DOI: https://doi.org/10.53350/pjmhs20221611231

ORIGINAL ARTICLE

Utilization of Immunohistochemistry in Gynecologic Pathology: An Experience at a Tertiary Care Hospital, Lahore-Pakistan

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ABSTRACT

Background: Immunohistochemistry i**s** an adjunct tool in Surgical Pathology. The fast growing use of immunohistochemistry in gynecologic Pathology has revolutionized the fields of tumor diagnostics & research.

Objective: The objective of the study was to share & discuss the experience of utility of immunohistochemistry in Gynecologic Pathology, at a tertiary care hospital in Lahore, Pakistan.

Patients & Methods: This was a retrospective, descriptive, cross sectional study, carried out at the Pathology Department of Fatima Jinnah Medical University, Lahore. All cases which were diagnosed after the application of immunohistochemistry during the study period from 1st July 2019 to 31st December 2020 were included in the study. Data included age of the patient, marital status, parity, clinical & radiological presentation, histopathological findings & differentials, list of immunohistochemical markers applied to the case with results & final histopathological diagnosis. Data was analyzed using SPSS version 17.

Results: A total of 196 cases were included in the study. The age of the patients ranged from 14 years to 82 years with a mean age of 41 ± 7 years. The commonest use of immunohistochemistry was for histological classification of the tumors of the female genital tract, identifying precancerous lesions, differentiating primary from metastatic CA & predicting response to chemotherapy via proliferative index Ki67. The most commonly used immunohistochemical markers were CK, CK7, CK20, CD 3, CD20, ER, PR, VIMENTIN, WT 1, Ki67, CD 117, SMA, INHIBIN, p53 & p63. Practical implication This study shares the experience of use of common immunohistochemical markers in different cases of gynecologic pathology, highlighting & discussing different panels for use in different scenarios, from which other pathologists may benefit.

Conclusion: Immunohistochemistry is an important ancillary tool in the evaluation of gynecologic pathology cases. However, it cannot replace conventional histopathology. It should always be used as an adjunct to histopathology, in the proper clinical & radiological context.

Keywords: immunohistochemistry, gynecologic pathology, ovarian carcinoma, leiomyoma, dysgerminoma,

INTRODUCTION

Immunohistochemistry is an adjunct tool in Surgical Pathology & Research.[1] It involves identification of certain antigens (proteins) expressed in cells of biological tissues by application of the target antigen-binding specific antibodies. The detection of these antigens plays a tremendous role in diagnostics, therapeutics and research.[2]

Over the past few years, the application of immunohistochemistry has revolutionized the field of Surgical Pathology. The ever growing use of immunohistochemistry has made possible correct & accurate diagnosis of tumors, the identification of precancerous lesions & the differentiation of primary versus metastatic tumors.[3] Immunohistochemistry also plays a pivotal role in diagnosing carcinomas of unknown primaries, defining prognostic factors of tumors & monitoring the therapeutic responses via targeted therapy.[4] Immunohistochemistry is also being increasingly used for screening of inherited cancer syndromes such as Mismatch Repair Proteins Immunohistochemistry in Hereditary Non Polyposis Colorectal Cancer Syndrome or Lynch Syndrome [5]

As with other specialties, the utilization of immunohistochemistry in gynecologic pathology has helped in better understanding of the tumor pathogenesis such as MYC immunohistochemistry in Burkitt Lymphoma,[6] tumor diagnostics such as positivity of lineage markers in poorly differentiated neoplasms[7] & prognostications e.g the expression of Estrogen receptors, Progesterone receptos & HER 2neu in breast carcinoma[8], thus contributing effectively to better patient management & survival.[5]

The rationale of the study is to provide a practical update about the application of different immunohistochemical markers in day to day gynecologic pathology. This study generously accepts the limitations in interpretation of some immunohistochemical results due to unavailability of certain immunohistochemical markers or equivocal staining pattern.

PATIENTS & METHODS

This is a retrospective, descriptive, cross sectional study, carried out at the Pathology department of Fatima Jinnah Medical University (FJMU), Lahore. This study was approved by the Institutional Ethical Review Board. Cases of gynaecologic pathology were received from Sir Ganga Ram Hospital, Lahore. All cases which were diagnosed after the application of immunohistochemistry, in the Pathology Department FJMU during the study period from 1st July 2019 to 31st December 2020 were included in the study. Gynecologic cases referred to the Pathology Department FJMU from other health centers for review and application of immunohistochemistry were also included in the study. Cases of gynecologic pathology which were not diagnosed despite the application of immunohistochemistry were excluded. These cases were referred to other health centers for an extended panel of immunohistochemistry & molecular studies for definitive diagnosis.

