

Diagnostic Utility of Peritoneal and Ovarian Fluid LDH and Cholesterol in Cystic Ovarian Tumours

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Abstract

Background: Ovarian cancer is the third most common cancer in Indian women. Biochemical analysis of ovarian and peritoneal fluid, especially lactate dehydrogenase (LDH) and cholesterol level is shown to be elevated in cases of malignancy. Peritoneal fluid LDH levels were also found to be of prognostic significance. To assess the diagnostic utility of LDH and cholesterol levels of ovarian cyst fluid and peritoneal fluid in cystic ovarian neoplasms by correlating with histopathological examination.

Material and Methods: Prospective cohort study carried out in a tertiary care hospital. Ovarian cyst fluid and/or peritoneal fluid in resected ovarian neoplasms were processed on automated biochemistry analyzers.

Results: Seventy-nine ovarian neoplasms with their corresponding ovarian cyst fluid and/or peritoneal fluid were studied. The accuracy rate for diagnosis of malignant tumors by ovarian fluid LDH and cholesterol levels was found to be 100% and 90.14% respectively, while that of peritoneal fluid LDH and cholesterol levels was 88.88% and 80.55% respectively.

Conclusion: Biochemical analysis (LDH and cholesterol) of peritoneal fluid can be used in pre-operative and post-operative management of ovarian malignancy. Ovarian fluid LDH was the most sensitive and specific parameter followed by peritoneal fluid LDH for differentiating ovarian malignancy from benign ovarian tumors.

Keywords: ovarian neoplasms; ovarian fluid; peritoneal fluid; biochemical; LDH; cholesterol

Introduction

Ovarian tumors are the most common neoplasms among women [1] and occur across all age groups from the intrauterine to the postmenopausal. Ovarian cancer is the third most common cancer in Indian women [2] amounting to the greatest number of deaths amongst the female genital tract cancers.[3, 4] Ovarian malignancies are usually asymptomatic in early stages and have no established screening test and are therefore detected at later stages causing increased mortality. [5, 6] The overall 5-year relative survival rate for ovarian cancers is 50% but in cases detected early, with localized disease it is 93%.[7] Thus, prompt investigations are required to detect malignancy at the early stage.

Ovarian tumors metastasize via peritoneal fluid and hence peritoneal fluid cytology is included in staging of ovarian tumor. Some research has shown use of biochemical analysis of peritoneal fluid in differentiating benign from malignant tumors.[8, 9] Biochemical analysis of peritoneal fluid, especially lactate dehydrogenase (LDH) and Cholesterol level is shown to be elevated in cases of malignancy. Few studies have shown peritoneal LDH and Cholesterol levels to be significantly raised in ovarian malignancies than those in control women. [8, 9] Some research has shown that diagnostic accuracy of peritoneal fluid LDH was 86% but when combined with cholesterol values it became 96% for differentiating

benign and malignant tumors.[9] Peritoneal fluid LDH levels were also found to be of prognostic significance. Thus, biochemical analysis of peritoneal fluid may assist in predicting the behaviour of ovarian tumors and thereby the prognosis.

In ovarian neoplasms, ovarian cyst fluids represent the micro-environment of the tumor cells. The biochemical profile of the ovarian fluid can thus be used to assess whether the tumor is benign or malignant. It has been shown that malignant ovarian tissues selectively take up lipidic emulsions composed of phospholipids and cholesterol esters and can concentrate them upto 8 times more than the normal or benign ovarian tissues.[10, 11, 12]

This study was undertaken to analyse the LDH and Cholesterol levels in cystic fluid and peritoneal fluid of cystic ovarian masses to assess its diagnostic utility. As most ovarian neoplasms are cystic in nature, ovarian fluid can be aspirated and analyzed for LDH and cholesterol. Aspiration of ovarian fluid in vivo risks upstaging of probable malignancy. Hence this procedure was done immediately after resection of the ovarian cystic mass and sent for biochemical analysis.

This study aimed to study the range of LDH and Cholesterol levels in ovarian cyst fluid and peritoneal fluid in cases of cystic ovarian neoplasms and correlate it with histopathological diagnosis.

