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Abstracts



**Blastocyst Implantation Biology Laboratory
Department of Physiology
All India Institute of Medical Sciences**

A Holistic Approach to Endometriosis

Linda C. Giudice

The Robert B. Jaffe MD Endowed Professor and Chair

Department of Obstetrics, Gynecology and Reproductive Sciences

University of California – San Francisco

Endometriosis affects millions of women worldwide. It is believed to be an estrogen-dependent, progesterone-resistant disorder that causes infertility and pelvic pain. It is associated with autoimmune disorders, including irritable bowel syndrome, chronic fatigue syndrome, and atopy, and is also associated with increased risk of malignancies, including endometriosis-associated ovarian cancer, non-Hodgkins lymphoma, dysplastic nevi and malignant melanoma. The time to diagnosis is protracted due to health care provider reluctance to proceed directly to surgery for a definitive diagnosis, as well as patients' belief that dysmenorrhea is normal and delaying seeking medical consultation for fear that a malignancy will be discovered. The personal toll is tremendous to patients with endometriosis, including living with a chronic disorder, pain that is not always well controlled and often unpredictable in course, disruption of personal relations and relationships, commonly undergoing multiple operations, facing the possibility of not being able to have a family, missing school and/or work because of pain, and having others not fully understand this complex and enigmatic disorder. While western medicine has addressed diagnosis and medical and/or surgical treatment of endometriosis, the consequences of the disorder and its associated syndromes underscore the need for a global, multi-disciplinary approach to the well being of the patient, at a holistic level. One approach is to establish Centers of Excellence in Endometriosis world-wide that would include gynecologists, surgeons, reproductive endocrinologists, dermatologists, immunologists, psychologists/counselors, sex therapists, pain management teams with anesthesiologists, physical therapists, nutritionists, massage, acupuncture, stress management, complementary therapies, and patient support groups. In addition, such Centers could be the nidus for clinical and basic research on this disorder, with a well-populated database of patient demographics, clinical data, and bio-specimens for research. Coordination of a patient's medical and surgical treatments is essential with the patient's mental, nutritional, spiritual and physical fitness and well being, in a setting that is woman-focused. Facilitating research on this disorder and its effects on women and their families has the goal to enable primary prevention of endometriosis, and, for existing disease, to effectively treat it on all levels of health and wellness.

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Suneeta Mittal
Professor & Head
Department of Obstetrics & Gynecology
WHO-CCR in Human Reproduction &
Chief, ART Centre
All India Institute of Medical Sciences

Endometriosis being defined as the presence of endometrial glands and stroma outside the uterine cavity is the leading cause of disability in reproductive age women, resulting in infertility and pelvic pain. This is the third leading cause of gynecological admissions. Endometriosis is a progressive disease which is both physically and mentally debilitating and the usual time interval between the onset of symptoms and confirmed diagnosis may average 6 years or more. Endometriosis is a fairly common condition. Approximately 1% women undergoing major gynecological surgery, 6-43% undergoing sterilization, 12-32% undergoing laparoscopy for pelvic pain, 21-48% undergoing laparoscopy for infertility are detected to have endometriosis. Among teenagers the need for laparoscopy for pelvic pain or dysmenorrhea is as prevalent as 50.6%, reportedly.

Gold standard for diagnosis is direct visualization of lesion by laparoscopy or laparotomy and biopsy. The lesions have varied appearance ranging from raised flame like, whitish, yellow brown, brown, translucent blebs, irregular islands to lesions with puckered surface. For histopathological confirmation both glands and stroma should be seen outside uterine cavity, sometimes with presence of fibrous tissue, blood and cysts. CA125 level is higher in endometriosis, especially in advanced stage disease. Levels >100 IU/ml indicate either the presence of extensive adhesions or ruptured endometrioma. CA125 has no diagnostic value, however pre-operative level is important and useful in selective women at high risk for bowel injury, which can be prevented by pre-op bowel preparation.

