ORIGINAL ARTICLE

AL-ANBAR MEDICAL JOURNAL
Anb. Med. J. 20(2): 200–206, 2024

Ovarian Epithelial Cancers: A Pathological Study of 164 Cases

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(Received : 21 May 2024; Accepted : 25 August 2024; First published online: 9 September 2024)

ABSTRACT

Background: Foremost, the diagnosis of ovarian carcinoma is light microscopic in nature. However high-graded tumors and some equivocal cases may require contemporary tests.
Objectives: To examine ovarian carcinoma types in Duhok-Iraq using morphology and available immunohistochemical panels.
Materials and methods: In this retrospective cross-sectional study, paraffin blocks of 164 ovarian carcinoma cases were retrieved from Duhok pathology centers between January 2012 and December 2022. Tumor microsections were taken from these paraffin blocks, stained with Hematoxylin and Eosin (H&E), and examined under the light microscope. After morphologic diagnosis, tumors were classified. High-grade and equivocal cases were subjected to different immunohistochemical panels depending on the light microscopical morphology and the available antibodies. The immunohistochemical technique applied was the automated immune-stainer (Dako-Denmark) on deparaffinized tumor sections using the avidin-biotin–peroxidase complex technique.
Results: The study cases included 125 primary and 39 metastatic carcinomas. All women were over 20 years with a mean age of 42.8 years. Among the primary tumors, high-grade serous carcinoma (33.5%) was the commonest, followed by mucinous carcinoma (22.6%), endometrioid carcinoma (14%), clear cell carcinoma (2.4%) and transitional cell carcinoma (1.2%). The remaining primaries comprised 4 (2.4%) Malignant Mixed Mullerian tumor. The 39 metastatic carcinomas were adenocarcinomas, average age was 36.9 years. Sites of origin included: 18 colonic (including appendix), 7 gastric, 4 endometrial, 5 breast, 3 pancreatobiliary, and 2 ampullary carcinomas.
Conclusion: High grade serous carcinoma topped the list of ovarian carcinoma cases (primary and metastatic). Diagnosis of most carcinoma cases was made using routine H&E light microscopic sections. Difficulties arise in high-grade and metastatic cases, particularly cases exclusive to an ovary. Metastatic cases have primarily emerged from the colon (including appendix), stomach, endometrium, pancreatobiliary, breast, and ampulla. Such cases need contemporary tests, like immunohistochemistry.
Keywords: Primary ovarian carcinoma; Epithelial ovarian cancer; Metastatic ovarian cancer; Immunohistochemistry.

DOI: 10.33091/amj.2024.150051.1732

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INTRODUCTION

Worldwide, ovarian cancer is a common gynecologic malignancy, and there is geographic variance in its prevalence [1]. In Iraq, this cancer is not uncommon, but when compared with benign and non-neoplastic female genital lesions, it forms a relatively uncommon pathology in the north of Iraq [2, 3]. According to the available data, the incidence of ovarian cancer among women is about 4/100,000 in Erbil and 2.6/100,000 in Duhok [4]. The importance of diagnosis lies in the fact that ovarian cancer harbors a high mortality rate because most cases are diagnosed at advanced stages as their symptoms are rather non-specific, particularly at early stages [1, 5].

A definite histologic diagnosis is crucial for ovarian malignancy as it is a heterogenous neoplasm and comprises a spectrum of distinct tumor types with different etiology, clinical course, response to therapy, and outcome [1]. According to the World Health Organization (WHO) 2022, ovarian neoplasms are classified into epithelial carcinomas (the most common) and non-epithelial neoplasms [1]. Epithelial tumors of the ovary are derived from ovarian surface epithelium. They are classified into serous, mucinous, clear cell, endometrioid, Brenner, mixed, and undifferentiated types. As well, these tumors are graded into benign, borderline, and malignant categories [6].

