ROLE OF INTERLEUKIN-8 IN PREDICTING ENDOMETRIOSIS IN PATIENTS WITH CHRONIC PELVIC PAIN & INFERTILITY

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ABSTRACT

To find out the levels of IL-8 in patients of chronic pelvic pain & infertility & assess its role in predicting endometriosis. It was a prospective study conducted over 50 patients of chronic pelvic pain & infertility undergoing diagnostic laparoscopy. During this procedure peritoneal fluid &blood samples were collected & prepared for ELISA, IL-8 estimation. Serum IL-8 levels of >22.16 pg/ml& peritoneal fluid level of >7.98 pg/ml were found to be 100% sensitive & specific .Thus they can be taken as cut-off value for predicting endometriosis. IL-8 was significantly elevated in the serum & peritoneal fluid in the patients

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of endometriosis diagnosed by diagnostic laparoscopy. Our study indicates that serum & peritoneal fluid IL-8 levels can discriminate patients with & without endometriosis and could serve as one of the non-invasive ideal markers to cut short the delay in the diagnosis & management of infertility and also lays down a foundation for further research in correlating IL-8 levels with disease severity.

KEYWORDS: Interleukin-8, Endometriosis, Chronic Pelvic Pain, Infertility.

INTRODUCTION

Endometriosis is a medical condition in female wherein, there is presence & growth of functioning endometrial tissue, containing glandular & stromal elements in areas outside the uterine cavity & are also influenced by hormonal changes & respond similarly as these cells do inside the uterus. These implants are most commonly found on the ovaries, the fallopian tubes, outer surfaces of the uterus or intestine, and on the surface lining of the pelvic cavity.

It is primarily a disease of reproductive years. Its prevalence varies from 6-10%[1], more commonly found in women with infertility & chronic pelvic pain(35%-50%).Almost 40% of women with infertility have endometriosis, while most cases of endometriosis are diagnosed in women aged around 25 to 35 years. Endometriosis has been reported in girls as young as 11 years of age and is rare in postmenopausal women. The average delay between the onset of pelvic pain & surgical confirmation is quite long & found to range from 8-12 years[2]. The average delay in diagnosis in patients presenting with infertility ranges from 3 to 6 years which is the most important issue to be considered regarding the management of this disease. To cut short this duration, we need to discover & implement a method for its earliest detection .Various cytokines & inflammatory markers like IL-2, IL-6, Ca-125, and TNF- α have been studied & remained the centre of research for a long time; IL-8 is one of them.

Studies further suggest that endometriosis is most common in taller, thin women with a low body mass index (BMI). Delaying pregnancy until an older age, never giving birth, early onset of menses, and late menopause all have been shown to increase the risk of endometriosis. It also is likely that there are genetic factors that predispose a woman to developing endometriosis, since having a first-degree relative with the condition increases the chance that a woman will develop the condition. Although a significant number of women with endometriosis remain asymptomatic, chronic pelvic pain, dysmenorrhea, heavy or irregular bleeding, dyspareunia & dyschezia (pain on defecation) are common symptoms.[3]

Etiopathogenesis of endometriosis is complex. Various evidences suggest that the altered immune system can be a causative factor. Moreover, several studies have shown that the certain cytokines and inflammatory markers in the peritoneal fluid of patients with endometriosis play an essential role in the pathogenesis& progression of the disease. Increased number & activity of macrophages in the peritoneal fluid of the patients with endometriosis have been documented. As a result there is increased inflammatory cytokines, growth factors & angiogenesis promoting substances. Increased concentrations of these cytokines are considered as a tool for the development of non-invasive diagnostic test. Interleukin-8 is one of them, produced by macrophages & other cell types such as epithelial cells and endothelial cells. IL-8 secretion is increased by oxidant stress , which thereby cause the recruitment of inflammatory cells thereby inducing further increase in oxidant stress mediators ,making it a key parameter in localized inflammation. Since endometriosis is assosciated with an inflammatory peritoneal environment, where multiple cytokines and growth factors are found at elevated levels, Interleukin-8 induces chemotaxis of neutrophils and is a potent angiogenic agent & stimulate proliferation of various other cells too.[4]

Diagnostic laparoscopy alongwith histopathology is the gold standard for diagnosis. Though it is a minimally invasive procedure but there are also potential complications and procedural costs. A non invasive diagnostic approach with serum IL-8 levels will be a boon with respect to cost, time & skill.