Material and Method

The study was performed in the Department of Pathology of a Tertiary care hospital for a period of 22 months after obtaining approval from the Ethics committee, Ref no. D020180199. Partially or completely cystic, unruptured ovarian tumors sent in formalin to the Department of Pathology with/without their corresponding peritoneal fluids were included in the study. Tissue sections from the ovarian tumour were taken as per conventional procedures of surgical grossing and histotechniques. The ovarian fluid was drawn from the intact ovarian cystic tumours. Centrifugation of the fluid-both ovarian and peritoneal was performed. If the fluids were non hemorrhagic after centrifugation, they were accepted for biochemical analysis. Biochemical analysis of LDH and Cholesterol levels was performed using fully automated biochemistry analyzers which were calibrated for fluid analysis. The coefficient of variation for LDH and Cholesterol parameter was 2.8% and 3.1% respectively. The results obtained were further subjected to appropriate statistical analysis using Microsoft Excel. Excel for z-test was used because of small sample size, ease of use and user friendliness.

Results

79 cases of ovarian neoplasms were studied in the prospective study of 22 months. On histopathological examination, 58 cases (73%) were benign, 6 cases (8%) were borderline and 15 cases (19%) were malignant ovarian tumors. Ovarian fluid was available in 71 cases, peritoneal fluid was available in 36 cases (Table 1).

Table 1: Ovarian and peritoneal fluid studied in the 3 categories of ovarian tumors.

Ovarian tumors (N)	Ovarian fluid	Peritoneal fluid
Benign (58)	51	21
Borderline (6)	6	5
Malignant (15)	14	10
Total (79)	71	36

N – number of tumors studied

Biochemical analysis for estimating LDH and Cholesterol levels was done in all fluids (Table 2). The results were then categorized as per the ovarian histopathology diagnosis.

LDH and cholesterol levels in peritoneal fluid and ovarian fluids showed similar variations in benign, borderline and malignant ovarian tumors. Levels of both LDH and cholesterol were higher in both fluids for borderline and malignant tumors as compared to benign tumors. However, borderline category showed significant overlap with both the benign and malignant ranges of LDH and cholesterol. Hence, the benign category was separated and the borderline category was clubbed together with the malignant group for further analysis. Average peritoneal and ovarian LDH and cholesterol levels were calculated in the benign and borderline + malignant ovarian tumors.

An attempt to assess the demarcation level i.e. cut-off level of fluid LDH and Cholesterol to differentiate from Benign and Borderline, Malignant entities was undertaken. For this, cut-off values were calculated by adding two standard deviations to the mean fluid value of the Benign group (Table 4).

In this study, an attempt was made to check if tumors could be classified as benign based on the Fluid LDH and Cholesterol levels. Cut-off value = Mean + 2 (SD)

To judge the practicality of the derived cut-off, all cases were classified into Benign and Borderline+Malignant groups based on their fluid LDH and Cholesterol levels and compared with the histologic diagnosis. The biochemistry results which were

Table 2: The range and mean of fluid LDH and cholesterol levels obtained categorized as per ovarian histopathology diagnosis.

Parameter	Category	Range	Mean	N
Fluid LDH (IU/L)				
Ovarian	Benign	177–654	537	51
	Borderline	784–4176	1650	6
	Malignant	1021–4288	2564	14
Peritoneal	Benign	476–1588	685	21
	Borderline	757–4010	1846	5
	Malignant	1314–4536	3421	10
Fluid Cholesterol (mg/dl)				
Ovarian	Benign	7–52.51	16	51
	Borderline	20.38–98	37.5	6
	Malignant	29.17–268	158	14
Peritoneal	Benign	12.68–119.45	49	21
	Borderline	44.09–148	77	5
	Malignant	35.53–265	150	10

Table 3: Comparison of mean LDH and cholesterol values in ovarian fluid and peritoneal fluid.

