well, which is statistically significant (p-value <0.05). There is evidence of inverse relationship between BCL-2 and Her 2 neu expression as all Her 2 neu positive cases remain negative for BCL-2.

Initially BCL-2 was studied as a prognostic marker in non-Hodgkin's lymphoma and is considered to be associated with favorable outcome.

BCL-2 expression is more common in breast carcinomas with positive ER/PR status, low histological grade, small tumor size, negative Her-2 neu status and hence points towards a favorable outcome. BCL-2 can be used as an independent prognostic marker and as its expression is upregulated by estrogen, it can be considered related to the Estrogen Receptor status. This study analyses the interaction of BCL-2 expression and Hormone receptor status of the tumor.

Our study shows an inverse relationship between BCL-2 expression and HER 2 neu status of the tumor. This correlation between the BCL-2 positivity and HER-2 neu overexpression shows that BCL-2 has an anti-proliferative role despite being anti-apoptotic. In Luminal B subtype (HER 2 neu positive) and HER 2 neu overexpression carcinomas there is a lower expression of BCL-2 which again proves the inverse relationship between BCL-2 expression and HER 2 neu status of the tumor.

No statistical relation was observed between BCL-2 expression and triple negative breast carcinoma. This makes us consider the fact that prognostic value of BCL-2 expression is mainly for Hormone receptor positive breast carcinomas. It is already mentioned that BCL-2 expression is positively correlated with the positivity of Estrogen receptors, so it is safe to assume that BCL-2 acts via the Estrogen receptor pathways. Hence BCL-2 doesn't have much prognostic value for the Estrogen receptor negative breast carcinomas.

Conclusion

BCL-2 is an independent prognostic marker in invasive breast carcinoma. Its expression in correlation with ER/PR positivity in Luminal A and B types of breast carcinomas mark a favorable outcome. This study establishes the importance of including BCL-2 immunohistochemistry for improving the prognostic stratification of breast carcinoma. Pro apoptotic anti-BCL2 therapy could be used to reinforce the effectiveness of hormonal therapy in ER/PR positive tumors.

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CASE REPORT

Importance Of Thorough Histopathological Examination Of Ovary In Case Of

Endometrioid Carcinoma In A Postmenopausal Female

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Abstract

The association between adult granulosa cell tumor of ovary and endometrial carcinomas is rare. The presence of an estrogen producing ovarian tumor can play an important role in the pathology of endometrium which can be simple hyperplasia with atypia and it may be an endometrial carcinoma. Most of these endometrial cancers are well-differentiated endometrioid adenocarcinomas that carry a good prognosis when detected early. Here we present a case of endometrioid carcinoma in which thorough histopathological examination revealed associated granulosa cell tumor in left ovary. Hence thorough examination of ovaries is very important in cases of endometrial carcinoma.

CASE REPORT: We report the case of a 65-year-old post-menopausal woman with endometrioid carcinoma. Her pre-operative radiological examination did not reveal any lesion in the ovary. She underwent a total abdominal hysterectomy and a bilateral salpingo-oophorectomy. Histopathologic examination confirmed well differentiated grade 1 endometrioid carcinoma uterus alongwith adult granulosa cell tumor of left ovary.

CONCLUSION: A good histopathologic analyses are important in making this diagnosis and can help to establish the role of endogenous hormones in the pathogenesis of endometrioid carcinomas as well.

Keywords: adult granulosa cell tumor, endometrioid carcinoma, histopathology, ovary

Introduction

It is well said by a professor of medicine Thomas McCrae that "More is missed by not looking than by not knowing".