Invariably, several tumors are diagnosed by time-honored light microscopy. However, classic Hematoxylin and Eosin (H&E) microsections appear to be challenging in high-grade tumors. Therefore, an upsurge in utilizing different contemporary methods has been created. These methods include immunohistochemistry (IHC), cytogenetic, and molecular techniques, which help in resolving certain diagnostic dilemmas that are associated with the presumed treatment implications and for prognostic purposes [7, 8].

In resource-limited settings, this study was conducted to investigate ovarian carcinoma types in Duhok-Iraq, relying on morphology and the available immune-profile in our pathology centers.

MATERIALS AND METHODS

This retrospective cross-sectional study described ovarian carcinomas received during 11 years (January 2012 until December 2022). Samples were received in the pathology departments of General Central Laboratories and Vin Private Laboratories in Duhok City, Iraq. The ethical status, consent for participation, and the study protocol were approved by the Institutional Ethical Committee of the College of Medicine, University of Duhok (348W).

After the exclusion of non-epithelial, unclassified, and non-representative tumors, 164 malignant epithelial tumors (carcinomas) were eligible for this study. For each case, paraffin blocks containing tumors were submitted. In the full section, H&E-stained, slides were reviewed under light microscopy. High-grade and vague cases were subjected to immunohistochemistry using panels of primary antibodies selected first according to the patient's age, ovarian involvement (unilateral or bilateral) with surface appearance, and tumor light microscopic features. IHC was performed on deparaffinized tumor sections using the avidin-biotin–peroxidase complex technique. Four micrometer sections were taken from each tumor tissue (with no or little necrosis). Antigen retrieval, staining platforms and detection methods were achieved using the automated immune-stainer technique (Dako-Denmark). According to the morphologic assessment, variable combinations of the following antibodies were used: Pankeratin (AE1/AE3), CA125, WT-1, p53, Pax8, and p16. Additional antibodies were then added to complement tumor typing and exclude mimickers. These antibodies included: ER, GATA3, SATB2, EMA, CK7, CK20, CDX2, Napsin A; CD45, CD20, CD3, CD10, Bcl6, TTF1, Gross Cystic Disease Fluid Protein 15 (GCDFP15), Mammoglobin, CK19.9, Calretinin, Inhibin, CD99, OCT3/4, CD117, CD30, AFP, Glypican3, HCG, MUC5AC, Vimentin, and hepatocyte nuclear factor-1β [7–14].

Interpretation of the microscopic findings was achieved by the pathologist authors. Cases were classified according to the 2022 WHO tumor classification of the female reproductive tract and complemented by immunohistochemical expression patterns as previously described [1].

We calculated the mean age of the patients ± standard deviation as well as the number and percentages of ovarian cancer cases using Microsoft Office Excel 2019.

RESULTS

Out of 164 carcinomas diagnosed in the ovary, 125 (76.2%) cases were primary and 39 (23.8%) were metastatic (Table 1). Among the primary tumors, high grade serous carcinoma [HGSC] (33.5%) was the most common (Figure 1), followed by (22.6%) mucinous carcinoma (Figure 2), (14%) endometrioid carcinoma (Figure 3), (2.4%) clear cell carcinoma (CCC), and (1.2%) transitional cell carcinoma. All women were above 20 years (average: 42.8 years ± 12.05 years). The remaining primary tumors (2.4%) included 4 cases of mixed epithelial and mesenchymal elements (carcinosarcoma) called Malignant Mixed Mullerian tumor (MMMT) with numerous psammoma bodies seen in the peritoneum (Figure 4); all women were above 40 years with a mean age of 63 ± 4.55 years).

The secondaries (n = 39) were metastatic adenocarcinomas. The age range was similar to that of primaries (average: 36.9 years; SD 12.14 years). Sites of origin comprised: 18 colonic (Figure 5) including appendiceal, 7 gastric (Figure 6), 4 endometrial, 5 breast (Figure 7), 3 pancreatobiliary, and 2 ampullary carcinomas.