Current research focuses on for non-surgical diagnosis. Role of peritoneal fluid/serum marker is under investigation. In young girls with non-cyclic pain of ≥ 6 months duration not responding to OCP/NSAID with normal US/Lab values - 78% endometriosis is the cause.

Major problem in endometriosis is Infertility, 25% to 50% of infertile women have endometriosis, while 30% to 50% of women with endometriosis are infertile. There is higher prevalence of endometriosis in infertile women (48%) compared with fertile women undergoing tubal sterilization (5%).

Infertility is caused by: anatomic distortion by adhesions and endometrioma, production of substances (Prostanoids, cytokines, growth factors) hostile to ovulation, fertilization, implantation, increased peritoneal fluid and activated anti-endometrial antibodies, macrophages and anovulation. There is no correlation between degree of pain & stage of disease

Management options include expectant management, surgical resection/ablation, ovarian suppression, a combination surgical and medical therapy, Controlled ovarian hyperstimulation ± intrauterine insemination and assisted reproductive technologies. In minimum & mild disease pregnancy rates are equal with expectant management compared to surgery. Suppressive hormone therapy and drug treatment is ineffective. Pregnancy following medical treatment vs. placebo or no treatment OR is 0.83 indicating no improvement in fertility on a *Cochrane Review*. Comparing Danazol vs. no treatment – pregnancy rates are 35% vs. 47%, and Buserelin vs. no treatment - 30% vs. 37%.

Medical treatment may be given pre-operative to decrease the size of implants or post operative for residual disease. Surgical Treatment is preferred approach in infertile patients with advanced endometriosis for mechanical clearance of adhesions and obstructive lesions, however it is much more expensive than medical treatment. Preferred treatment is a step wise approach.

IVF is indicated in women with moderate to severe endometriosis, in whom medical or surgical therapy has failed and in mild to moderate endometriosis in the presence of other factors contributing to infertility. IVF outcome is a controversial issue. A meta-analysis involving 22 studies and 2377 cycles calculated:

Endometriosis versus tubal factor OR 0.56 (54% reduction in pregnancy rate)

Severe versus mild disease OR 0.60 (36% reduction)

Another meta-analysis to evaluate the ovarian reserve and ovarian responsiveness to ovarian stimulation and assisted reproduction outcomes in patients with ovarian endometrioma showed a similar overall pregnancy rate - OR - 1.17. Decreased ovarian responsiveness to ovarian stimulation in patients with ovarian endometrioma may be due to a reduced number of follicles in these patients compared with controls. The quality of oocytes is poor, with higher granulosa cell apoptosis. Eggs from oocyte donors with endometriosis result in reduced pregnancy rates, but oocyte recipients with endometriosis have same implantation rates. There is normal pinopode formation seen in endometriotic women, but there is deficient expression of $\alpha\beta3$ integrin with increased estradiol levels and elevated levels of aromatase enzyme in the endometrium with decreased *ebaf* expression.

There are several controversies which will be discussed. The conclusion is that the presence of endometriomas *per se* may negatively influence ovarian function and may impose difficulties and risks during oocyte retrieval. The magnitude of the negative effect on ovarian reserve is unknown and there are no definite data clarifying whether treatment of endometriomas increases (or decreases) the chances of success using IVF. Larger prospective randomized studies are required.

In the absence of tubal occlusion or severe male factor infertility, laparoscopy may still be considered for the treatment of endometriosis even after multiple IVF failures. Three randomized controlled trials (n=165) have shown that the live birth rates were significantly higher in women receiving the GnRH agonist compared to the control group (OR 9.19). Administration of GnRH agonists for a period of 3-6 months prior to IVF or ICSI in women with endometriosis increases the odds of clinical pregnancy by fourfold.