The association between adult granulosa cell tumors of the ovary and endometrial carcinomas is rare.[1,2] Adult granulosa cell tumors are the most common estrogenic ovarian tumors.[3] Unopposed effect of estrogen plays an important role in various endometrial pathology. The endometrium can show features of simple hyperplasia with some degree of atypia or complex hyperplasia or it can be a well differentiated endometrial carcinoma. The incidence of endometrial carcinoma with associated granulosa cell tumor ranges from 5 to 25%. The wide variation can be attributed to the difference in opinion for differentiating complex atypical hyperplasia from well differentiated adenocarcinoma.[1,2,4-6]

Case Report

A 65-year-old, para 5, presented with chief complaints of post-menopausal bleeding and dull, intermittent lower abdominal pain. She had no history of post-coital or contact bleeding, weight loss, anorexia, urinary symptoms and vaginal discharge. There was no family history of a similar illness. An endometrial tissue biopsy was done and it revealed a moderately differentiated adenocarcinoma of endometrium.

A clinical diagnosis of post-menopausal bleeding secondary to endometrial carcinoma was made. MR Pelvis and MRCP revealed retroverted and bulky uterus with heterogeneously enhancing T2/STIR hyperintense and T1 iso-hypointense large polypoidal mass arising from endometrium and extending further to involve >50% of the myometrium, suggestive of FIGO Stage 1b. No significant lymphadenopathy was noted. Bilateral ovaries were unremarkable. No free fluid in Pouch of Douglas was noted. The urine analysis, renal function test and electrocardiography results were normal.

She underwent a total abdominal hysterectomy and a bilateral salpingo-oophorectomy. Also cholecystectomy was done alongwith removal of external iliac and common pelvic lymph nodes. The intra-operative findings included a bulky uterus that measured 8 cm × 5 cm with an enlarged, malignant-looking endometrium. Her bilateral fallopian tubes and ovaries appeared to be healthy. All the surgical specimens were sent for histopathological examination.

Grossly, the uterus was bulky measuring 8 x 5.5 cm. On cutting open, the endometrial cavity showed an infiltrating greyish white firm growth measuring 4x2.2 cm [Figure 1]. Right ovary measured 3x1.5 cm and left ovary measured 3.5x2.5 cm. Both fallopian tubes measured 4cm in length. The right ovary and both tubes were unremarkable. The left ovary showed a small cystic cavity measuring 2x1cm filled with greyish brown friable tissue.

Figure 1: Gross photomicrograph of uterus with ovaries showing the uterine mass

Microscopic examination of the uterine mass showed a well-differentiated grade 1 endometrioid carcinoma invading the adjacent myometrium (pStage 1b N0) [Figure 2]. Histologic sections of the left ovary showed a granulosa cell tumor containing coffee bean cells growing in solid sheets and call exner bodies [Figure 3]. Right ovary and both fallopian tubes were unremarkable.

Adenomyomatous hyperplasia of gall bladder was noted on histologic examination of gall bladder. Pelvic lymph nodes were found to be reactive with sinus histiocytosis. Sections from omentum were uninvolved by tumor.

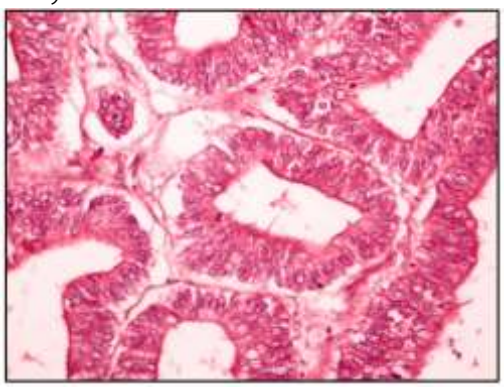

Figure 2: Photomicrograph of uterine mass showing endometrioid carcinoma (H&E, x40)

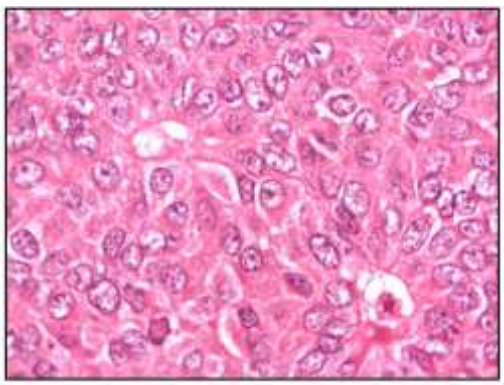

Figure 3: Photomicrograph of ovarian mass showing granulose cell tumor (H&E, x40)

