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ORIGINAL RESEARCH ARTICLE

CLINICOMORPHOLOGICAL STUDY OF OVARIAN LESIONS

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ABSTRACT

This prospective study was conducted at College of Medical Sciences-Teaching Hospital (CMS-TH) during December 2008 to May 2010. One hundred and fifty cases were analyzed. Age of the patients, parity, presenting symptoms, types of surgery, clinical diagnosis and complications were retrieved from case sheets. Surface epithelial tumors were more frequently observed above 30 years of age, (62.07%) cases. During 1st to 3rd decades of life, 62.8% germ cell tumors were seen; and sex cord tumors were seen in age group 41-60 years only. For all age groups, benign tumors were common than malignant tumors. There were 10.7% unmarried patients, 5.33% nulliparous, 35.55% of parity 1 to 2; 4% pregnant and 20 % postmenopausal women. There were 86.67% cases neoplastic, and 13.33% non-neoplastic ovarian lesions; 93.85% benign, 5.38% malignant and 0.77% borderline tumors. Surface epithelial tumors were the most common tumors (53.84%) followed by germ cell tumors (43.85%), constituted 46.7% and 38% among all ovarian lesions. The commonest benign tumors were serous cystadenomas and mature cystic teratomas, constituted 40% cases each. Dysgerminoma was the common malignant neoplasm (2.31%), all were seen in adolescents. Most of the other malignant neoplasms were observed above 40 years of age. Seventy percent (70%) of non-neoplastic lesions were hemorrhagic corpus luteum cysts. The commonest presenting symptom was pain in the lower abdomen (82%) followed by abdominal mass/ or distension (48.7%). Constitutional symptoms were observed in malignant cases only. Grossly, majority of the ovarian lesions were of size ranging 5 to 15 cm; and 89.93% cystic lesions. There were 48.7% lesions in the right ovary and 45.3% in the left ovary; 6% bilateral ovarian lesions, all of which were observed in benign and non-neoplastic lesions. The common complication observed was torsion (6.7%) followed by rupture of the cysts (6%).

Key Words: *Benign, Malignant, Neoplastic, Non-neoplastic, Ovarian lesions.*

INTRODUCTION

Ovarian lesions have diverse histopathologies reflecting the different cell origin of the lesions. Neoplasm of the ovary is the most fascinating tumor of women in terms of its histogenesis, clinical behavior and malignant potentiality. It has been mentioned that neoplasms of the ovary were the most frequent tumors representing about 42.4% of all gynecological malignancies.[1]

Ovarian cancer is the most frequent cause of death from gynecological cancer and the fourth most frequent cause of death from cancer in women in Europe and the United States.[2] The age adjusted incidence rate of invasive ovarian cancer in the United States observed by Surveillance, Epidemiology and End Results Program (SEER), a source of cancer statistics review during 1975 to 2006 was 14.82/ 100,000 women of all races, and the mortality rate was 9.15/ 100,000 women.[3] The age-adjusted rate of ovarian cancers in India was 4.9 per 100,000 women during 1993-1997.[4]

The incidence of ovarian neoplasm was 16.7% among total gynecological admissions in the study of Nepal.[5] Ovarian neoplasms have become increasingly important not only because of the large variety of neoplastic entities but more because they have gradually increased the mortality rate in females.[6]

OBJECTIVE OF THE STUDY

Clinical and histopathological correlation of ovarian lesions, and to identify symptoms that could lead to early diagnosis of ovarian carcinoma.

MATERIAL AND METHODS

A hospital based, descriptive and prospective study was conducted in College of Medical Sciences-Teaching Hospital (CMS-TH), Bharatpur, Chitwan during a period of December 2008 to May 2010. 150 cases were randomly selected meeting the inclusion criteria. The inclusion criteria were that all symptomatic patients with ovarian lesions those who required surgical intervention, and asymptomatic patients with ovarian lesions which were detected during ultrasonogram or other clinical surgical procedures. The exclusion criteria was that two ovarian lesions of different type, in same ovary or both ovaries.