DISCUSSION

Ovarian cancer is a common gynecologic cancer and the leading cause of cancer death worldwide. Generally, the high mortality rate of ovarian cancer stems from its difficult early diagnosis, leading to the detection of most cases at advanced stages, which complicates its management. Invariably, several tumors involving the female genital tract are diagnosed by the time-honored morphological analysis, using the classic H&E microsections. Over the years, there has been an upsurge in the utilization of various IHC markers, especially in resolving certain diagnostic dilemmas, associated with significant treatment implications in gynecologic onco-pathology [1]. The current study emphasized the efficiency of IHC typing as a tool for a more precise characterization of the origin and differentiation of human neoplasms. Primary ovarian carcinoma cases (79.8%) overnumbered the metastatic ones in this study. Of the primaries, HGSC topped the list (33.5%). Other primary carcinomas included mucinous (22.6%), endometrioid (14%), clear cell (2.4%), and transitional cell car-

Table 1. Types and distribution of ovarian cancer cases with age intervals per years (n = 164). Malignant Mixed Mullerian tumor = MMMT.

Tumor type N (%) Surface epithelial: n = 121 (73.8)	20–30 N (%)	31–40 N (%)	41–50 N (%)	51–60 N (%)	61–70 N (%)	> 70 N (%)
High-grade serous carcinoma 55 (33.5)	2 (1.2)	2 (1.2)	11 (6.7)	31 (18.9)	9 (5.5)	–
Mucinous carcinoma 37 (22.6)	3 (1.8)	7 (4.3)	12 (7.3)	7 (4.3)	5 (3)	3 (1.8)
Endometroid carcinoma 23 (14)	3 (1.8)	2 (1.2)	6 (3.7)	8 (4.9)	4 (2.4)	–
Clear cell carcinoma 4 (2.4)	–	2 (1.2)	2 (1.2)	–	–	–
Transitional cell carcinoma 2 (1.2)	–	–	2 (1.2)	–	–	–
MMMT: n = 4 (2.4)	–	–	–	1 (0.6)	3 (1.8)	–
Secondary: n = 39 (23.8%)	2 (1.2)	15 (9.1)	8 (4.9)	8 (4.9)	6 (3.7)	–

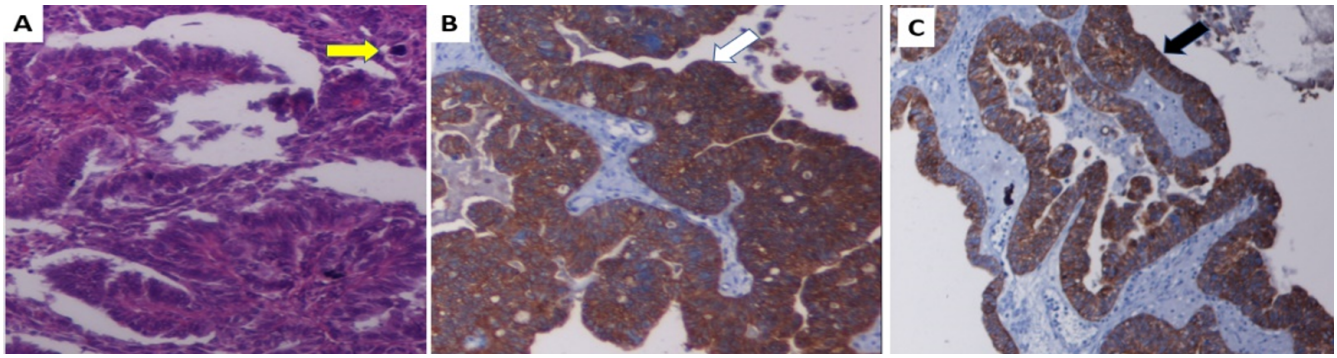

Figure 1. Ovarian serous carcinoma showing psammoma bodies (yellow arrow, panel A) with positive CK7 (white arrow, panel B) and CA125 (black arrow, panel C) (A: H&E stain, X400, B: IHC, X400; C: IHC, X200).