Discussions

Endometrial carcinoma is the most common invasive neoplasm of the female genital tract and it accounts for 7% of all invasive cancer in women.[7] Unopposed excessive estrogen stimulation plays an important role in its pathogenesis.[8] The risk factors include obesity, nulliparity with a history of infertility, late menopause, unopposed estrogen therapy, tamoxifen therapy, and the use of sequential oral contraceptive pills. Excess estrogen from any of these sources can produce continuous stimulation of the endometrium, resulting in endometrial hyperplasia which can be simple hyperplasia with atypia or it may lead to endometrial cancer.[7-8]

Adult granulosa cell tumors account for approximately 95% of all granulosa cell tumors and is considered as feminising mesenchymomas of the ovary. It is the most common estrogen producing tumor.[3] It is associated with isosexual pseudoprecocity in young patients. In adults, the typical clinical manifestation is uterine bleeding.[6] The continuous and unopposed estrogen secretion by this tumour may causes the pathologic modifications which range from cystic hyperplasia to atypical hyperplasia to invasive adenocarcinoma. The best estimate of the incidence of associated endometrial carcinomas is approximately 5%.[1,2,4-5] These carcinomas associated with granulosa cell tumors are more frequent in postmenopausal females. In a retrospective study conducted by Ottolina et al, it was found that endometrial carcinoma/atypical hyperplasia were commonly observed in ovarian granulosa cell tumor patients over 40 years of age. Hyperplasia was observed in 29.2% of patients and endometrial cancer was found in 7.5% of patients.[5]

The granulosa cell tumor does not present with palpable ovarian enlargement in 10–15% of the cases.[3] It is not detected even by radiological investigations. In these cases, it is apparent only at the time of a hysterectomy for another indication, such as atypical endometrial hyperplasia or carcinoma. This was seen in our case as well where even the radiological investigations does not reveal any abnormal findings in ovary.

Thus, in patients with well-differentiated endometrioid adenocarcinomas, both gross and microscopic examination of ovaries is important. Since these are the estrogen secreting ovarian tumor, they can be a predisposing factor for endometrial carcinoma in these patients. This was the case with our patient, who had a well-differentiated, nuclear grade 1 endometrioid adenocarcinoma and adult granulosa cell tumor in left ovary. Though no lesion was found in ovary on radiological examination, proper gross and histopathological examination revealed the presence of granulosa cell tumor in ovary.

Conclusion

Endometrioid adenocarcinoma associated with an adult granulosa cell tumor is rare. A good histopathologic analyses are important in making this diagnosis. It can help to establish the role of endogenous hormones in the pathogenesis of endometrioid carcinomas as well.

This also emphasises on importance of bilateral salpingo-oophorectomy done in cases of endometrial carcinoma.

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APPENDIX 1
INFORMATION FOR AUTHORS

All authors listed in a paper submitted to Asian Archives of Pathology (AAP) must have contributed substantially to the work. It is the corresponding author who takes responsibility for obtaining permission from all co-authors for the submission. When submitting the paper, the corresponding author is encouraged to indicate the specific contributions of all authors (the author statement, with signatures from all authors and percentage of each contribution can be accepted). Examples of contributions include: designed research, performed research, contributed vital new reagents or analytical tools, analysed data, and wrote the paper. An author may list more than one type of contribution, and more than one author may have contributed to the same aspect of the work.

Authors should take care to exclude overlap and duplication in papers dealing with related materials. See also paragraph on Redundant or Duplicate Publication in "Uniform Requirements for Manuscripts Submitted to Biomedical Journals" at http://www.icmje.org/index.html.