Sampling

All cases of ovarian lesions with above mentioned criteria, found in cystectomy, ovariotomy, oophorectomy, salpingo-oophorectomy, total abdominal hysterectomy with bilateral or unilateral salpingo-oophorectomy specimens registered for histopathological examination were selected for the study. 150 cases of surgically resected ovarian lesions were analyzed; grossly and histologically for the present study. The cases included were those, who were operated at CMS-TH or were operated somewhere else but the specimens were received and processed in Department of Pathology of CMS-TH. Age and parity of the patients, presenting symptoms, types of surgery, complications and clinical diagnosis were retrieved from case sheets, and histopathological analysis of ovarian lesions was performed. The data were collected on a proforma. The symptoms were grouped into: abdominal symptoms, gastrointestinal symptoms, urinary symptoms, constitutional symptoms such as loss of weight and appetite. The other presenting symptoms were also noted.

All the tissues received were fixed in 10% buffered formalin overnight. The gross examination of the specimens was carried out and adequate representative sections were taken according to the guidelines by Rosai,[7] with special emphasis on solid foci, areas adjacent to the ovarian surface and from papillary projections. The sections were then routinely processed under standardized conditions for paraffin embedding, then cut into 5 micron or even thinner when necessary and stained with Hematoxylin and Eosin (H & E) stain using standard procedure. Special stains were used whenever necessary such as- Mayer's Mucicarmine, Periodic Acid Schiff (PAS, and Masson's Trichrome. World Health Organization (WHO) Classification of ovarian tumors[8] was used for classifying the tumors.

Statistical tools

Data were entered in excel master sheet with coding of the variables. The Statistical Package for the Social Sciences (SPSS) software version 16.0 was used for evaluation. Frequencies, percentages, mean and median were calculated. Findings were presented as tables, bar diagrams and pie-charts. Results were reviewed.

RESULTS

Out of 150 cases included, 130 cases (86.67%) were ovarian neoplasms, and 20 cases (13.33%) non-neoplastic ovarian lesions (Figure 1)

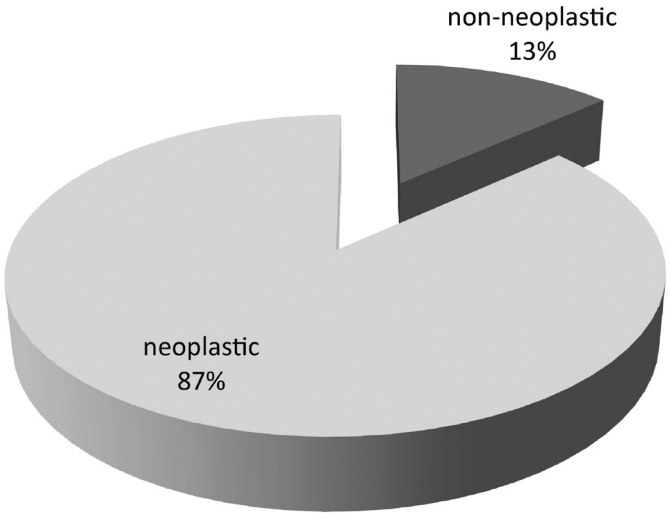


Figure 1: Incidence of neoplastic and non-neoplastic ovarian lesions

Age of the patients

The age range of patients was 12 to 70 years with a median age of 38 years and mean age of 37.39 years for all ovarian lesions. Most ovarian lesions; 115(76.67%) cases were seen between 21 to 50 years of age. (Table 1)

Table 1: Age of patients with ovarian lesions

Age of patients(Years)	Number of cases(n)	Percentage
≤ 20	14	9.3
21-30	37	24.7
31-40	41	27.3
41-50	37	24.7
51-60	15	10.0
>60	6	4.0
Total	150	100

Benign, malignant and borderline tumors in different age groups

For all age groups, benign neoplasms were more common than malignant neoplasms. One hundred and twenty-two (122) cases of benign neoplasms were observed in all age groups, which comprised 93.85% among ovarian neoplasms and 81.33% among all ovarian lesions.

Different histological types of ovarian lesions in various age groups

Surface epithelial tumors were more frequently observed above 30 years of age, (54/87) 62.07% cases. Germ cell tumors were more common (27/43) 62.8% in ≤20 to 30 years of age, and sex cord tumors were present in only age group between 41 to 60 years. Eighty-five percent (17/20) cases of non-neoplastic lesions were observed in age group of 21-40 years (Table 2)

Out of 7 malignant cases; 3 cases of dysgerminoma were observed in adolescent age group. The other malignant neoplasms were found above 40 years of age except 1 case of endodermal sinus tumor which was seen in patient of 23 years of age (Figure 2).