In extensive endometriosis, mifepristone is useful for intractable pain, although effects on lesions found to be minimal. Six months therapy (50mg daily) resulted in alleviation of pelvic pain and cramping in all subjects. Doses range from 50-100 mg daily and adverse effects include hot flashes, fatigue, nausea & transient liver enzyme changes. Mifepristone is effective in improving symptoms and causing regression of endometriosis in absence of significant side effects

Recurrences are common, unless definitive surgery is done. The risk is 5-20% /yr or 40% at 5 yrs. Rate of recurrence increases with duration of follow up and occurrence of previous surgery. Postoperative low dose OCP lower recurrence after 1yr but not after 3 yrs and recurrence rate after postoperative GnRH agonist is 37%.

There is no proven way but decrease in number and volume of menstrual cycle by - OCP for several years or having several pregnancies may reduce the risk. The role of diet and exercise is also important.

Endometriosis – Imaging

Raju Sharma
Department of Radio-Diagnosis
All India Institute of Medical Sciences

Endometriosis is a disorder that affects women in the reproductive age group, and is the result of functioning endometrium located outside the uterus. Radiologists are often involved in the diagnosis & work-up of this disease either when they are asked to exclude endometriosis in a woman with pelvic pain or infertility, or when they are considering endometriosis in the differential diagnosis of an adnexal mass. The imaging modalities used in the evaluation of this entity include Ultrasonography and Magnetic Resonance Imaging, while laparoscopy is considered as the gold standard. Both trans-abdominal and trans-vaginal sonography can be used. Majority of endometriomas are seen as hypoechoic focal, often multilocular ovarian masses, with low level internal echoes and echogenic wall foci. They are bilateral in up to half the cases. Magnetic Resonance Imaging has greater specificity for the diagnosis of endometriotic cysts and they typically present as T1 hyperintense masses and demonstrate shading on T2W images. The differential diagnosis includes dermoids, mucinous neoplasms and hemorrhagic cysts. Adhesions are the other important feature of endometriosis, and can sometimes be recognized on MRI as spiculated low intensity stranding that obscures organ interfaces. Unusually endometriotic implants can occur in the gastrointestinal tract, urinary tract and chest. Barium enema, excretory urography and CT are then used for the diagnosis but the findings are non-specific.

Histopathology of Endometriosis

Seema Kaushal, Sandeep R. Mathur, Siddhartha Datta Gupta
Department of Pathology
All India Institute of Medical Sciences

Cullan in 1920 first documented the presence of endometrial tissue outside endometrial cavity before which this condition was largely unrecognized. However it was Sampsons whose contributions in early nineties made endometriosis a recognized entity. Since then research in this field has grown tremendously.

Term endometriosis is derived from words *endo* (inside) and *metra* (womb) and is defined as the presence of endometrial tissue outside endometrium and myometrium. Although considered as primarily a disease of the reproductive years it can affect any woman, from premenarche to postmenopause with worldwide prevalence of 10 -15 % . Main symptom is pelvic pain in various manifestations(dysmenorrhea, chronic pelvic pain, dyspareunia, dyschezia, dysuria), infertility , non specific signs and symptoms are other common presenting features.

Controversies regarding the etiology and pathogenesis of endometriosis have been there ever since this entity was recognized and questions still remains unanswered. Many theories have emerged from time to time.

Although endometriosis has been reported from many sites including brain, common sites involved are ovaries (most common), fallopian tube, the posterior and anterior cul-de-sac, uterine ligaments (broad or round ligament of the uterus), pelvis, intestines (most commonly the rectosigmoid), urinary bladder and ureters .

Grossly endometriotic foci appear as punctuate, red blue, brown or white spots or patches. They may form nodules or cysts. Cysts are more common in ovaries where they are also known as endometriomas or chocolate cysts. Another entity which has been recently described as a separate type of endometriosis is polypoid endometriosis. It presents as polypoidal mass lesion and simulates malignancy clinically. Role of tamoxifen has been proposed as causative agent in polypoid endometriosis.

Histopathology is gold standard for diagnosis of endometriosis. Although fairly straightforward in most cases, it can present as diagnostic challenge because of many variations or absence of one or more components. Typical findings are of presence of endometrial glands or stroma. Usually both are seen, but diagnosis can be made when only one component is present. Hemosiderin laden macrophages are also commonly seen and their presence in absence of characteristic glands and stroma is considered as an important diagnostic clue.