The submitted manuscripts will be reviewed by the members of the Editorial Board or the expert reviewers. At the discretion of the Editorial Board, the manuscripts may be returned immediately without full review, if deemed not competitive or outside the realm of interests of the majority of the readership of the Journal. The decision (reject, invite revision, and accept) letter will be coming from the Editorial Board who has assumed responsibility for the manuscript's review. The editor's decision is based not just on technical merit of the work, but also on other factors such as the priority for publication and the relevance to the Journal's general readership. All papers are judged in relation to other submissions currently under consideration.

Categories of Manuscripts

1. Letters to the Editor

The letters to the editor are the reactions to any papers published in AAP. These letters will be reviewed by the Editorial Board and sent to the authors of the original paper with an invitation to respond. Letters and eventual responses will be published together, when appropriate.

- *Word Count: 300 – 500 words (excluding references and figure or table legends)*
- *Abstract: Not required*
- *References: Maximum of 10*
- *Figure or Table: Maximum of 1 (if needed)*

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The original articles are the researches describing the novel understanding of anatomical pathology, clinical pathology (laboratory medicine), forensic medicine (legal medicine or medical jurisprudence), molecular medicine or pathobiology. Systematic reviews, meta-analyses and clinical trials are classified as articles. The articles should be clearly and concisely written in the well-organised form (see ***Organisation of Manuscripts***): abstract; introduction; materials and methods; results; discussion; and conclusions. The manuscripts that have passed an initial screening by the Editorial Board will be reviewed by two or more experts in the field.

- *Word Count: 3,000 – 5,000 words (excluding abstract, references, and figure or table legends)*
- *Structured Abstract (see **Organisation of Manuscripts**): 150 – 200 words*
- *References: Maximum of 150*
- *Figures or Tables: Maximum of 6*

3. Review Articles

The review articles are generally invited by the Editor-in-Chief. They should focus on a topic of broad scientific interest and on recent advances. These articles are peer-reviewed before the final decision to accept or reject the manuscript for publication. Therefore, revisions may be required.

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- *Unstructured Abstract: 150 – 200 words*
- *References: Maximum of 150*
- *Figures or Tables: Maximum of 4*

4. Case Reports

AAP limits publication of case reports to those that are truly novel, unexpected or unusual, provide new information about anatomical pathology, clinical pathology (laboratory medicine) or forensic medicine (legal medicine or medical jurisprudence). In addition, they must have educational value for the aforementioned fields. The journal will not consider case reports describing preventive or therapeutic interventions, as these generally require stronger evidence. Case reports that involve a substantial literature review should be submitted as a review article. The submitted case reports will undergo the usual peer-reviewed process.

- *Word Count: 1,200 – 2,000 words (excluding abstract, references, and figure or table legends)*
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- *References: Maximum of 20*
- *Figures or Tables: Maximum of 4*

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Case illustrations are aimed to provide education to readers through multidisciplinary clinicopathological discussions of interesting cases. The manuscript consists of a clinical presentation or description, laboratory investigations, discussion, final diagnosis, and up to 5 take-home messages (learning points). Regarding continuous learning through self-assessment, each of the case illustrations will contain 3 – 5 multiple choice questions (MCQs) with 4 – 5 suggested answers for each question. These MCQs are placed after the final diagnosis and the correct answers should be revealed after the references. The questions and take-home messages (learning points) are included in the total word count. The manuscripts that have passed an initial screening by the Editorial Board will be reviewed by two experts in the field.

- *Word Count: 1,000 – 2,000 words (excluding references and figure or table legends)*
- *Abstract: Not required*
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- *Figures: Maximum of 2*
- *Tables: Maximum of 5*

6. Technical Notes

The technical notes are brief descriptions of scientific techniques used in the anatomical pathology, clinical pathology (laboratory medicine), forensic medicine (legal medicine or medical jurisprudence), molecular medicine or pathobiology. The submitted manuscripts are usually peer-reviewed.