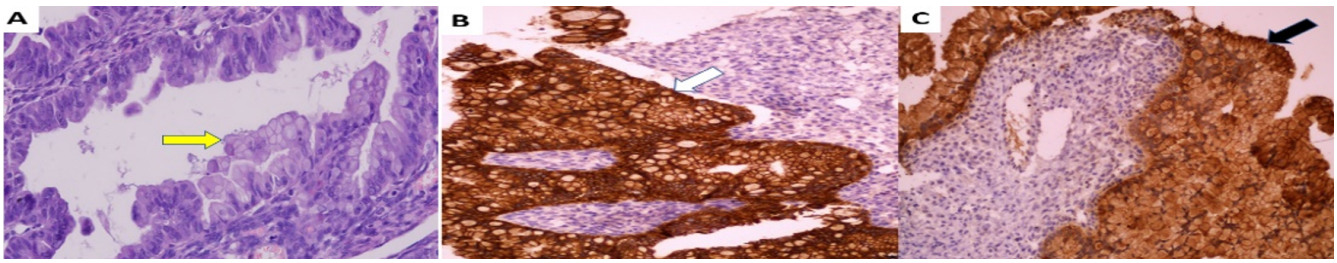

Figure 2. Primary ovarian mucinous carcinoma rich in mucin-filled cells (yellow arrow, panel A) with positive CK7 (white arrow, panel B) and CEA (black arrow, panel C). (A: H&E, X400; B: IHC, X400; C: IHC, X400).

cinomas (1.2%). Studies have shown that primary tumors are more common than metastasized ovarian cancers. The most common type is epithelial, but of different combinations [1, 9, 15, 16].

From the diagnostic point of view, morphology takes priority and forms the most practical option for ovarian cancer classification because of its close association with survival compared with IHC and molecular tests [10]. However, diagnosis is challenging in high-grade tumors, as their categorization appears to be difficult on a morphologic basis. A well-known difficulty among pathologists is differentiating primary ovarian carcinoma from metastatic adenocarcinoma and mesothelioma. Clinically, bilateral ovarian involvement with irregular ovarian surface, vascular invasion, and absence of ovarian borderline tumors favors metastatic tumors [11, 12]. Here, positivity for Pankeratin (AE1/AE3) is of little impact as it is expressed in almost all primary and metastatic carcinomas as well as in mesothelioma, but its negativity helps to exclude carcinomas. The consequent expressions of different antibodies, like CK7, WT1, CA125, ER with negativity for CK20, CDX2, and TTF1, although of little value in differentiating high grade serous neoplasms from mesothelioma and mucinous tumor, but outweigh the primary carcinomas and keep the metastatic ones away [7–13].

A common difficulty among pathologists is differentiating primary ovarian mucinous carcinoma from metastatic colorectal carcinoma. For such a task, immune-expression of some markers, like CEA, CK7 and CK20, may be confusing as they give variable results. However, cases expressing CK20, CEA, CDX2, and β-Catenin while negative for CK7 and PAX8 are considered of colorectal origin, while those positive for CK7, PAX8, WT1, CA125, ER (with or without PR) and negative for CK20, are presumed to be primary ovarian carcinomas [7, 11, 12]. On the other hand, diagnosis of metastatic pancreatobiliary adenocarcinomas can be boosted with CA19.9+ (although not so specific), whereas those of breast origin is