Data of all the cases was entered on a predesigned proforma. Data included age of the patient, marital status, parity, clinical presentation, radiological findings, histopathological findings & differentials, list of immunohistochemical markers applied to the case with results & final histopathological diagnosis. Data was analyzed using SPSS version 17.

RESULTS

A total of 196 cases were included in the study. The age of the patients ranged from 14 years to 82 years with a mean age of 41 ± 7 years.

Ovaries: Twenty one cases of adnexal masses had differentials of ovarian carcinoma. A panel of CK 7, CK 20, ER, PR, Vimentin, WT1 & p53 were applied. CK 7+ve & CK 20-ve in all the cases favored carcinoma of ovarian origin. 10 cases proved to be Serous carcinoma with WT 1 +ve, p53 mutational type, ER focally +ve, PR –ve, Vimentin –ve (Figure 1). The reverse profile in 8 cases favored a diagnosis of Endometriod Adenocarcinoma. Three cases of ovarian carcinoma turned out to be CK 7+ve/CK 20-ve, all other markers were negative. These cases were diagnosed Clear Cell carcinoma based on histopathology & ruling out other histological types immunohistochemically.

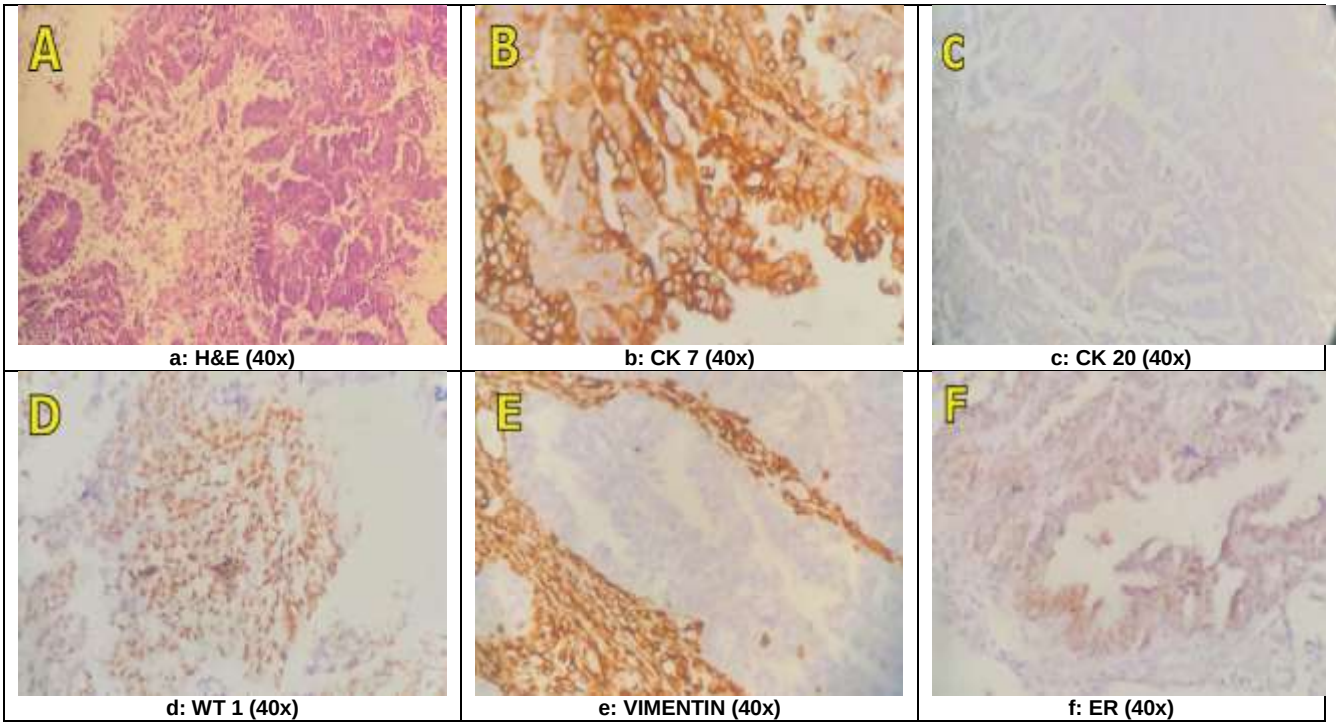

Figure 1: The expression of CK 7, CK 20, WT1, Vimentin and ER in serous carcinoma of ovary by IHC a: H&E, b: CK 7 positive, c: CK 20 negative d: WT 1 positive e: Vimentin negative f: ER mild positive