Table 2: Different histological types of ovarian lesions in various age groups

Age of patients (years)	Ovarian neoplasms			Non-neoplastic lesions
	Surface epithelial tumors	Germ cell tumors	Sex cord-stromal tumors	
≤ 20	4	8	0	2
21-30	12	19	0	6
31-40	17	13	0	11
41-50	24	12	1	0
51-60	9	3	2	1
>60	4	2	0	0
Total (n=150)	70(46.7%)	57(38%)	3(2%)	20(13.3%)

Clinical presentation of patients

There were 12(8%) patients without symptoms which were detected on routine pelvic examination, during ultrasonogram or other clinical surgical evaluation procedures such as appendectomy, cesarean section or total abdominal hysterectomy. Most of the patients presented with abdominal symptoms, such as 123(82%) patients presented with pain in the lower abdomen. The pain was acute, occurring for less than a week before surgery in 19(12.7%) patients due to either torsion or rupture of the ovarian cysts. Seventy-three (48.7%) patients presented with abdominal mass / or distension.

Constitutional symptoms such as, loss of appetite was observed in 3(2%) patients, and weight loss in 2(1.33%) patients which were present in malignant cases only. Many patients had more than one symptom. The other presenting symptoms were menstrual abnormalities, abnormal vaginal bleeding, urinary symptoms and GIT symptoms. (Figure 2)

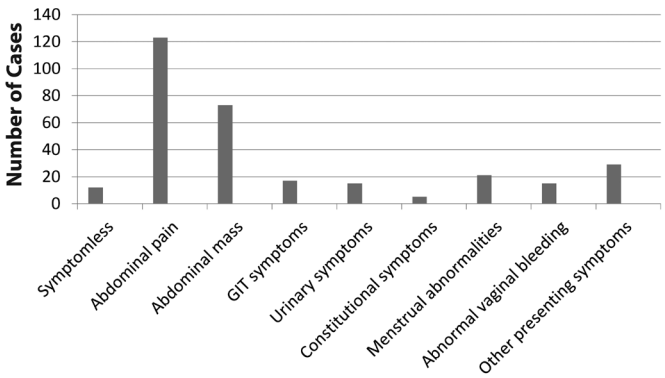


Figure 2: Clinical presentation of patients

Marital status and parity

There were 16(10.7%) unmarried patients in the present study, and 134(89.3%) patients married. Among married patients, 8 (5.33%) were nulliparous, 53(35.33%) patients of parity one or two, 49(32.67%) patients of parity three to four, and 24 (16%) patients of parity five or more. 30(20%) patients were postmenopausal women, and 6(4%) patients pregnant at the time of diagnosis.

Size

Grossly, the ovarian lesions were of variable size ranging from 3 to 28 cm with mean maximal diameter of 7.96 cm. The majority of the ovarian lesions (both unilateral and bilateral) in this study were of size ranging from 5 to 15 cm.

Consistency

The consistency of ovarian lesions varied from cystic, partially solid and partially cystic to solid. Out of 150 ovarian lesions, most of them were cystic, 134 (89.30%).

Content

Sixty-one (40.70%) ovarian lesions show serous fluid on cut section. In 52 (34.70%) lesions, content was greasy; composed of sebaceous material and contains tufts of hair, some cases with teeth. In 4(2.7%) cases of cyst, papillary projections protruding into the cavity were observed; and 11(7.33%) lesions were solid, gray-white and firm.

Laterality

Among 150 cases, 73(48.7%) lesions were found in the right ovary and 68(45.3%) in left ovary. There were 9(6%) bilateral ovarian lesions, out of which 8(88.89%) cases were neoplastic (benign cystic teratomas) which comprised 6.15% of all ovarian neoplasms and 1(11.11%) case of non-neoplastic ovarian lesion.

