ORIGINAL ARTICLE

Role of Tumor Markers (Ca-125 And Ca19-9) in the Screening of Endometriosis- Case Control Study

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Abstract:
Background and Objectives: Endometriosis is a considerable burden to patients in terms of healthcare costs and quality of life. Identification of reliable biomarkers to screen for it would be a great boon. *Aim:* To evaluate the role of autoimmune markers (CA-125 and CA19-9) in the screening of endometriosis.*Material and Methods:* This case-control study was conducted at a tertiary care centre over a span of two years. Peripheral blood samples were collected and a quantitative estimation of CA-125 and CA19-9 was performed on them, in a blinded format. The parameters used to analyze the data were: age, education, body mass index, occupation, marital status, parity, family history of endometriosis, nature of menstrual cycle, obstetric history, ultrasonographic findings, laparoscopic findings, and levels of CA-125 and CA19-9. Data was analyzed using SPSS 17.0 software. For significance testing in cross-tabulation tables – chi square test, z-test, and proportion 'p' tests were used. *Results:* Statistically significant correlation was observed between cases of endometriosis and history of infertility, family history of endometriosis, symptomatology (chronic pelvic pain, dysmenorrhoea, dyspareunia), USG findings, hystero/laparoscopic findings, and most importantly, raised levels of CA-125 and CA19-9.*Conclusion:* Serum levels of CA-125 and CA19-9 separately are not very sensitive tests for detection of endometriosis, but are quite specific . Estimation holds promise for future screening and diagnosis of endometriosis, while being minimally invasive.

Keywords: Endometriosis, Biomarkers, CA-125, CA19-9

Introduction:

Presence of normal endometrial glands and stroma in locations other than the uterine cavity is called as endometriosis[1]. It can affect any woman from menarche to menopause, regardless of race, ethnicity, or parity[2].

Women with chronic pelvic pain will commonly have endometriosis . Its prevalence has been estimated to be 1-2% of reproductive age women, and it is more common in women with infertility (15-25%)[3]. Endometriosis often coexists with fibroid, adenomyoma, and autoimmune disorders[4]. This leads to a considerable burden in terms of healthcare costs and quality of life[5]. The disease is detected by laparoscopic visualization of endometriotic lesions, which requires surgical intervention with associated costs and risks[6]. The use of biomarkers such as CA-125 is not sensitive enough to detect the disease in early stages[7]. Therefore, the need to develop biomarkers for non-invasive detection of minimal to mild endometriosis has become a priority in endometriosis research[8].The most accepted theory to explain the aetiopathogenesis of endometriosis is Sampson's Retrograde Menstruation (RM) theory[5] – However, only 10-15% women with RM develop the disease. Mihalyi et al. (2010) reported six plasma biomarkers - Interleukin-6 (IL-6), IL-8, Tumor Necrosis Factors α (TNF-α), high sensitivity C-Reactive Protein (hs-CRP), CA-125, and CA19-9, for non-invasive diagnosis of endometriosis[9]; however, they are not very sensitive. Serum CA-125 has been associated with the presence of many gynaecological disorders, including endometriosis[1]. The most important clinical use of this serum marker has been in monitoring the course of ovarian cancer in response to treatment. Serum CA19-9 levels are elevated in patients with benign ovarian tumors[4] and in those with ovarian chocolate cysts. Serum CA19-9 levels in women with endometriosis fell significantly after treatment for endometriosis, when compared with the basal levels before treatment is observed by Bulun et al in their study[10].Based on these observations, we had conducted an evaluation of pre-operative sera from a series of women undergoing diagnostic laparoscopy for benign gynaecological conditions to determine the serum levels of CA-125 and CA19-9. The specific aim of the study was to verify the clinical usefulness of these markers i.e., CA-125 and CA19-9, in the diagnosis of endometriosis, either by themselves, or combined. Early symptom

recognition, particularly in adolescence, will lead to timely intervention, accuracy of diagnosis, effective treatment, and adequate referral as needed, which will ultimately assist in reducing the associated morbidity of endometriosis.