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- *Abstract: Not required*
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- *Figures or Tables: Maximum of 2*

Organisation of Manuscripts

1. General Format

The manuscripts written in English language are preferable. However, Thai papers are also acceptable, but their title pages, abstracts, and keywords must contain both Thai and English. These English and Thai manuscripts are prepared in A4-sized Microsoft Word documents with leaving 2.54-cm (1-inch) margins on all sides. All documents are required to be aligned left and double-spaced throughout the entire manuscript. The text should be typed in 12-point regular Times New Roman font for English manuscript and 16-point regular TH SarabunPSK font for Thai manuscript.

The running titles of English and Thai manuscripts are placed in the top left-hand corner of each page. They cannot exceed 50 characters, including spaces between words and punctuation. For the header of English paper, the running title will be typed in all capital letters. The page number goes on the top right-hand corner.

Footnotes are not used in the manuscripts, but parenthetical statements within text are applied instead and sparingly. Abbreviations should be defined at first mention and thereafter used consistently throughout the article. The standard abbreviations for units of measure must be used in conjunction with numbers.

All studies that involve human subjects should not mention subjects' identifying information (e.g. initials) unless the information is essential for scientific purposes and the patients (or parents or guardians) give written informed consent for publication.

2. Title Page

The title page is the first page of the manuscripts and must contain the following:

- The title of the paper (not more than 150 characters, including spaces between words)
- The full names, institutional addresses, and email addresses for all authors (If authors regard it as essential to indicate that two or more co-authors are equal in status, they may be identified by an asterisk symbol with the caption "These authors contributed equally to this work" immediately under the address list.)
- The name, surname, full postal address, telephone number, facsimile number, and email address of the corresponding author who will take primary responsibility for communication with AAP.
- Conflict of interest statement (If there are no conflicts of interest for any author, the following statement should be inserted: "The authors declare that they have no conflicts of interest with the contents of this article.")

3. Abstract

A structured form of abstract is used in all Original Article manuscripts and must include the following separate sections:

- *Background: The main context of the study*
- *Objective: The main purpose of the study*
- *Materials and Methods: How the study was performed*
- *Results: The main findings*
- *Conclusions: Brief summary and potential implications*
- *Keywords: 3 – 5 words or phrases (listed in alphabetical order) representing the main content of the article*

4. Introduction

The Introduction section should clearly explain the background to the study, its aims, a summary of the existing literature and why this study was necessary or its contribution to the field.

5. Materials and Methods

The Materials and Methods section must be described in sufficient detail to allow the experiments or data collection to be reproduced by others. Common routine methods that have been published in detail elsewhere should not be described in detail. They need only be described in outline with an appropriate reference to a full description. Authors should provide the names of the manufacturers and their locations for any

specifically named medical equipment and instruments, and all chemicals and drugs should be identified by their systematic and pharmaceutical names, and by their trivial and trade names if relevant, respectively. Calculations and the statistical methods employed must be described in this section.

All studies involving animal or human subjects must abide by the rules of the appropriate Internal Review Board and the tenets of the recently revised Helsinki protocol. Hence, the manuscripts must include the name of the ethics committee that approved the study and the committee's reference number if appropriate.

6. Results

The Results section should concisely describe the findings of the study including, if appropriate, results of statistical analysis which must be presented either in the text or as tables and figures. It should follow a logical sequence. However, the description of results should not simply repeat the data that appear in tables and figures and, likewise, the same data should not be displayed in both tables and figures. Any chemical equations, structural formulas or mathematical equations should be placed between successive lines of text. The authors do not discuss the results or draw any conclusions in this section.

7. Discussion

The Discussion section should focus on the interpretation and the significance of the findings against the background of existing knowledge. The discussion should not repeat information in the results. The authors will clearly identify any aspects that are novel. In addition, there is the relation between the results and other work in the area.