Complications

The common complication of the ovarian lesions in the present study was torsion in 10(6.67%) cases. The patients presented with acute abdominal pain in 7(70%) cases, and torsion was observed at the time of operation. Rupture of the cysts was observed in 9(6%) cases during ultrasonogram of patients who presented with acute abdomen or was observed at the time of operation. Ascites was seen in 3(2%) cases. (Figure 3)

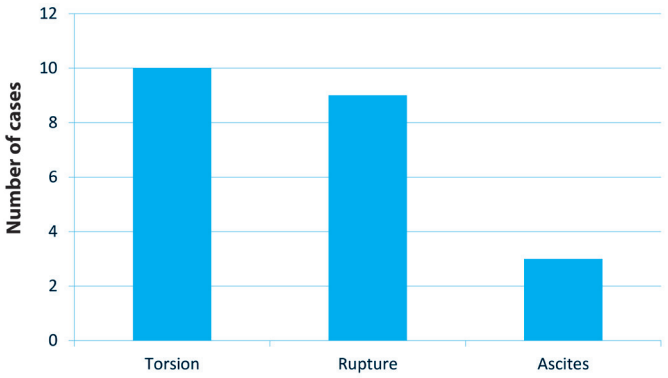


Figure 3: Complications in ovarian lesions

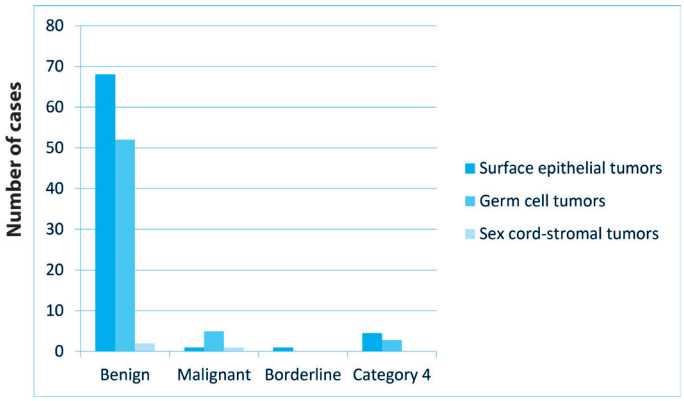


Figure 4: Prevalence of different histological types of ovarian neoplasms

Histopathological analysis

1. Non-neoplastic ovarian lesions

Out of 20 non-neoplastic ovarian lesions, majority of cases 14(70%) were hemorrhagic luteal cysts, 3(15%) cases endometriosis, 2(10%) cases follicular cysts and 1(5%) case tubo-ovarian abscess.

2. Ovarian neoplasms

Among 130 cases of ovarian neoplasms; benign was the most common neoplasm, 122 cases (93.85%); 7 malignant cases which constituted 5.38% and only 1(0.77%) case of borderline tumor (Figure 4).

These neoplasms were further subdivided into three major categories according to WHO[8] classification: surface epithelial-stromal tumors, germ cell tumors and sex cord-stromal tumors (Table 3).

There were 70(53.85%) cases of surface epithelial tumors which constituted 68(52.3%) cases of benign category. The main bulk of benign surface epithelial tumors were serous cystadenomas 52(40%). Only 1(0.77%) case of papillary serous cystadenocarcinoma and 1(0.77%) case of borderline mucinous tumor was observed in the present study. Mucicarmine stain was positive in the borderline mucinous tumor.

Fifty-seven (43.84%) cases of germ cell tumors were observed, among which the majority 52(40%) cases were benign mature cystic teratomas; which represented the main bulk of all ovarian neoplasms in the present study along with serous cystadenomas, both found in equal frequency. The malignant germ cell tumors observed were 3(2.31%) cases of dysgerminoma, and 2(1.54%) cases of endodermal sinus tumor, in which PAS stain showed PAS positive hyaline globules. Thus, malignant germ cell tumors were the most common malignant ovarian neoplasm in the present study, comprising 71.43% of all malignant ovarian neoplasms.

The present study had observed 3 cases of sex cord-stromal tumors, comprising 2.31% of ovarian neoplasms. These were 2(1.54%) cases of benign fibroma which was confirmed by Masson's Trichrome stain, and 1(0.77%) malignant adult granulosa cell tumor.