Material and Methods:

This case-control study was conducted at a tertiary care centre over a span of two years. Prior clearance was obtained from the ethics committee. A total of 107 patients were screened based on their clinical symptoms (chronic pelvic pain, dysmenorrhoea, dyspareunia, and infertility) and they underwent a diagnostic laparoscopy after obtaining written informed consent.Two independent cohorts (Cases and Controls) were recruited from this screening, based on their laparoscopic findings and after segregating them as per the inclusion and exclusion criteria. Patients with documented evidence of endometriosis on laparoscopy were classified as cases, while those with no evidence of endometriosis on a diagnostic laparoscopy were classified as controls were included in the study.Patients with genital tuberculosis, pelvic inflammatory disease, fibroids, polycystic ovary syndrome, ovarian cancer, and endometrial cancer were excluded from the study. Laparoscopic findings diagnostic of endometriosis were taken as: Powder burn patches on utero-sacral ligaments, in the pouch of Douglas, and other pelvic and abdominal viscera, genital tract adhesion (peri-tubal and adnexal), ovarian chocolate cysts (unilateral and/or bilateral) Peripheral blood samples were collected and centrifuged to obtain sera. The quantitative detection of CA-125 and CA19-9 was performed on Elecsys analyzer using a commercially available chemi-luminescent immunometric assay by Roche Diagnostics GmbH (Germany) . The concentrations of CA-125 and CA19-9 were expressed as IU/ml. Both the surgeon and the serologist were unaware of the classification of cases and controls, making this a blinded evaluation. The obtained data was then tabulated into two groups of cases and controls. The parameters used to analyze the data were: age, education, body mass index, occupation, marital status, parity, family history of endometriosis, nature of menstrual cycle, obstetric history, ultrasonographic findings, laparoscopic findings, and levels of CA-125 and CA19-9. Data was analysed using the Statistical Package (SPSS) 17.0 software. For significance testing in cross-tabulation tables, chi square test, z-test and proportion 'p' tests were used.

Results:

One hundred seven patients were screened from the gynaecology out-patient department, and 60 were selected after undergoing a diagnostic laparoscopy. They were further divided into two groups: Study group with 30 cases of endometriosis, and Control group with 30 patients having no evidence of endometriosis. CA-125 & CA19-9 levels from the sera of these patients were checked and compared with each other, as well as with different parameters.The age distribution between both the age groups lies between 25 to 30 years with a mean age of 27 years in the cases and 26 years in the control group. The 'p' value is >0.05 and hence is not statistically significant.

Table No. 1: Distribution by Age

Parameter	Case (n=30)		Control (n=30)		t value	P value
	Mean	SD	Mean	SD		
Age (years)	27.10	5.63	26.07	4.11	0.81	>0.05

Table No. 2: Distribution by History of Infertility

H/O Infertility	Case	Control	Total
Primary	16	2	18
Secondary	5	0	5
None	9	28	37
Total	30	30	60

The study group had 21 cases of infertility, which is 70 percent of the cases. The control group had 2 cases of infertility, which is 6.6 percent of the control population. The chi-square value worked out to 25.65, and 'p' value worked out to <0.0001, suggesting that this finding is statistically highly significant. There were 6 cases in the study population with a family history of endometriosis, which is 20 percent of the study population. No one in the

Table No. 3: Distribution by Family History

Family History	Case	Control	Total
Mother	2	0	2
Sister	4	0	4
None	24	30	54
Total	30	30	60

control population had a family history of endometriosis. The chi-square value worked out to 6.67 and the 'p' value worked out to <0.05 which suggests that this finding is statistically significant. The incidence of chronic pelvic

Table No. 4: Distribution by Symptomatology

Symptomatology	Case (n=30)	Control (n=30)	Z value	P value
Chronic pelvic pain	29 (96.67)	4 (13.33)	11.87	<0.0001
Dysmenorrhoea	20 (66.67)	7 (23.33)	3.75	<0.001
Dyspareunia	11 (36.67)	0	4.17	<0.0001

pain in the study population was 96.6 percent while in the control population was 13.33 percent. The 'p' value worked out to <0.0001 which is statistically highly significant. The incidence of dysmenorrhoea in the study population was 66.6 percent while in the control population was 23.33 percent. The 'p' value worked out to <0.001 which is statistically significant. The incidence of dyspareunia in the study population was 11 percent while in the control population was zero. The 'p' value worked out to <0.0001 which is statistically highly significant.The above graph

Figure 1 : Distribution by USG findings

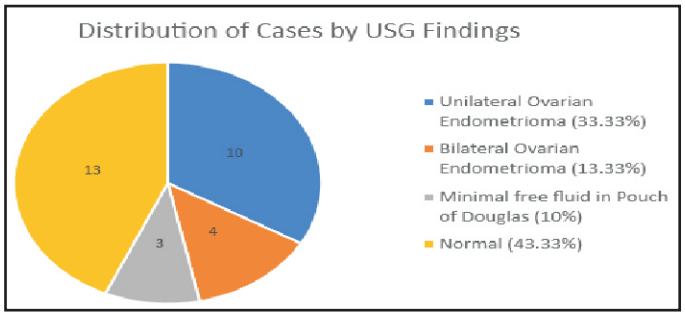
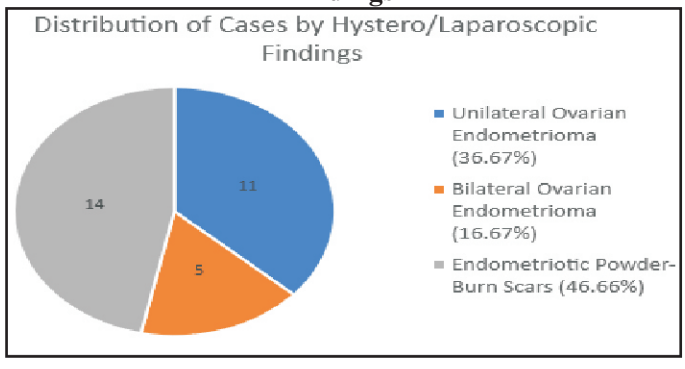