Fluid	Parameter	Benign	Borderline + Malignant
Ovarian fluid	Mean LDH (IU/L)	537 (N=51) ± 2SD (63.7)	2564 (N=20)
	Mean Cholesterol (mg/dl)	16 (N=51) ± 2SD (7.42)	158 (N=20)
Peritoneal fluid	Mean LDH (IU/L)	685 (N=21) ± 2SD (304.12)	3421 (N=15)
	Mean Cholesterol (mg/dl)	49 (N=21) ± 2SD (31.41)	150 (N=15)

Table 4: Cut-off values for LDH and cholesterol in ovarian and peritoneal fluids.

Parameters	Mean + 2SD	Cut-off value
Ovarian fluid LDH	537 + 2SD (63.7)	664 IU/L
Ovarian fluid Cholesterol	16 + 2SD (7.42)	30 mg/dl
Peritoneal fluid LDH	685 + 2SD (304.12)	1293 IU/L
Peritoneal fluid Cholesterol	49 + 2SD (31.41)	112 mg/dl

not concurrent with the histopathology diagnosis were tabulated in Table 5 and Table 6 and were indicated as False Positive (FP) and False Negative (FN).

Table 5: Comparing the false results obtained on ovarian fluid biochemistry analysis.

Diagnosis based on Ovarian fluid biochemical analysis cut-off	LDH	Cholesterol	Histologic diagnosis
Benign	51 FN-nil	54 FN-5	51
Borderline + Malignant	20 FP-nil	17 FP-2	20
Total	71	71	71

On comparison, as there was no false results obtained on Ovarian LDH, it appeared to be of relatively greater significance in diagnosis (Table 5).

Table 6: Comparing the false results obtained on peritoneal fluid biochemistry analysis.

Diagnosis based on peritoneal fluid biochemical analysis cut-off	LDH	Cholesterol	Histologic diagnosis
Benign	21 FN-2	26 FN-6	21
Borderline + Malignant	15 FP-2	10 FP-1	15
Total	36	36	36

There was 1 false positive and 6 false negative results in peritoneal fluid Cholesterol analysis.

Efficacy parameters i.e. sensitivity, specificity, accuracy, negative predictive value (NPV) and positive predictive value (PPV)

were then calculated for these biochemical tests using appropriate statistical formulae (Table 7).

Table 7: Comparison of sensitivity, specificity, accuracy, PPV and NPV of ovarian fluid LDH and cholesterol with that of peritoneal fluid.

Parameter	Formulae	Ovarian fluid	Peritoneal fluid
LDH			
Sensitivity	TP / TP + FN	100%	86.66%
Specificity	TN / TN + FP	100%	90.47%
Accuracy	TP + TN / TP + TN + FP + FN	100%	88.88%
NPV	TN / FN + TN	100%	90.47%
PPV	TP / TP + FP	100%	86.66%
Cholesterol			
Sensitivity	TP / TP + FN	75%	60%
Specificity	TN / TN + FP	96.07%	95.23%
Accuracy	TP + TN / TP + TN + FP + FN	90.14%	80.55%
NPV	TN / FN + TN	90.74%	76.92%
PPV	TP / TP + FP	88.23%	90%

The sensitivity, specificity and accuracy of both investigations were comparable to each other although all parameters of LDH were marginally higher in both fluids as compared to Cholesterol.

In order to evaluate whether detecting malignancy by using peritoneal fluid biochemical analysis is equivalent to ovarian fluid biochemical analysis, chi square test was performed. As $p > 0.05$ (0.093), there was no significant statistical difference in LDH measurement in peritoneal fluid versus ovarian fluid for detecting malignancy. Similarly, there was no significant statistical difference in Cholesterol measurement in peritoneal fluid versus ovarian fluid for detecting malignancy [$p > 0.05$ (0.344)].

In order to determine whether the LDH and cholesterol levels in ovarian fluid as well as peritoneal fluid were significantly more in Borderline+Malignant category as compared to Benign ovarian category, tests of significance were applied. Unpaired students t-test was used for LDH and Cholesterol in peritoneal fluid and large sample z-test was used for LDH and Cholesterol levels in ovarian fluid and p value was calculated. As p value < 0.001 was obtained for ovarian as well as peritoneal fluid LDH and cholesterol, these tests showed that Borderline+malignant tumors have statistically significant higher LDH and cholesterol levels than benign tumors.