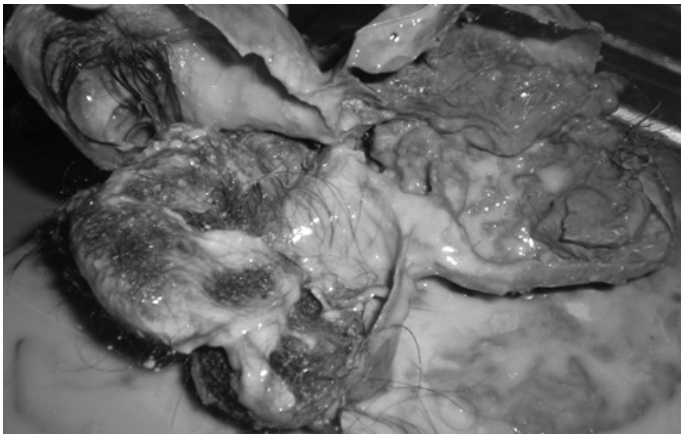
Table 3: Different histological types of ovarian neoplasms

Histological type	Benign		Malignant		Borderline	
Surface epithelial tumors n=70 (53.84%)	**No.** **68**	**%** **52.3**	**No.** **1**	**%** **0.77**	**No.** **1**	**%** **0.77**
Serous cystadenoma	52	40	0	0	0	0
Seromucinous cyst	2	1.54	0	0	0	0
Serous cystadenofibroma	1	0.77	0	0	0	0
Mucinous cystadenoma	9	6.92	0	0	0	0
Brenner tumor	4	3.07	0	0	0	0
Papillary Serous cystadenocarcinoma	0	0	1	0.77	0	0
Borderline mucinous tumor	0	0	0	0	1	0.77
Germ cell tumors n=57 (43.85%)	**52**	**40**	**5**	**3.85**	**0**	**0**
Mature cystic teratoma	52	40	0	0	0	0
dysgerminoma	0	0	3	2.31	0	0
Endodermal sinus tumor	0	0	2	1.54	0	0
Sex cord-stromal tumors n=3 (2.31%)	**2**	**1.54**	**1**	**0.77**	**0**	**0**
Fibroma	2	1.54	0	0	0	0

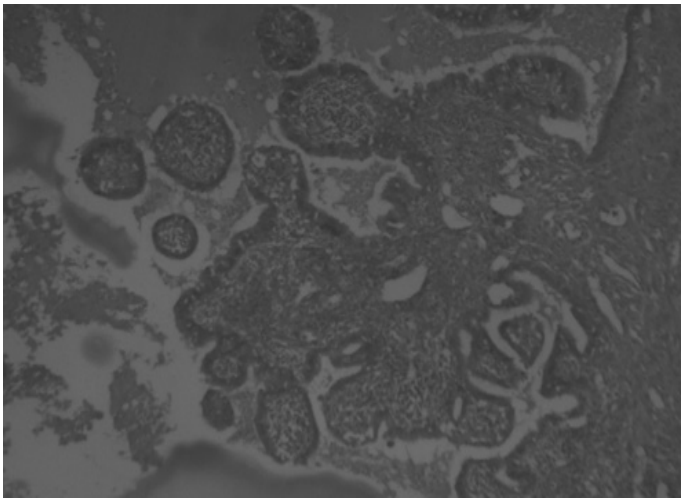
Adult granulosa cell tumor	0	0	1	0.77	0	0
TOTAL (n=130)	**122**	**93.84**	**7**	**5.39**	**1**	**0.77**

Gross picture of Borderline Mucinous Tumor of ovary showing mucinous content in the cystic cavities.

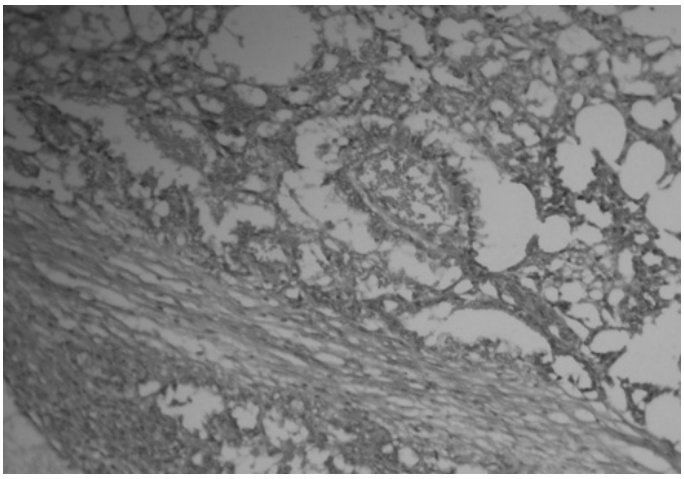
Gross picture of Mature Cystic Teratoma of ovary containing greasy material and tufts of hair