The differential diagnosis of 2 ovarian masses rested between a non Hodgkin lymphoma & a dysgerminoma. A panel of OCT ¾, CD 117, CD3, CD20 & Ki67 were applied. Both cases turned out to be CD 117 +ve, OCT ¾ +ve, with variable Ki67. CD3 & CD 20 were –ve. The cases were diagnosed as dysgerminoma.

The morphology of 3 ovarian masses favored a diagnosis of non Hodgkin lymphoma. A panel of CD3, CD 20, CK, CD 117, Tdt & Ki67 was applied. All 3 cases were CD 20 +ve, CD 3-ve, CK -ve, CD 117-ve. One case showed 100% Ki67 & Tdt –ve.It was diagnosed as Burkitt lymphoma. The other case was Tdt +ve, Ki67 90 % .It was diagnosed as Acute lymphoblastic lymphoma, B cell type. The third case was Tdt –ve, Ki67 60%. It was diagnosed diffuse large B cell lymphoma.

The morphology of 3 ovarian masses favored a diagnosis of mixed germ cell tumor .An immunohistochemistry panel of CK, CD 117, CD 30, AFP, OCT ¾, highlighted the dysgerminoma & non dysgerminoma components.

Five ovarian masses showed morphology of well differentiated Granulosa cell tumor. The diagnosis was confirmed by vimentin +ve, inhibin +ve, CD 99+ve, WT 1 focally +ve, CK variable staining, EMA –ve, synaptophysin -ve, CD 45 –ve.

Eight adnexal masses showed a benign spindle cell neoplasm. 6 masses were SMA +ve, inhibin –ve. They were diagnosed leiomyoma.2 cases were SMA –ve, inhibin +ve. They were diagnosed as Ovarian Fibroma. One Fibroma presented with Meig's Syndrome.

Three cases had mucinous adenocarcinoma in the ovaries as well as colon. 2 cases were CK 7 strong +ve , CK 20 focally +ve. They were diagnosed as ovarian primaries. One case was CK 7-ve, CK 20 strong & diffuse +ve. It was diagnosed as colonic primary with ovarian metastasis.

Fallopian Tube: The fallopian tube of one ovarian mass harbouring serous carcinoma showed serous tubal intraepithelial carcinoma (STIC).STIC was confirmed by p53 mutational expression, WT 1 strong +ve & full thickness Ki67 expression.

Uterus: Thirteen cases of uterine carcinoma were applied a panel of CK 7, CK 20, ER, PR, Vimentin, p53 & WT1. The immunohistochemical profiles of endometrioid, serous & clear cell Carcinomas were the same as stated in the ovarian section, but uterine Serous carcinoma were WT 1 –ve.

Seven cases of endometrial stromal sarcoma low grade were encountered. The diagnosis was confirmed by CD 10+ve, ER +ve, PR +ve, SMA –ve, Desmin –ve, CD 34-ve, CD 117 variable expression & low Ki67.

Two uterine masses had a malignant spindle cell morphology. They were SMA +ve, Desmin +ve, CD 34 –ve, CD 10-ve, CD 117 –ve. ER, PR & ki67 had variable expression. They were diagnosed as uterine leiomyosarcomas.

Two uterine masses had a malignant epithelial as well as stromal component. They were CK +ve, Vimentin +ve. They were diagnosed as carcinosarcoma.

A patient had bilateral ovarian masses along with a uterine mass. The tumors were CK 7+ve, CK 20 -ve, WT 1 strong & diffuse +ve, p53 mutational type. ER was focally +ve. Vimentin was –ve. A diagnosis of primary ovarian serous CA with metastasis to uterus was made.

Cervix & Vagina: Twenty cases of cervical & one vaginal biopsy showed squamous cell carcinoma. The diagnosis was confirmed by CK +ve, p63 +ve, CK 7 variable expression.