http://doi.org/10.33091/amj.2024.150051.1732

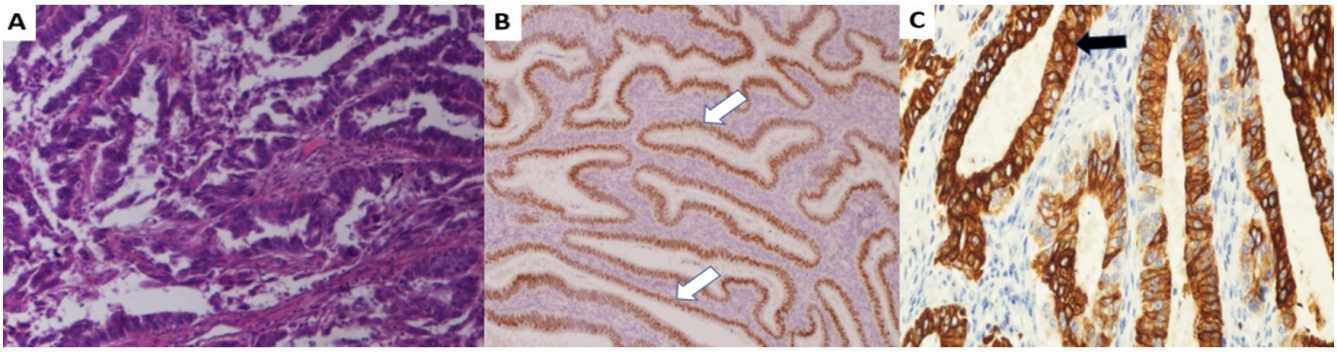

Figure 3. Primary ovarian endometrioid carcinoma with positive ER (white arrows) and CK7 (black arrow). (A: H&E, X200; B: IHC, X200; C: IHC, X400).

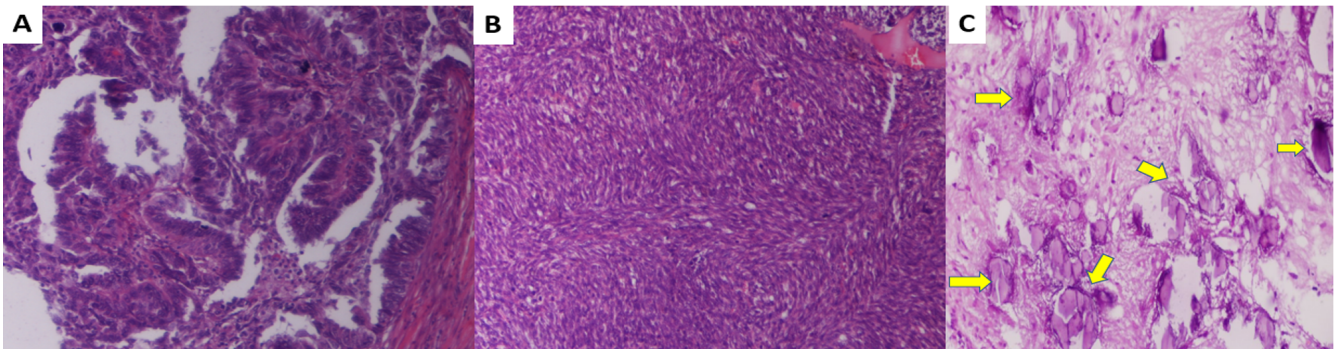

Figure 4. Primary ovarian malignant mixed Mullerian tumor showing the carcinomatous compartment (serous carcinoma) in A and the sarcoma compartment (fibrosarcoma) in B with multiple psammoma bodies seen in the peritoneum, marked by yellow arrows in C (H&E, A: X400, B and C: X250).