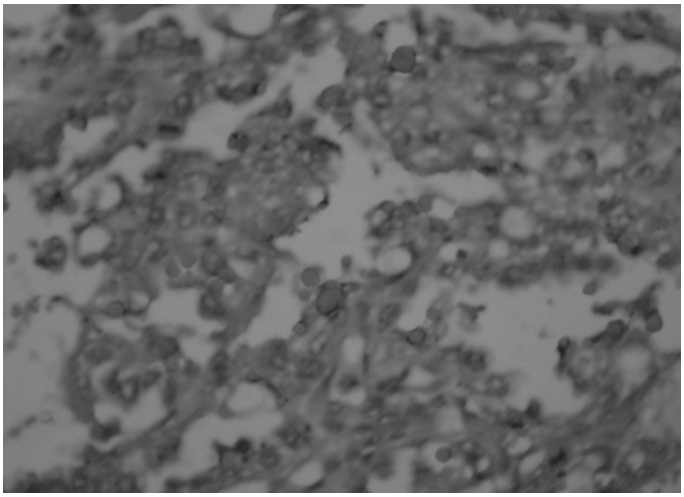
Microscopic photo of Papillary Serous Cystadenocarcinoma. H&E stain.



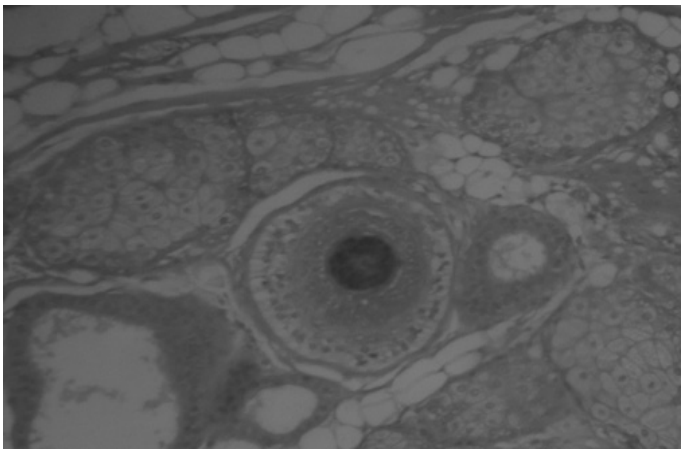
Microscopic photo of Endodermal Sinus Tumor showing Schiller-Duval Body. H&E stain.



Microscopic photo of Endodermal Sinus Tumor showing PAS positive hyaline globules. PAS stain.



Microscopic photo of Mature Cystic Teratoma showing mature hair follicle, sebaceous glands, sweat glands and adipose tissue. H&E stain.



DISCUSSION

The present study had observed 86.87% cases of ovarian neoplasms and 13.33% non-neoplastic ovarian lesions. There was similar observation by Bhattacharya et al.[9] and Okugawa et al.,[10] 92.59% of ovarian neoplasms while 7.41% of non-neoplastic ovarian lesions; and 72% of ovarian neoplasms and

28% of non-neoplastic lesions respectively. In contrast, Gupta et al.[11] observed 41.20% of ovarian neoplasms and 58.80% of non-neoplastic ovarian lesions.

Hemorrhagic corpus luteum cysts (70%) were the commonest non-neoplastic ovarian lesions observed in the present study. This observation contrasts with the studies of Yasmin et al.[12] in which follicular cysts and corpus luteum cysts were common, constituting 27.8% and Gupta et al.[11] observed 80.2% of follicular and corpus luteum cysts. Okugawa et al.[10] observed endometriosis (71.52%) as the most common non-neoplastic ovarian lesions.

In the present study, majority of tumors were benign (93.85%) among ovarian neoplasms. The frequency of benign tumors was similar to other studies conducted in Nepal,[5,13] and Pakistan.[12] However, The frequency of benign tumors was relatively lower in the studies conducted in Japan 10 (51%), Pakistan 14 (68.7%), and India 11 (72.9%).

In studies of Yasmin et al.,[12] Khan et al.,[15] and Kayastha;[5] the majority of the benign category of ovarian neoplasm were serous cystadenomas followed by mature cystic teratomas. Jha et al.[13] had observed mature cystic teratomas as the most common benign ovarian neoplasm. However, the present study had observed equal frequency of mature cystic teratomas and serous cystadenomas in benign category, 40% of each among all ovarian neoplasms.