Glands in endometriosis are of endometrioid type with appearance ranging from inactive glands to proliferative or secretory type. Simple and complex hyperplasias, various metaplasias including ciliated, eosinophilic, hobnail, squamous, and mucinous metaplasia can be seen. Hormonal changes leading to decidual reaction, Arias-Stella reaction, atrophic glands and fibrous replacement of glands have also been observed. Also postmenopausal and treatment related changes should be kept in mind before excluding the diagnosis of endometriosis.

Stromal endometriosis is the term used for presence of endometriotic stroma in absence of glands while atypical endometriosis is the term used for presence of cytological atypia in endometrial glands.

Endometrial stroma can present as a lone component of endometriosis, can be abundant or very scant presenting just as a periglandular cuffing. CD10 immunostaining should be done whenever there is doubt regarding nature of stroma. Smooth muscle metaplasia is commonly seen in stroma. Fibrosis, elastosis, foamy histiocytes and myxoid change are other variations observed in stroma which can cause diagnostic problems.

Inflammatory and reactive changes including infected cysts, pseudoxanthomatous salpingitis, florid mesothelial hyperplasia and Leisegang rings have also been observed in endometriosis. However, rare endometriosis should be kept in consideration while looking for etiology of these lesions.

Other rarer findings in endometriosis include necrotic pseudoxanthomatous nodules, vascular invasion, perineural invasion. Peritoneal leiomyomatosis, glial implants of ovarian teratomas, and nodules of splenosis have also been reported.

With so many variations reported in literature we aimed at studying them in our cases. All the cases with histological diagnosis of endometriosis in last five years were subjected to histomorphological analysis. A total of 90 cases were analyzed. Most common site of involvement was ovary (73.3%) followed by supporting structures of uterus (7.7%), uterine serosa (6.6%), soft tissues (5.5%), fallopian tube (3.3%), cervix (1.1%), urinary bladder (1.1%) and peritoneum (1.1%). Endometrial stroma was seen in 94.4% cases while endometrial glands were seen in 63%. Atrophic glands with flattened cuboidal lining were seen in 44.4%, proliferative glands in 12.2% and secretory glands in 6.6 %. Hemosiderin laden macrophages were seen in 53.5% cases. Cystic dilation of glands (7.7%), decidualized stroma (3.3%), ciliated (3.3%), mucinous (2.2%) and hobnail (1.1%) metaplasia of glands were also seen. From our study it emerged that ovary is the most common site of endometriosis. Atrophic glands are the most common type of glands seen followed by proliferative and secretory glands. Decidualized stroma and ciliated, mucinous, hobnail metaplasias of glands are uncommon.

Endometriosis – Molecular Strategy

M.A. Khan, M. Goyal, A.R. Najwa, Jayasree Sengupta, D. Ghosh
Department of Physiology
All India Institute of Medical Sciences

Physiology

Endometriosis is a complex disorder with varied pathogenetic mechanisms involved in implantation of endometrium ectopically and its progression both in terms of pathogenesis and clinical presentation. The present clinical staging does very little about guiding therapy, predicting prognosis, fertility outcome, treatment response and chances of recurrences.

Molecular approaches for diagnosis of a disease and guiding therapies are not new but all of the hitherto been described approaches are essentially candidate-based. The biological system is not essentially linear; there are thousands of proteins interacting in networks with time and space contexts along with redundancy, thus resulting in complex emerging properties. In the present time, there are available tools and techniques of generating high throughput genomics and proteomics data. Such data mining and their analyses towards pattern discovery and prediction provide a promise for improvising novel approach to address complex disorders like cancer and endometriosis. The idea of molecular classification proposed by Golub et al. (Science 286: 531-537, 1999) for acute myeloid leukemia (AML) has provided an objective tool which enabled cancer biologist to classify the disease on the basis of molecular characteristics and thereby treating it. This classification scheme involves defining the subtypes of a disease on the basis of genomic expression pattern, i.e. class discovery and then to assign an unknown sample to already defined classes, i.e. class prediction. The gene expression pattern is analyzed for each class or subtype of disease and several informative genes are selected based on their differential expression.