Discussion

In ovarian neoplasms, ovarian cyst fluids represent the micro-environment of the tumor cells. The biochemical profile of the ovarian fluid can thus be used to assess whether the tumor is benign or malignant. It has been shown that malignant ovarian tissues selectively take up lipidic emulsions composed of phospholipids and cholesterol esters and can concentrate them upto 8 times more than the normal or benign ovarian tissues.[10, 11, 12] Also, apart from being a source of energy for the malignant cells, many derivatives of the fatty acid pathway are signaling molecules or second messengers involved in initiation, development and dissemination of ovarian malignancy.[13] Lactate dehydrogenase (LDH) is a major enzyme of the glycolytic pathway present in all body tissues and elevated levels are associated with proliferative activity, speed of tumor growth and the tumor cell burden.[11, 15] Malignant tissues have a high glycolytic activity and previous studies have shown that ovarian cyst fluids show higher levels of LDH in cancerous tumors as compared to benign tumors.[15, 16]

Using quantitative mass spectrometry, iTRAQ MS, one study identified 837 proteins in cyst fluid from benign, carcinoma stage I, and carcinoma stage III. Fluid from ovarian cysts connected directly to the primary tumor harbor many possible new tumor-specific biomarkers. It identified 87 differentially expressed proteins and validated two candidates to verify the iTRAQ method. Several of these proteins were of interest and required validation in a larger setting.[27]

The present study was conducted to assess the utility of ovarian and peritoneal fluid biochemical analysis in ovarian neoplasms using routine biochemistry analysers which are conventionally used in daily laboratory practice.

79 cases were studied over a period of 22 months. Ovarian fluid was available in 71 cases and peritoneal fluid was available in 36 cases. The sample size of peritoneal fluid was less as the clinicians did not consider it essential to send peritoneal fluid sample for analysis in the cases in which they were confident of its benign nature.

Ovarian tumours of borderline category had biochemistry readings overlapping with the benign and malignant tumours. The biochemistry cut-off for benign tumours was well established. Due to this, it was proposed that we can separate out the benign tumours from others based on biochemical readings. A study on large sample size is necessary to corroborate this observation. A study on larger sample size may also render a cut-off values for exclusive malignant tumours. The present study may be considered as a Pilot-study in this regard.

The ovarian mean LDH values (537 IU/L in benign group and 2564 IU/L in borderline+malignant group) in the present study were found to be similar to those in the study by Parker MF et al. (555.9 IU/L in benign group and 2321 IU/L in borderline+ malignant group).[16] Ovarian fluid LDH was found to be statistically significantly elevated in the malignant group as compared to the benign tumors in the present study ($p<0.001$) which was similar to findings by Parker MF et al ($p=0.001$).

The present study showed the mean ovarian fluid cholesterol to be 16 mg/dl in benign group and 158 mg/dl in the malignant group. The LDH and cholesterol levels in ovarian fluid were found to have a high sensitivity, specificity, accuracy, NPV and PPV for diagnosing malignancy. However, as none of the previous studies have assessed these parameters, it was not possible to compare them.

Since ovarian tumors spread by trans-coelomic spread, the peritoneal fluid is in close proximity to cancer cells and forms their micro-environment.[17] Various studies have analyzed peritoneal fluid biochemically and found that the levels of cholesterol and LDH are higher in malignant peritoneal fluids,[18, 19, 20] especially in cases of ovarian cancers.[9, 13, 17, 21, 23, 25, 26, 24]

The mean LDH value (685 IU/L) of peritoneal fluid in the Benign group category in the present study was comparable to those from Gulec UK et al and Boran N et al. The mean LDH (3421 IU/L) in the malignant group in the present study was also much higher as compared to the benign group, similar to the findings in other studies.[9, 15, 21, 22] However, one study by Alexandrakis MG et al [22] did not show much difference in peritoneal fluid LDH levels between the benign and malignant groups (Table 8).