The frequency of malignant ovarian neoplasms was 5.39% in the present study. This result is lower than what had been reported by Shaikh et al.,[14] (31%); and Gupta et al.,[11] (22.9%).

There is a great variation in the frequency of different categories of malignant neoplasms in various studies with the present study. Jha et al.[13] had observed serous cystadenocarcinoma (46.2%) as the most common malignant ovarian neoplasm. Yasmin et al.,[12] observed granulosa cell tumors and endometrioid carcinoma (28.5%) each as the most common malignant ovarian neoplasm. In contrast, the most common malignant ovarian neoplasm in the present study was dysgerminoma 3 cases (42.86%) followed by yolk sac tumor 2 cases (28.57%) among all malignant neoplasms.

Only 0.77% of borderline tumor was observed in the present study, which was lower than the studies by Gupta et al.,[11] and Okugawa et al.,[10] both studies had observed 4% of borderline ovarian tumors.

As far as the overall histological variety is concerned, the present study had observed most common tumors originating from the surface epithelium (53.84%). The frequency of surface epithelial tumors is approximately similar to the studies conducted in Nepal[13] and India.[9,11] However, the frequency of surface epithelial tumors was above 70% in the study of Kayastha,[5] Sarwar,[16] and Yasmin.[12]

Following surface epithelial tumors, the second common ovarian neoplasm was germ cell tumor which constituted 43.85% in the present study. This result is comparable with the study carried out at Tribhuvan University Teaching Hospital, Nepal[13] in which germ cell tumors were the second common tumors (42.2%); and is also comparable with different series of studies.[10,11,12,14,15]

The other observation in the present study was, only 3 (2.31%) cases of sex cord-stromal tumors among all ovarian neoplasms. The frequency of sex cord-stromal tumors was relatively lower than those observed by Jha et al.,[13] (3.1%); Shaikh et al.,[14] (5.03%); Khan et al.,[15] (5.15%) and Pai et al.,[17] (6%).

The most common sex cord-stromal tumors observed in the present study was, 2 (1.54%) cases of fibroma among all ovarian neoplasms. Similar to this study, fibroma was found as the commonest sex cord-stromal tumors in the studies by Khan et al.,[15] (2.58%), and Pai et al.,[17] (2.51%). There was different observation by Shaikh et al.,[14] Piura et al.,[18] and Schneider et al.,[19] in which they observed granulosa cell tumor as the most common sex cord-stromal tumors. Jha et al.,[13] reported thecoma as the commonest sex cord-stromal tumors, which constituted 1.86% of all ovarian neoplasms.

Similar to the present study, Jha et al.,[13] also had observed most of the ovarian tumors occur in women of reproductive age group, and peak incidence of ovarian tumors was observed between the age group of 21-40 years. The present study observed peak incidence of ovarian lesions (76.67%) in the age group of 21-50 years.

Okugawa et al.,[10] observed that incidence of non-neoplastic lesions peaked in 21-45 years age group, and were rarely encountered in the postmenopausal age group. This result is comparable to the present study in which, non-neoplastic lesions were observed mostly (85%) in the age group of 21-40 years, and only 1 (5%) case was observed in the postmenopausal women.

Bhattacharya et al.,[9] and Khan et al.,[15] observed benign neoplasms mostly in between 21-40 years of life. The present study had observed benign neoplasms mostly in age group between 21-50 years.

Malignant ovarian neoplasms were common in elderly, after 40 years of age, except malignant germ cell tumors which were found in young age group in present study. This observation is comparable with the studies in Nepal[5,13] and other neighboring countries-India[9] and Pakistan.[15]

Similar to Jha et al.,[13] in which malignant surface epithelial tumor was observed above the age of 40 years; the present study had observed a case of malignant surface epithelial tumor in a patient above the age of 40 years.

The present study constituted 66.67% (8/12) of cases of germ cell tumors in the age group below 20 years. Amatya et al.,[20] also had similar observation; 73.7% of germ cell tumors of all ovarian neoplasms in childhood and adolescents. Mature cystic teratomas accounted for 41.67% (5/12) of all ovarian neoplasms in 1st two decades of life and 52.46% (32/61) were seen in between 21 to 40 years of age group in the present study. Jha et al.,[13] observed 63.6% of mature cystic teratomas in 1st two

decades of life. Amatya et al.,[20] observed 76.27% of mature cystic teratomas in childhood and adolescents; in both studies the frequency of mature cystic teratomas in those age group were higher than in the present study. The 100% of malignant germ cell tumors were observed in 1st two decades of life in the present study. The similar observation was made by Jha et al.,[34] In contrast to the present study and Jha et al.,[13] there were only 23.23% malignant germ cell tumors in childhood and adolescents in study of Amatya et al.[20]

The present study had observed sex cord-stromal tumors in age group of 41-60 years. In the Pakistani study,[15] these tumors were common in the age range from 30-70 years and majority was seen in 4th decade of life.