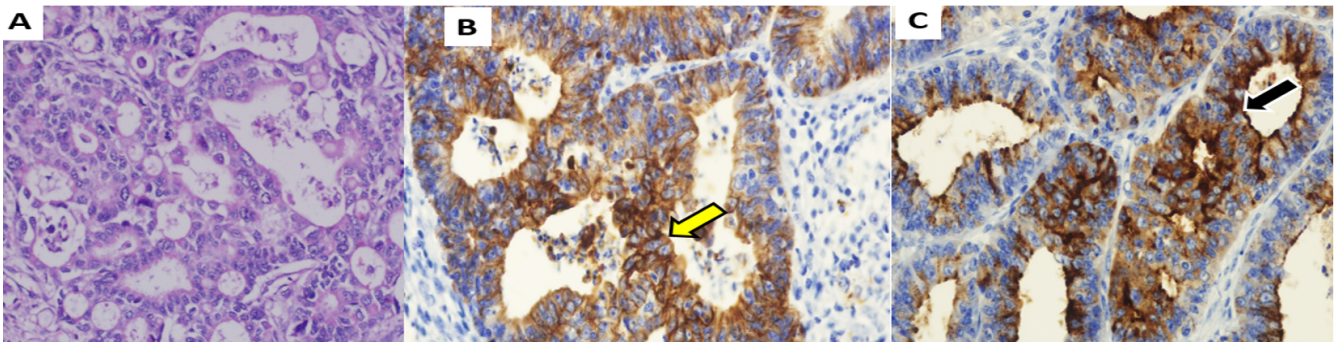

Figure 5. Metastatic colonic adenocarcinoma to the ovary with positive CK7 (yellow arrow) and CK20 (black arrow) [A: H&E, X250; B: IHC, X400; C: IHC, X400].

reinforced by the expression of Gross Cystic Disease Fluid Protein 15 (GCDFP15), mammaglobin, and GATA3. Additionally, GATA3 has to be taken into account in suspected metastatic mammary or urothelial carcinomas as GATA3 has multiple specificities because this marker is not only exclusive to breast and urothelial carcinomas but also seen in renal tumors, germ cell tumors (GCT), mesotheliomas, paragangliomas, and even ovarian carcinoma [13–15]. Here, it is worth mentioning that high-grade tumors may give false expressions to the well-known markers. Tumors with equivocal results are generally put within a group of unknown sources. In this study, no equivocal cases were described.

Another practical point here is differentiating primary carcinomas from mesothelioma, as both tumors display WT1 immuno-expression despite their different therapeutic and prognostic approaches. This is particularly important when comparing effusion samples with extensively infiltrative high-grade malignant tumors, as both exhibit morphological similarities and are characterized by frequent psammoma bodies and strong WT1 expression. It is interesting to note that some authors rank the combination of WT1 and p53 expressions as the first option in pilot prioritization for diagnosing primary ovarian HGSC. Diffuse WT1 and p53 immunostaining reinforce the diagnosis of HGSC. We refer to p53-mutated

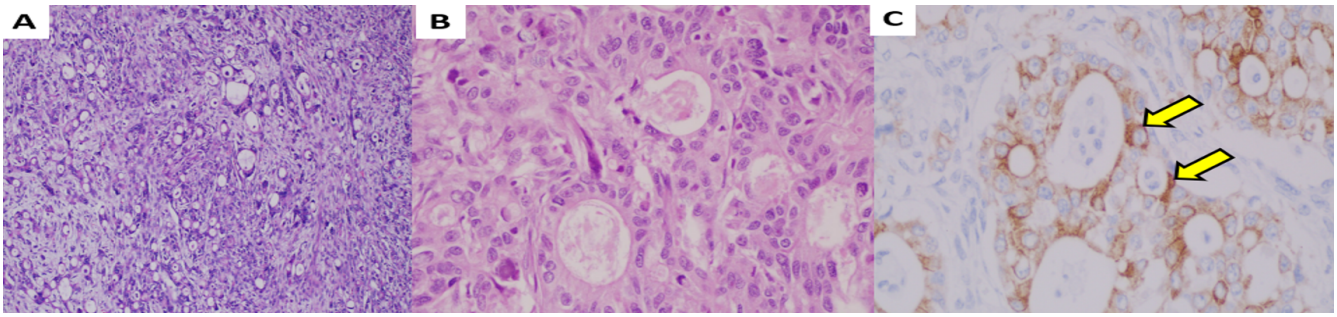

Figure 6. Metastatic gastric carcinoma to the ovary with positive MUC5AC (yellow arrows) [H&E, A: X100; B: X400; C: IHC, X400].