Table 8: Comparison of mean peritoneal fluid LDH in malignant and non-malignant groups.

Study	Year	Mean Peritoneal LDH (IU/L) Non-malignant	Mean Peritoneal LDH (IU/L) Malignant
Present study (N=36)		685	3421
Gulec UK et al (N=99)	2012	508.6	945.1
Boran N et al (N=50)	2000	766	851
Schneider D et al (N=30)	1997	215	978
Alexandrakis MG et al (N=36)	2000	458.9	461.3
McGowan L et al (N=33)	1973	128	630.7

The sensitivity, specificity, NPV, PPV and accuracy of peritoneal fluid LDH for detecting malignancy in the present study (86.66%, 90.47%, 88.88%) were found to be similar to the study by Schneider D et al. (87%, 93%, 88%).[23] The present study ($p<0.001$) also showed that the LDH levels in peritoneal fluid were significantly higher in malignant ovarian tumors as compared to benign tumors which was similar to previous studies by Gulec UK et al, Schneider D et al and Halperin R et al.[21, 23, 25]

The mean cholesterol values of peritoneal fluid of the present study were found to be in accordance to those in the study by McGowan L et al.[9] The mean values of cholesterol in peritoneal fluids were higher in the malignant group compared to the benign group in the present study as well as other studies.[9, 18, 19, 20, 22] (Table 9)

Table 9: Comparison of mean peritoneal fluid cholesterol in malignant and non-malignant groups.

Study	Year	Mean cholesterol (mg/dl) Benign	Mean cholesterol (mg/dl) Malignant
Present study (N=36)		49	150
McGowan L et al (N=33)	1973	64	158.4
Rana S V et al (N=50)	2005	40.56	117.19
Ekpe EEL et al (N=75)	2015	33.20	103.10
Mayank et al (N=74)	2018	6.7	100.85
Alexandrakis MG et al (N=36)	2000	52.8	89.4

The efficacy of peritoneal fluid cholesterol in detecting malignancy was assessed and found to be similar to other studies.[18, 19, 20, 25]

The present study showed that the cholesterol levels in peritoneal fluid were significantly higher in malignant ovarian tumors ($p<0.001$) as compared to benign ovarian tumors which was similar to other studies by Ekpe EEL et al, Rana SV et al, Halperin R et al and McGowan L et al.[9, 18, 19, 25]

There was minimal variation in the mean cholesterol values in peritoneal fluid and efficacy parameters of other studies which can be attributed to the fact that all other systemic malignancies were also included in those studies whereas the present study was restricted to ovarian malignancy (Table 10).

Table 10: Comparison of sensitivity, specificity, NPV, PPV and accuracy of peritoneal fluid cholesterol levels.

Parameter	Present study	Mayank et al (N=74)	Zhu H et al (N=743)	Rana SV et al (N=50)	Ekpe EEL et al (N=75)
Year		2018	2015	2005	2015
Sensitivity	60%	65%	82%	88%	94.6%
Specificity	95.23%	100%	90%	100%	94.7%
NPV	76.92%	–	–	89%	94.7%
PPV	90%	–	–	100%	94.6%
Accuracy	80.55%	–	–	94%	94.7%

The sensitivity of ovarian fluid LDH (100%) was higher than ovarian fluid cholesterol (75%). Similarly, the sensitivity of peritoneal fluid LDH (86.66%) was higher than peritoneal fluid cholesterol (60%).

Conclusion

Although the sample size of the present study was small, Ovarian fluid LDH was the most sensitive and specific parameter followed by peritoneal fluid LDH for differentiating ovarian Borderline+Malignant from Benign ovarian tumors. This study analysis warrants a more detailed research on a larger sample size to confirm the findings. Biochemical analysis (LDH and cholesterol) of Peritoneal fluid can be used in in Pre-operative, operative (frozen) or post-operative management of ovarian tumours. Extensive analysis on larger number of malignant ovarian tumours is necessary to determine and standardize a mean cut-off value to be of diagnostic significance.

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