Material & Methods

Our present study was conducted at Department of Obstetrics & Gynecology of G.S.V.M Medical College, Kanpur in collaboration with C.D.R.I Lucknow. A total of 50 patients were enrolled in the study who presented with either or both features of chronic pelvic pain & infertility. All enrolled patients underwent Diagnostic Laparoscopy for diagnosis and samples of peritoneal fluid & serum were collected separately. Samples were analyzed with ELISA Kit for IL-8 at C.D.R.I, Lucknow.

STUDY DESIGN: Prospective Study PATIENTS SELECTION CRITERIA

INCLUSION CRITERIA

- Age: 15-45 years menstruating females
- Chronic pelvic pain of more than 6 months duration
- Infertility of more than one year of unprotected intercourse.

Exclusion Criteria

- patients with infertility with other known causes.
- Postmenopausal women

• Patients with chronic pelvic pain with other proven causes

Control group

• Patients with other gynecological complaints apart from infertility

RESULTS

A total of 50 patients were enrolled in the study who presented with either or both features of chronic pelvic pain and in infertility. The results of the present study are based on the levels of IL-8 in serum and peritoneal fluid and their co-relation with endometrisis and its severity.

In this study the maximum endometriotic cases were found between 20-25 years of age & the mean age was 26 with SD ± 3.02 .

S.No.	Findings	No. of cases Present	No. of cases Absent	
1	Chronic Pelvic Pain	39	11	
2	Endometriosis	27	23	
3	Infertility	50	0	

 Table I: Distribution Of Total Cases (n=50) According

 To Chronic Pelvic Pain, Endometriosis And Infertility

**In table 1 we see that our patients presented with one or more complaint and therefore numbering in these groups is overlapping.

ſ	Group Statistics							
	Endometriosis N(50) Mean Std. Std. Error of Deviation Mean							
ſ	Serum IL-8	Present (n _a)	27	67.9589	20.2404	3.8953		
	SLevels	Absent (n _b)	23	17.3085	2.1930	0.4573		

Table 2: Level Of Serum II-8 In EndometrioticCases And Control Group

t = 11.924 & p<0.0001

In table ii the levels of serum IL-8 were found to be statistically raised in endometrisis cases as compared to those without endometrisis .Independent t test also showed statistically significant results (p < .001).

Sample size		50
Positive group	Diagnosis = 1	27
Negative group	Diagnosis = 0	23

 Table 3: Roc Curve Analysis For Evaluation Of Serum II-8 As A Predictor Of Endometriosis

Area under the ROC curve (AUC)

Area under the ROCcurve (AUC)	1
Standard Error	0.000
95% Confidence interval	0.929 to 1.000
Significance level P (Area= 0.5)	< 0.0001



Criterion	Senstivity	95% CI	Specificity	95% CI	+LR	-LR
	100.00	87.2- 100.0	0.00	0.0- 14.8	1.00	
>22. 16789	100.00	87.2- 100.0	100.00	85.2- 100.0		0.00
>96. 03747	0.00	0.0- 12.8	100.00	85.0- 100.0		1.00

Fig.1: Linear Graph Correlating Sensitivity And Specificity Of Serum IL-8 For Predicting Endometriosis

Group Statistics							
Endometriosis N (50)			Mean	Std. Deviation	Std. Error of Mean		
Perirtoneal	Present (n _a)	27	17.8346	1.7840	0.3433		
IL-6 Levels	Absent (n _b)	23	6.1856	1.5232	0.3176		

Table 4: Level Of Peritoneal Fluid IL-8 In Endometriotic Cases And Control Group

t=24.590,p<0.0001

In TABLE IV we see that the levels of peritoneal fluid IL-8 were significantly raised in cases of endometriosis when compared to those without it (t=24.590 and p<0.0001).