Figure 2 : Distribution by Hystero/Laparoscopic findings



shows the positive findings on an Ultrasonography (USG), as found in the cases of endometriosis. The entire control group has normal USG with no positive findings.
as found in the cases of endometriosis. The entire control group showed no positive findings on the Hystero/ Laparoscopic evaluation.

Table No. 5:Comparison of CA-125 levels in case and control groups

Parameter	Case (n=30)		Control (n=30)		t Value	P Value
	Mean	SD	Mean	SD		
CA-125 level	46.7	28.7	17.4	10.7	5.23	<0.0001

Table No. 6: CA-125 level wise distribution of cases in case and control group

CA-125 level	Case	Control	Total
>35 (Abnormal)	13	4	17
=35 (Normal)	17	26	43
Total	30	30	60

The study population showed 13 out of 30 cases to have an abnormal CA-125 value which worked out to 43.33 percent sensitivity and 86.67 percent specificity. The 'p' value for this test was calculated to be <0.01 which makes this finding statistically highly significant. The positive predictive value of this test was calculated to be 76.47 percent and the negative predictive value is 60.46 percent.

Table No. 7: Comparison of CA19-9 levels in case and control group

Parameter	Case (n=30)		Control (n=30)		t Value	P Value
	Mean	SD	Mean	SD		
CA-19.9	48.8	26.2	14.7	15	6.17	<0.0001

The study population showed 14 out of 30 cases to have an abnormal CA19-9 value which worked out to 46.67 percent sensitivity and 80% specificity. The 'p' value was calculated to be <0.05 which makes this finding statistically significant. The positive predictive value of this test was calculated to be 70 percent and the negative.
predictive value is 60 percent

Table No. 9:Combination of CA-125 & CA19-9 wise distribution of cases in case and control group

CA-125 & CA19-9	Case	Control	Total
Abnormal	17	6	23
Normal	13	24	37
Total	30	30	60

Chi-square = 8.53, P<0.005, Sensitivity = 56.67%, Specificity = 80% PPV = 73.91%, NPV = 64.86%

Discussion:

The present study was conducted to study and determine the role of biomarkers, specifically CA-125 and CA19-9 in the diagnosis of Endometriosis. The correlation of endometriosis with age, infertility, family history, symptomatology, ultrasonographic and laparoscopic findings was also studied. A total of 30 study and control subjects were enrolled in each group based on the inclusion and exclusion criteria. The present study found no significant correlation of incidence of endometriosis with age. A mean age of 26 years was found in the study group, while a mean age of 27 years was found in the control population. Gajbhiyeet. al. reported a mean age of 20-39 years in case as well as control groups.The present study demonstrated a significantly higher incidence of infertility (70%) in cases of endometriosis. Carlo Bullettiet. al.[12] demonstrated that women with endometriosis suffered from infertility 6 to 8 times more as compared to women without endometriosis. They also demonstrated that women with endometriosis had a lower fecundity (0.02 – 0.1 per month) as compared to women without endometriosis (0.15 – 0.20 per month). This correlates with our findings of a high incidence of infertility in women suffering from endometriosis.The present study demonstrated a higher incidence of endometriosis (20%) in women with a family history of endometriosis and more so in first degree relatives. This correlates well with the findings of KazemNouriet. al.[13] who found the incidence of endometriosis higher (43.4%) in women with a family history of endometriosis. Out of these women 6 percent were first degree relatives. Although the present study shows a higher incidence of endometriosis in women with a higher body mass index, the finding is not statistically significant as the 'p' value is >0.5. The present study describes the incidence of symptoms in women suffering from endometriosis as follows:Chronic pelvic pain 96.67%, Dyspareunia 36.67%, Dysmenorrhoea 66.67% The Endometriosis Association[18] in USA in 2009, described the incidence of symptoms in women suffering from endometriosis as follows:Dysmenorrhea 95%, Menorrhagia 65%, Dyspareunia 64%, No symptoms 1% KazemNouriet. al.[13] in their study in 2010, demonstrated the incidence of symptoms in women suffering from endometriosis as follows: Dysmenorrhea 82.5%, Severe dysmenorrhea (visual analogue scale = 7) 25% Chronic pelvic pain 20%, Severe chronic pelvic pain (visual analogue scale = 7) 17.5% Dyspareunia 16.3%, Severe dyspareunia (visual analogue scale= 7) 30% This also supports our findings of an increased incidence of pelvic complaints in women suffering from endometriosis. The present study also compares the diagnostic accuracy of ultrasonography versus hystero-laparoscopy in the diagnosis of endometriosis. Women who showed demonstrable signs of endometriosis on hystero-laparoscopy were subjected to an ultrasonography prior to the procedure, 56.67 percent women showed an abnormal ultrasonography, most of which were cases of moderate to severe endometriosis. 43.33 percent women showed a normal ultrasonography, a majority of which were eventually found to have mild to moderate endometriosis. Mettleret. al.[19] correlated the laparoscopic findings with the histology findings in 164 women who underwent a targeted biopsy from suspicious lesions. The histological reports of the biopsies confirmed the presence of endometriosis in 138 patients (84.1%) but in 26 patients (15.9%) no evidence of endometriosis was observed. This correlates with our study which also states that laparoscopy is a better diagnostic modality as compared to ultrasonography. The present study examines the correlation of endometriosis with serum levels of CA-125 and CA19-9 independently and together as a combined test. We have demonstrated a mean CA-125 level of 46.67 IU/ml in the study group and 17.4 IU/ml in the control population. The sensitivity of CA-125 was 43.33 percent and the specificity was 86.67 percent. The positive predictive value of CA-125 was 76.47 percent and the negative predictive value was 60.46 percent which is statistically highly significant. We have demonstrated a mean CA19-9 level of 48.8 IU/ml in the study group and 14.7 IU/ml in the control population. The sensitivity of CA19-9 was 46.67 percent and the specificity was 80 percent. The positive predictive value of CA19-9 was 70 percent and the negative predictive value was 60 percent which is statistically highly significant. As a combination, the tests give a sensitivity of 56.67 percent and a specificity of 80 percent. The positive predictive value of the combination of these tests was 73.91 percent and the negative predictive value is 64.86 percent. Cheng

Y.M. et. al.[18] evaluated the utility of CA-125 in the diagnosis of endometriosis. The mean serum CA-125 levels (IU/ml) for American Society of Reproductive Medicine stages I, II, III, and IV endometriosis were 18, 40.3, 77.1, and 182.4, respectively. They found that CA-125 levels were significantly increased with advanced stages of endometriosis and that they were significantly higher in patients with more extensive adhesions to the peritoneum, omentum, pelvic organs, or with ruptured endometrioma. When they set a cut-off of 65 IU/ ml, the test gave a sensitivity of 76 percent, a specificity of 71 percent, a positive predictive value of 76 percent, and a negative predictive value of 93.2 percent. They thus concluded that women with preoperative CA-125 levels higher than 65 IU/ml are at high risk for severe pelvic adhesions and advanced stages of endometriosis. This correlates well with our study with shows a significantly higher level of CA-125 in women later diagnosed on laparoscopy with endometriosis. Chen et. al[21] discussed the sensitivity and specificity of serum CA-125 for the diagnosis of endometriosis. The sensitivity and specificity were 61.1% and 87.5% respectively. Elevated CA-125 (>35 U/ml) was noted in 65 out of 75 women (86.70%) with advanced endometriosis, but in only 15 out of 56 women (26.8%) with minimal to mild endometriosis. This study supports our findings, however the sensitivity and specificity of the serum levels of CA-125 and CA19-9 are lower than depicted by our study. E. Somiglianaet. al.[22] in 2004, performed a study to verify the clinical value of serum CA-125 and CA19-9 levels, either by themselves or combined, in the detection of endometriosis.. Sensitivity and specificity of CA-125 were 27 percent and 97 percent, respectively and were higher than those related to CA19-9. Concomitant use of the two tests led to a sensitivity and a specificity of 42 percent and 71 percent respectively. This study further examines the serum levels of CA-125 and CA19-9 in correlation with the grades of severity of endometriosis and supports our findings.

Conclusion:

Ultrasonography detects a high number of cases of endometriosis but is inadequate in doing so for mild cases, whereas Hystero-Laparoscopy is capable of detecting mild as well as severe cases. Serum levels of CA-125 and CA19-9 separately detect a reasonable number of cases, but as a combination are much more sensitive & specific for this purpose. Thus, a combination of the estimation of serum levels of CA-125 & CA19-9 holds promise for future screening and diagnosis of endometriosis, while being minimally invasive.

Conflict of Interest - Nil
Sources of Support - Nil

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