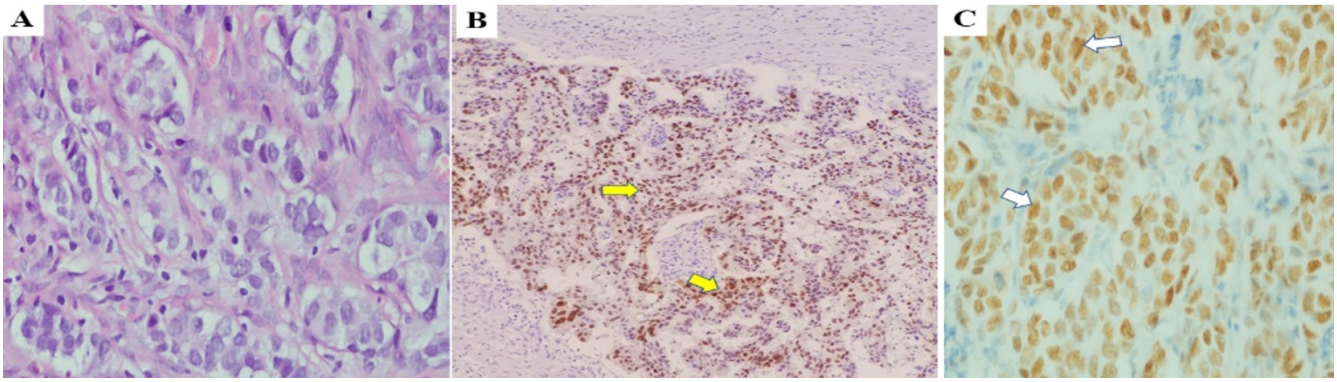

Figure 7. Metastatic breast carcinoma to the ovary with positive GATA3 (yellow arrows) and Estrogen receptors (white arrows) [A: H&E, X400; B: IHC, X100; C: IHC, X400].

type expression as intense diffuse p53 staining in more than 70% of tumor cells or complete absence (null-type), while focal immunoreactivity indicates its "wild-type" expression [7].

In this study, endoscopy and histologic samples from the primary sites detected the origin of 39 carcinoma cases with unknown sources, which proved to be metastatic. IHC tests were needed to show that some tumors had come from the colon (CK20+, CEA+, CDX2+, β-cateninin+, CK7-, PAX8-), the breast (CK7+, GCDFP15+, Mammoglobin+), the pancreas and bile ducts (CK7-, CK20-, CK19.9+), the ampullae (CK20+, CK7-, CDX2-, MUC2+), the uterus (Vimentin+, Pankeratin+, CK7+, ER+, CK20-), and the stomach (CK7+, CK20+, MUC5AC). Here, it is worth mentioning that a decrease in MUC5AC expression is accompanied by the progression of gastric cancer and an increased malignant property of the gastric mucosa [1, 13, 14].

In this study, primary ovarian CCC was relatively low (2.4%). However, it is crucial to be differentiated from other primary tumors like serous and endometrioid neoplasms from metastatic CCC of the kidney, as primary CCC is relatively non-sensitive to chemotherapy [7]. Indeed, here CA125 is of limited value, as most primary ovarian carcinomas express CA125, but WT1 is diffusely positive in the serous neoplasms which variably express to ER and PR, while endometrioid carcinoma is consistently positive for ER and PR, unlike CCCs which are generally negative for ER, PR, and renal cell carcinoma expresses CD10 in addition to Vimentin and PAX8. As well, it is noteworthy that the CCC cases are consistently positive for Napsin A and hepatocyte nuclear factor-1β (HNF-1β) [6].