Sample size		50
Positive group	Diagnosis = 1	27
Negative group	Diagnosis = 0	23

Table 5: Roc Curve Analysis For Evaluation OfPeritoneal Fluid IL-8 As A Predictor OfEndometriosis

Area under the ROC curve (AUC)

Area under the ROC curve (AUC)	1
Standard Error	0.000
95% Confidence interval	0.929 to 1.0
Significance level P (Area= 0.5)	< 0.0001



Criterion	Senstivity	95% CI	Specificity	95% CI	+LR	-LR
≤2. 37485	100.00	87.2- 100.0	0.00	0.0-14.8	1.00	
>7. 98123	100.00	87.2- 100.0	100.00	85.2- 100.0		0.00
>21. 11343	0.00	0.0- 12.8	100.00	85.0- 100.0		1.00

Fig. 2: Linear Graph Correlating Sensitivity And Specificity of Peritoneal IL-8 in Predicting Endometriosis

DISCUSSION

IL-8 has been found to be increased in inflammatory conditions. IL-8 is a pleiotropic cytokine which is produced by a variety of cell types, including monocytes, lymphocytes, fibroblasts, endothelial cells, keratinocytes and mesangial cells. The cytokine, which appears to mediate numerous physiological and pathologic processes, acts on a wide variety of cells and regulates immune responses, angiogenesis, acute phase-responses of the liver, hematopoiesis, neuronal function and osteoclastogenesis &may also have important functions in reproductive physiology including the regulation of ovarian steroid production, folliculogenesis and early events related to implantation. Older studies have shown that both normally placed and ectopic endometrium produce IL-8. This observation may limit the role of serum IL-8 to be used as a predictor for the presence of endometriosis.[5]

Serum and Peritoneal fluid samples of all 50 cases were collected and IL-8 were measured via ELISA kit. Among 50 cases enrolled, 27 patients were diagnosed to have endometriosis on laparoscopy whereas 23 patients were disease negative. Patients with Endometriosis (n=27) had an average serum IL-8 of 67.958 pg/ml with a SD of ± 20.2404 pg/ml while those without Endometriosis (n = 23) had an average serum IL-8 of 17.3085 pg/ml with SD of + 2.1930 pg/ml. The independent t test shows that difference is statistically highly significant (t=11.924 & p< 0.001)(TABLE II). In our study that patients with Endometriosis were found to have had a significantly higher serum levels of IL-8.ROC curve analysis was used to assess the diagnostic ability of serum IL-8 for detecting Endometriosis. An area under the curve of 1 was found which implied that serum IL-8 is an excellent discriminating marker for diagnosing Endometriosis(TABLE III). A serum IL-8 of > 22.16789 pg/ml was found to be 100% sensitive and 100% specific and thus can be used as a cut-off value.

In previous study by Ohata Y et al 2008 found that the serum concentration of IL-8 in patients with endometrioma was significantly higher than in patients with benign ovarian cyst. The serum IL-8 threshold (25 pg/mL) had a higher sensitivity (71.4%) for diagnosing ovarian endometrioma.^[6]

Iwabe T 1998 found that the level of IL-8 in serum was significantly higher in patients with endometriosis than in patients without endometriosis.^[7]

On comparing the means of peritoneal fluid(PF) level of IL-8 in the Endometriosis positive and negative groups, patients with Endometriosis (n=27) had an average PF IL-8 of 17.8346 pg/ml with a SD of +1.7840 pg/ml while those without Endometriosis (n=23) had an average PF IL-8 of 6.1856 pg/ml with SD of \pm 1.5232 pg/ml. To analyse this data independent t test was also done and the difference found to be statistically highly significant (t= 24.590 and p <0.0001)(TABLE IV). Thus it can be concluded from our study that patients with endometriosis had a significantly higher PF levels of IL-8. ROC curve analysis was used to assess the diagnostic ability of PF IL-8 for detecting Endometriosis. An area under the curve of 1 was found which implied that PF IL-8 is a good discriminating test for diagnosing Endometriosis(TABLE V). PF IL-8 of >7.98123 pg/ml provided a sensitivity and specificity of 100% and can be used as a cut-off value.