The present study had shown variability in the presentation of ovarian lesions. Many had more than one symptom at the time of presentation. There was similar observation by Kayastha.[5] The commonest symptom was pain in lower abdomen (82%), followed by abdominal mass or distension (48.7%) in the present study. This observation is comparable with the studies conducted in Nepal,[5] and in Pakistan,[12] in which abdominal pain (84%) followed by abdominal mass or distension (10.5%); and abdominal pain (70.59%) followed by abdominal mass/ or distension (14.71%) respectively were the common symptoms. However, in the studies conducted by Choudry et al.,[21] and Bhattacharya et al.,[9] the clinical presentation in majority of patients was abdominal mass and/or distension that led to diagnosis. In the present study, constitutional symptoms like loss of weight (3%) and loss of appetite (2%) were present only in malignant neoplasms. This observation was similar to the study conducted by Wasim et al.[22]

In the present study, 7(70%) cases of torsion presented with acute abdomen. There was similar observation by Cass et al.,[23] (73.5%) and Ong et al.,[24] (65.4%)

Jha et al.,[13] observed that 50% of metastatic tumors were bilateral and 18.3% of bilateral ovarian neoplasms were metastatic. The bilateral tumors were common in malignant neoplasms (55%). The data from India[9] and Nepal[13] had observed that bilaterality was common in malignant ovarian neoplasms, 37.6% and 66.67% respectively indicating bilaterality is significant risk factor for malignancy. However, the present study observed bilaterality in 8 cases (6.15%) of all ovarian neoplasms, and all of them were in benign category. This variation in the result in the present study may be due to less number of malignant cases observed in the present study. Among bilateral ovarian tumors, 88.89% were observed in mature cystic teratomas. This observation is similar to Jha et al.,[13] in which 66.67% bilaterality was observed in mature cystic teratomas.

Harlap et al.,[25] had observed; women having 0 or 1 sibling were at higher risk of ovarian neoplasm than women with 2 or more siblings. The data from Nepal by Kayastha[5] had observed 20% ovarian tumors occurred in nulliparous women and 38.9% in low parity (1-2) women. In the present study, 35.33% (53/150) women were of low parity (1-2), and only 5.33% (8/150) women were nulliparous in contrast to study of Kayastha.[5] There were 16 (10.67%) cases of unmarried women in the present study which was approximately similar to study by Kayastha[5] in which unmarried women constituted 11.6%.

The commonest complication in the present study was torsion of ovarian lesions observed in 10(6.67%) cases followed by rupture of cysts in 9(6%) cases. Kayastha[5] had also observed torsion as the most common complication (12.6%). Cass et al.,[23] had observed high frequency (33.33%) of torsion in ovarian lesions. In contrast to present study, Bhattacharya et al.,[9] had observed adhesions to the surrounding structures as the most common complication (10.98%); torsion being the second common complication. Torsion was present in 9(90%) cases of neoplasms and 1(10%) case of non-neoplastic ovarian lesions in the present study. Cass et al.,[23] had observed 53% of torsion in ovarian neoplasms and 47% in non-neoplastic lesions. Torsion was more common on the left side (60%) than on the right side (40%) in the present study. In contrast, Cass et al.,[23] observed 60% of torsion was on right side, and 40% on left side. Cass et al.,[23] observed frequent (70.6%) torsion involving mature cystic teratomas. This observation is less frequent in the present study (20%), and studies by Ong et al.,[24] (27.1%) and Ayhan et al.,[26] (4.9%).

CONCLUSION

The most common presenting symptom of ovarian lesions is pain in lower abdomen followed by abdominal distention/ mass though vague, and non-specific presentation is observed. However, constitutional symptoms are present in malignant neoplasms only. In the present study, bilaterality is observed in benign tumors only in contrast to other studies. This variation in the result may be due to small sample size, and less number of malignant cases observed in the present study.

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