Uncommonly, MMMTs can be identified in the ovary, similar to their corresponding uterine counterparts, wherein epithelial and mesenchymal markers can be applied to reinforce the carcinomatous and sarcomatous components [16]. In this study, 4 primary ovarian cases were addressed as MMMT; all were high grades with suboptimal cytoreduction. The carcinomatous elements overwhelmed the sarcomatous components in 2 cases, while the opposite was true in the other 2 cases. In 3 cases, the carcinomatous component was HGSC, while the fourth was endometrioid carcinoma. The sarcomatous component comprised a variable admixture of mesenchymal sarcomas and fibrosarcoma with heterologous (cartilaginous and bony) elements. Cases with advanced tumor stages, high grades, and predominant sarcomatous components are likely to behave more ominously with a poor response to chemotherapy. Thus, optimal cytoreduction seems to have some impact on survival [16]. Differentiation from endometrial cases and other primary ovarian epithelial and metastatic carcinomas as well as germ cell tumors may be challenging, not only morphologically but also immunohistochemically, as MMMTs may share some immune-profile with these tumors like SALL4, OCT3/4, glypican3, in addition to other carcinomatous (Keratins, EMA), and sarcomatous (Vimentin) markers. Additionally, positivity for Glypican3 raises the possibility of MMMT [16]. The carcinomatous compartment in the current study cases expressed Pankeratin, EMA, CK7, CA125, and WT1, but Vimentin and all germ cell markers were negative.

http://doi.org/10.33091/amj.2024.150051.1732

The limitations of the study included the absence of clinical data related to the patients, particularly concerning secondary tumors, which might impact the final results. A larger-sized sample may give a more obvious view of the study cases. This is in addition to the limitations of the immunostaining technique applied. In Duhok Pathology Centers, immunostaining on tissue slides is performed according to the leaflet supplied with the Kit. Understanding the variability of the buffer, PH, and the consumed time for each step, clarifies the need for a change in the processing of the immunostaining technique applied.

CONCLUSION

The application of different panels of immunomarkers has a great impact on the diagnosis and categorization of high-grade carcinomas and those settled in the ovary from unknown sources. High-grade serous carcinoma topped the list of ovarian carcinoma cases (primary and metastatic). Most carcinomas are diagnosed by the routine H&E light microscopic sections. Metastatic epithelial tumors reach the ovary from variable (adjacent and distant) sources, like the colon (including appendix), stomach, endometrium, pancreatobiliary, breast, and ampulla. Such cases need contemporary tests, like IHC.

ETHICAL DECLARATIONS

Acknoweldgements

The authors greatly appreciate the technical assistance and the acquisition of study materials provided by pathology technicians in General Central Laboratories and Vin Private Laboratories, Duhok, Iraq.

Ethics Approval and Consent to Participate

The ethical status, consent for participation, and the study protocol was approved by the Institutional Ethical Committee of the College of Medicine, University of Duhok (348W). Informed consent was waived from the patients owing to the retrospective nature of the study.

Consent for Publication

Not applicable (no individual personal data included).

Availability of Data and Material

Data generated during this study are available from the corresponding author upon reasonable request.

Competing Interests

The authors declare that there is no conflict of interest.

Funding

No funding.

Authors' Contributions

Pity IS: Idea of research project, overseeing, coordination of case analyses, interpretation of pathological data, and writing manuscript; Kareem GM: Collection and preparation, immunohistochemistry and interpretation of some pathological cases; Musa DH: Surgical specimens (40%), collection of clinical data, and organization of the research; Suleiman AY: Writing part of introduction; Amedi SMS: Surgical specimens (30%); Rasoul HM: Surgical specimens (30%); Abdalwahab YH: Photographing of pathological and immunohistochemical microscopic pictures; Hayder DB: Helped in organization of cases and statistics; All authors: Final writing, reading, and editing issues. The authors read and approved the final version of the manuscript.

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