In a study conducted by Rafat Gazvani et al 1998 studied cytokine levels in peritoneal fluid obtained during laparoscopy and found that the concentration of IL-8 in the peritoneal fluid was higher in women with endometriosis compared to women without (P=0.02).^[8]

In a previous study done by Ryan IP et al 1995

investigated eighteen women with laparoscopic findings of mild to severe endometriosis, and nine women with no visual evidence of pelvic pathology. Peritoneal fluid IL-8 levels were determined using an ELISA. Interleukin-8 concentrations were compared among women with and without endometriosis. Interleukin-8 was detectable in the PF of a majority of women (67%). Interleukin-8 concentrations were higher in the PF of women with endometriosis than in matched normal controls.^[9]

Calhaz Jorge C et al 2003 found thatPatients with moderate/severe stages had IL-8 significantly higher than controls (P = 0.008) and marginally higher than patients with minimal/mild endometriosis (P = 0.053). Concentrations of IL-8 were significantly higher in patients than in controls in the luteal phase.^[10]

Aydin Arici et al 1996 found that the peritoneal fluid levels were elevated in patients with endometriosis and its level is in correlation with the severity of the disease. The mean concentration of IL-8 in samples obtained from control patients (n = 28) was 4.8 +/-0.5 pg/ml; from patients with minimal-mild endometriosis (n = 24) was 27.5 +/-2.6 pg/ml; and from patients with moderate-severe endometriosis (n = 21) was 530.2 +/-65.1 pg/ml.^[11]

CONCLUSION

In the present study there was a significant elevation in the levels of serum IL-8 and Peritoneal fluid IL-8 in patients with endometriosis. The levels of serum IL-8 were found to be statistically raised in Endometeriosis cases as compared to those without Endometriosis (t = 11.924 and p < 0.001). Independent t test also showed statistically significant results (p<0.0001). Hence, it can be concluded from our study that IL-8 levels were significantly higher in patients with endometriosis. ROC curve analysis further supports that serum IL-8 can be used to identify patients of Endometriosis from those who don't have it. Serum IL-8 of >22.16789 pg/ml provided a sensitivity and specificity of 100% and can be used as a cutoff value and it also shows that evaluation of serum IL-8 can be a mode of non-invasive test for detecting endometriosis.

The levels of peritoneal fluid IL-8 were also found to be significantly raised in cases with endometriosis as compared to those without endometriosis (t=24.590 and p<0.0001). Independent t test also showed statistically significant results (p<0.0001). Thus it can be concluded from our study that patients with endometriosis had significantly higher PF levels of IL-8.ROC curve analysis also showed that peritoneal fluid IL-8 can be used to identify patients with Endometriosis from those who don't have it.PF IL-8 of >7.98123 pg/ml was found to be 100% sensitive and 100% specific and thus can be used as a cutoff value.

As our study is showing elevated levels of IL-8 in serum & peritoneal fluid in patients of endometriosis it can serve a basis for its correlation with severity of the disease too.

In our study we compared our results with previous studies in literature which were conducted either on Caucasian or African population. Our study is unique and based on Indian population and is one of its kind. The results obtained from the present study though conducted on a small sample can serve as a basis for further evaluation and research. The above study was conducted with proper monitoring and care and they are all tested and analyzed carefully, but for the reliable data further studies with larger sample size might be required to establish it as a non-invasive diagnostic tool for detecting endometriosis as well as for predicting its correlation with severity of the disease.

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Conflict of interest. Nil.

Ethical statements. All the procedures followed in study were in accordance with the ethical standards of institution, and ethical committee of institution had critically evaluated the study and its methodology and given the approval before study was started.

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