Vulva, Vagina & Labia: Five cases of labial/vulval growth had spindle cell morphoplogy with low grade features. Three cases were SMA +ve, desmin +ve, ER +ve, PR +ve.CD 34 expression was variable. They were diagnosed as aggressive angiomyxoma. Two cases were CD 34 –ve, SMA –ve, Desmin –ve, ER +ve, PR +ve. They were diagnosed as angiomyofibroblastoma.

DISCUSSION

This study reviews & shares our experience of use of different immunohistochemical markers in day to day gynecologic pathology at our department. Our department had a limited number of immunohistochemical markers available during the study period. We tried to utilize the limited available immunohistochemical markers to the best of our knowledge & experience to reach at a conclusive diagnosis in all the cases. All the immunohistochemical markers were applied & interpreted in the proper clinico-radiological context of the cases, with especial emphasis on their histopathological diagnosis or differentials. In cases where the immunohistochemical results were discordant with the histopathological findings, the histopathological diagnosis were favored & notes of caution were written at the ends of the histopathological reports.

The commonest use of immunohistochemistry was for the histological classification of ovarian & uterine carcinoma. The commonest used panel was CK 7, CK 20, ER, PR, vimentin, WTI, & p53.CK 7 +v/CK 20 -ve favored female genital tract origin. Endometroid adenocarcinomas were ER, PR & VIMENTIN +ve, Ovarian serous Carcinomas were WT1 +ve & p53 mutational type. Uterine serous CA were WT 1 –ve & p53 mutational type. Clear cell CAs were CK 7+ve. All other markers –ve. Our results are concordant with the results of Kuhn E[9] who used similar panel for the diagnosis. However, he used additional markers i.e Napsin A for clear cell CA, PAX 8 for ovarian origin & p16 for serous CA. We did not have these markers available at our department.

In case of serous CA involving both the uterus & ovaries, WT 1 positivity was used to confirm ovarian primary with uterine metastasis. Shimizu M[10] reported 97% of ovarian serous carcinomas & 20% of uterine serous carcinomas were WT 1 positive.

The morphology of dysgerminoma overlapped with a non Hodgkin lymphoma in two cases. A panel of OCT ¾, CD 117, CD 3, CD 20 & Ki67 was applied. OCT ¾ confirmed germ cell lineage .CD 117 positivity & CD 3 & CD 20 negativity confirmed the diagnosis of dysgerminoma. Our results are strengthened by the study of Rijlaaesdam M A[11] who used OCT ¾ for confirming germ cell lineage.

For mixed germ cell tumors, a panel of OCT ¾, CD 117, CD 30, AFP, GFAP, inhibin & CD117 was used. OCT ¾ confirmed germ cell origin, CD II7 highlighted the dysgerminoma component, AFP was positive in the yolk sac component, CD 30 was positive in embryonal carcinoma. GFAP highlighted the glial component in the teratoma. The study by Kaur B[12] showed similar results with these immunohistochemical markers. However, she also used SALL4 & NANOG for germ cell origin & PLAP for dysgerminoma. We did not have these markers available at our department.

For ovarian lymphomas, we used a panel of CD3, CD 20, CK, CD 117, Tdt & Ki67.All cases were CD 20 +ve, CD 3-ve, CK –ve, CD117 –ve, confirming B cell non Hodgkin lymphoma. One case was Tdt +ve, Ki67 was 90% . It was diagnosed as B cell Acute Lymphoblastic Lymphoma (B-ALL). One case was Tdt –ve, Ki67 100%. It was diagnosed as Burkitt lymphoma. Joel A & colleagues[13] in India showed similar results. They applied a panel of CD 45, CD 20, CD 3, CD 79a, CD 10, Tdt, EMA, CD 68 & Ki67 to an ovarian mass. It was CD 45 +ve, CD 20 +ve, Tdt +ve, Ki67 90%. All other markers were negative. It was diagnosed B cell Acute Lymphoblastic Lymphoma (B-ALL). Similar to our findings, Al-Maghrebi H[6] reported bilateral ovarian Burkitt lymphoma which was CD79a +ve, CD20 +ve, PAX-5 +ve, CD10 +ve while CD3 -ve, CD5 -ve, , CD23 -ve, TdT -ve and Cyclin D1-ve . Ki67 was 100% .

We used a panel of vimentin, inhibin, CD 99,WT 1, CK, EMA, CD 45 & synaptophysin for sex cord stromal tumors. Granulosa cell tumors were found to be Vimentin +ve, inhibin +ve, WT 1+ve, CD 99 +ve, CK variable staining, EMA –ve, synaptophysin -ve & CD 45 –ve. The results of our study are strengthened by the findings of Guleria P [14] who demonstrated similar immunohistochemical profile of granulosa cell tumors. However, they also used calretinin in the panel, which we did not have.