8. Conclusions

The Conclusions section should state clearly the main summaries and provide an explanation of the importance and relevance of the study reported. The author will also describe some indication of the direction future research should take.

9. Acknowledgements

The Acknowledgements section should be any brief notes of thanks to the following:

- *Funding sources*
- *A person who provided purely technical help or writing assistance*
- *A department chair who provided only general support*

- *Sources of material (e.g. novel drugs) not available commercially*

Thanks to anonymous reviewers are not allowed. If you do not have anyone to acknowledge, please write "Not applicable" in this section.

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The Vancouver system of referencing should be used in the manuscripts. References should be cited numerically in the order they appear in the text. The authors should identify references in text, tables, and legends by Arabic numerals in parentheses or as superscripts. Please give names of all authors and editors. The references should be numbered and listed in order of appearance in the text. The names of all authors are cited when there are six or fewer. When there are seven or more, only the first three followed by "et al." should be given. The names of journals should be abbreviated in the style used in Index Medicus (see examples below). Reference to unpublished data and personal communications should not appear in the list but should be cited in the text only (e.g. A Smith, unpubl. Data, 2000).

- *Journal article*
  1. Sibai BM. Magnesium sulfate is the ideal anticonvulsant in preeclampsia – eclampsia. Am J Obstet Gynecol 1990; 162: 1141 – 5.
- *Books*
  2. Remington JS, Swartz MN. Current Topics in Infectious Diseases, Vol 21. Boston: Blackwell Science Publication, 2001.
- *Chapter in a book*
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11. Tables

The tables should be self-contained and complement, but without duplication, information contained in the text. They should be numbered consecutively in Arabic numerals (Table 1, Table 2, etc.). Each table should be presented on a separate page with a comprehensive but concise legend above the table. The tables should be double-spaced and vertical lines should not be used to separate the columns. The column headings should be brief, with units of measurement in parentheses. All abbreviations should be defined in footnotes. The tables and their legends and footnotes should be understandable without reference to the text. The authors should ensure that the data in the tables are consistent with those cited in the relevant places in the text, totals add up correctly, and percentages have been calculated correctly.

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The legends should be self-explanatory and typed on a separate page titled “Figure Legends”. They should incorporate definitions of any symbols used and all abbreviations and units of measurement should be explained so that the figures and their legends are understandable without reference to the text.

If the tables or figures have been published before, the authors must obtain written permission to reproduce the materials in both print and electronic formats from the copyright owner and submit them with the manuscripts. These also follow for quotes, illustrations, and other materials taken from previously published works not in the public domain. The original resources should be cited in the figure captions or table footnotes.

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All illustrations (line drawings and photographs) are classified as figures. The figures should be numbered consecutively in Arabic numerals (Figure 1, Figure 2, etc.). They are

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14. Components

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The Letter to the Editor manuscripts consist of the following order:

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- *Conclusions*
- *Acknowledgements*
- *References*
- *Table (s)*
- *Figure Legend (s)*
- *Figure (s)*

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- *Conclusions*
- *Acknowledgements*

- *References*
- *Table (s)*
- *Figure Legend (s)*
- *Figure (s)*

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- *Acknowledgements*
- *References*
- *Correct Answers to MCQs*
- *Table (s)*
- *Figure Legend (s)*
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- *Title Page*
- *Introduction*
- *Main text*
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The authors of the accepted manuscripts will receive proofs and are responsible for proofreading and checking the entire article, including tables, figures, and references. These authors should correct only typesetting errors at this stage and may be charged for extensive alterations. Page proofs must be returned within 48 hours to avoid delays in publication.

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In many cases, the authors will be invited to make revisions to their manuscripts. The revised manuscripts must generally be received by the Editorial Board within 3 months of the date on the decision letter or they will be considered a new submission. An extension can sometimes be negotiated with the Editorial Board.

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