The originally described method utilizes microarray data and it is known that biological functions and transcriptomics are linked by translation of proteins and their complex interaction. Therefore high throughput proteomics approach may compliment in the process of molecular classification. The extensive researches elucidating protein-protein interactions, utilizing yeast two-hybrid system, mass spectrometry and protein arrays have generated enormous data, and some of them are available online. With the availability of interactomes bank and powerful computational and mathematical modeling tools, it is now possible to predict the function of a newly discovered protein. Several available softwares provide user-friendly interface for analyzing genomics and proteomics data and to identify the involved pathways in a particular disease.

Apart from the top-down model as described above, the pattern of molecular expression profiles may also be used in a bottom-up approach *in-silico* for generating hypotheses on pathogenesis and can provide new dimensions to the understanding of natural history of development and progression of endometriosis and other complex disorders.

Pre-emptive medicine is an emerging concept of predicting, anticipating and preventing diseases and disease-sequels before the irreversible tissue damage takes place. Analysis of data from genomic and proteomic profiles, as discussed above, may help in such predictions and may provide a lead time for designing preventive strategies. The inputs required for such studies are though enormous but these do hold promise of richer dividends in terms of possibility of marker discovery for early diagnosis and prognosis. Presently evidence based medicine, which largely evolved from epidemiological studies, is observing a paradigm shift from population-based uniform therapeutics approach to tailored-therapeutics or personalized medicine depending on the individual's molecular phenotype.

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Future Trends in Endometriosis Research

Linda C. Giudice

The Robert B. Jaffe MD Endowed Professor and Chair

Department of Obstetrics, Gynecology and Reproductive Sciences

University of California – San Francisco

Endometriosis is an estrogen-dependent, progesterone-resistant disorder where endometrial-like tissue forms lesions primarily on the pelvic peritoneum, bowel, and ovaries, as well as at distant sites. It affects about 10% of women of reproductive age and up to 50% of women with pelvic pain and/or infertility. The pathogenesis of the disorder and the pathophysiology of associated infertility and pelvic pain are not well understood, and diagnosis and therapies are challenging. Surgical diagnosis is the current gold standard, and patients often require repetitive procedures due to recurrence of symptoms after surgical therapy. In addition, most current medical therapies are not suitable long-term due to either their lack of efficacy or unacceptable side-effects. Thus, there are several key areas of research that can benefit progress in the diagnosis, treatment, and prevention of endometriosis and associated disorders and enhance the quality of life of women affected by endometriosis. These include: developing a non-invasive or minimally invasive test to diagnose the presence or absence of disease, and if present, the extent (stage) of disease; developing a classification system that has relevance to prognosis for fertility and pain control; understanding the pathogenesis and pathophysiology of the disorder; understanding the epidemiology of the disorder; and developing targeted therapeutics for pain relief and treatment of infertility. In order to achieve these goals, investigators from multiple disciplines need to pursue a systems biology approach to this research agenda, including well annotated databases for epidemiologic research, well annotated bio-specimen banks for optimal experimental design and interpretation of results relevant to pathogenesis and pathophysiology, improved animal models for elucidating pathogenesis and pathophysiology, advanced “omics” and technologies for diagnostics and understanding cellular processes and signaling cascades and to develop targeted therapeutics, and integration of seemingly disparate disciplines, such as infectious disease, immunology, regenerative medicine, genetics/epigenetics, and endocrine disruptors, into the current research paradigms. The field of endometriosis research is in need of a catalyst to spark the next scientific revolution in prevention, diagnosis, and treatment of this enigmatic disorder. The time is now for this to occur, as current science and technology are well poised to make this a reality.

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