Adnexal masses which showed a benign spindle cell morphology were applied SMA, Desmin & inhibin. Leiomyomas were SMA +ve, Desmin +ve, INH –ve. Fibromas had the reverse immunohistochemical profile. Our results are similar to the case report by Bharti [15], who used similar IHC panel. She also used caldesmon for smooth muscle lineage, which we did not have.

In cases of mucinous adenocarcinoma involving both the colon & ovaries, CK 7/CK 20 was used. CK 7 strong +ve , CK 20 focally +ve favored ovarian primary. CK 7-ve, CK 20 strong & diffuse +ve favored colonic primary with ovarian metastasis. Our results are in concordance with Fletcher et al.[16], who used additional markers as well. They used CDX2 & ß catenin for colonic primary & MUC5AC for ovarian primary.

We used p53 mutational expression, WT 1 +ve, & Ki67 full thickness expression to demonstrate serous tubal intraepithelial carcinoma in a fallopian tube. Weinberger [17] used the same panel for serous tubal intraepithelial carcinoma.

During our study period, we regret we could not differentiate between endometrioid adenocarcinoma of uterus from primary endocervical adenocarcinoma. Sternberg [18] used the panel ER, PR, vimentin, p16, m CEA for this scenario. They reported that endometriod adenocarcinomas were ER +ve, PR +ve, vimentin +ve, p16 –ve, m CEA –ve. Endocervical adenocarcinoma had the reverse profile. We had ER, PR & vimentin, but did not have p16 & m CEA, so this panel could not be used.

Cases of uterine mesenchymal lesions were applied a panel of ER, PR, CD 10, SMA, desmin, CD 34, CD 117 & Ki67. Endometrial stromal sarcoma low grade was diagnosed by the following panel : CD 10+ve, ER +ve PR +ve, SMA –ve, Desmin –ve, CD 34-ve, CD 117 variable expression & low Ki 67. Cases of leiomyosarcoma were SMA +ve, desmin +ve, CD 34 –ve, CD 10-ve, CD 117 –ve. ER, PR & ki67 expression was variable. Subbaraya S [19] & Hwang H [20] showed same results with similar immunohistochemical panel in these cases.

Uterine masses with malignant epithelial & stromal components were CK +ve as well as vimentin +ve. They were diagnosed carcinosarcoma. Pillarisetty [21] used CK & EMA for epithelial component & vimentin & SMA for stromal component.

Cervical squamous cell carcinomas were confirmed by CK +ve, p63 +ve, CK 7 variable expression. Li H [22] et al used p63, p40, CK 7, CK 5/6 & MUC5AC for differentiating cervical squamous cell carcinoma from cervical adenocarcinoma. They found out that cervical squamous cell carcinomas were p63+ve, p40+ve, CK 7 variable expression & MUC5AC –ve. Cervical adenocarcinomas showed the reverse profile. We could not use this panel due to non-availability of p40, CK 5/6 & MUC5AC.

CONCLUSION

IHC is a very important diagnostic tool in the evaluation of surgical pathology specimens. Use of immunohistochemistry has immensely advanced the fields of tumor diagnostics, prognostics, targeted therapy, screening for inherited cancer syndromes & research. However, it cannot replace conventional histopathology. It should always be used as an adjunct to histopathology, in the proper clinical & radiological context.

REFERENCES

1. Troxell M L, Lanciault C. Practical Applications in Immunohistochemistry: Evaluation of Rejection & Infection in Organ Transplantation. Arch Pathol Lab Med.2016;140(9):910-925.
2. Kim S W, Roh J, Park C. Immunohistochemistry for Pathologists: Protocols, Pitfalls, and Tips. J Pathol Transl Med.2016;50(6):411-418.
3. Rekhi B. Role of immunohistochemistry in gynec oncopathology including specific diagnostic scenarios with associated transplant implications. Indian J Pathol Microbiol.2020;63(5):70-80.
4. Kar A. Role of immunohistochemistry in gynecological malignancies. Oncol J India.2018;2(1):1-2
5. Buza N. Immunohistochemistry in gynecologic carcinomas: Practical update with diagnostic and clinical considerations based on the 2020 WHO classification of tumors. Semin Diagn Pathol. 2022 Jan;39(1):58-77.
6. Al-Maghrabi H, Meliti A. Primary bilateral ovarian Burkitt lymphoma; a rare issue in gynecologic oncology. J Surg Case Rep. 2018 May 18;2018(5):1-3
7. Omar EOR, Husain NE, Ismail A.The role of immunohistochemistry in the work up of malignant neoplasm of unknown primary origin at Khartoum Oncology Hospital. Asian Pacific Journal of Cancer Care.2021;6(4);441-447.
8. Łukasiewicz, S.; Czeczelewski, M.; Forma, A.; Baj, J.; Sitarz, R.; Stanisławek, A. Breast Cancer—Epidemiology, Risk Factors, Classification, Prognostic Markers, and Current Treatment Strategies—An Updated Review. Cancers 2021;13(1): 1-30.
9. Kuhn E, Ayhan A. Diagnostic immunohistochemistry in gynaecological neoplasia: a brief survey of the most common scenarios. Journal of Clinical Pathology 2018;71(2):98-109.
10. Shimizu, M., Yamanaka, K., Azumi, M. et al. A case of synchronous serous ovarian cancer and uterine serous endometrial intraepithelial carcinoma. J Ovarian Res.2021; 14 (87):1-5 .
11. Rijlaarsdam MA, van Herk HA, Gillis AJ, Stoop H, Jenster G, Martens J, van Leenders GJ, Dinjens W, Hoogland AM, Timmermans M, Looijenga LH. Specific detection of OCT3/4 isoform A/B/B1 expression in solid (germ cell) tumours and cell lines: confirmation of OCT3/4 specificity for germ cell tumours. Br J Cancer. 2011;105(6):854-63.
12. Kaur B. Pathology of malignant ovarian germ cell tumors. Diagnostic Histopathology.2020;26(6):289-297.
13. Joel A, Ganesan P, Sundersingh S , Radhakrishnan V, Ganesan T S, Sagar T G. Primary B-cell Lymphoblastic Lymphoma of the Ovary. Indian Journal of Medical and Paediatric Oncology.2018;39(3):418-420
14. Guleria P, Kumar L, Kumar S, Bhatla N, Ray R, Singhal S, Meena J,Mathur SR.A clinicopatholgical study of granulosa cell tumors of the ovary: Can morphology predict prognosis? Indian J Pathol Microbiol 2020;63(1):53-59
15. Bharti, Sushma, Khera, Sudeep, Sharma, Charu, Balakrishnan, Arsha. primary ovarian leiomyoma masqueraded as ovarian fibroma, Journal of Family Medicine and Primary Care.2021;10(9): 3494-3497
16. Zaloudek C. Tumors of the female genital tract. In Fletcher C D M. (ed).Diagnostic Histopathology of Tumors.4th edition. Elsevier; 2013.p.683
17. Weinberger V, Bednarikova M, Cibula D, Zikan M. Serous tubal intraepithelial Carcinoma (STIC)-clinical impact & management. Expert Review of anticancer therapy. 2016;16(12):1311-1321.
18. Longacre T A. Uterine Corpus. In : Stacey E Mills.(ed) Sternberg's Diagnostic Surgical Pathology.6th edition. Wolters Kluwer;2015.p.4923
19. Subbaraya S, Murthy SS, Devi G S. Immunohistochemical and Molecular Characterization of Endometrial Stromal Sarcomas. Clin Pathol. 2020;13(1):1-8.
20. Hwang H, Matsuo K, Duncan K, Pakzamir E, Pham HQ, Correa A, Fedenko A, Mhawech-Fauceglia P. Immunohistochemical panel to differentiate endometrial stromal sarcoma, uterine leiomyosarcoma and leiomyoma: something old and something new. J Clin Pathol. 2015;68(9):710-717.
21. Pillarisetty L S, Mukkera S, Mannem M, Patel J, Nagalla S, Ammu A, Alahari L. A Rare Case of Transformation of Endometrial Carcinoma Into Uterine Carcinosarcoma. J Clin Gynecol Obstet . 2021;10(3):81-85
22. Li H, Jing X, Yu J, Liu J, Zhang T, Chen S, Zhang X. A combination of cytokeratin 5/6, p63, p40 and MUC5AC are useful for distinguishing squamous cell carcinoma from adenocarcinoma of the cervix. Diagn Pathol. 2020 ;104(1):1-9