INTRODUCTION

Endometriosis is a disease defined by the presence of endometrial glands and stroma located outside the uterine cavity. These ectopic implants can be found throughout the pelvis, on and within the ovaries, abutting the uterine ligaments, occupying the rectovaginal septum, invading the intestinal serosa, and along the parietal peritoneum. Endometrial implantation at distant sites such as the pleura, lung, within surgical scars, and along the diaphragm also has been reported ***(Boyle and Torrealday, 2008).***

Although the exact prevalence of this disease remains unknown, it is believed to affect between 3% and 10% of women of reproductive age with an increased prevalence of up to 30% or more in infertile women ***(Boyle and Torrealday, 2008).***

It results often in subfertility and pain, occurs mainly in women of reproductive age (16–50 years) and has a progressive character in at least 50%, but the rate and risk factors for progression are unknown ***(Mihalyi et al., 2010).***

Endometriosis usually presents with painful symptoms, such as menstrual period pain (dysmenorrhea), pain on intercourse (dyspareunia), or non menstrual pelvic pain. It also may be found incidentally at surgery (e.g. for sterilization) or during investigation for infertility ***(Kocakoc et al., 2008).***

Endometriosis can be classified into four stages: minimal, mild, moderate and severe. More advanced endometriosis can be deeply invasive behind the cervix and invade into the rectovaginal septum, obliterating the pouch of Douglas partially or completely, or can present as ovarian endometriotic cysts (endometrioma) ***(Mihalyi et al., 2010).***

There are no sufficiently sensitive and specific signs and symptoms or diagnostic tests for the clinical diagnosis of endometriosis, and no diagnostic strategy is supported by evidence of effectiveness ***(Mounsey et al., 2006).***

The diagnosis of endometriosis can be suspected in women with pelvic pain and/or subfertility, although endometriosis may be completely asymptomatic. Clinical presentation with abdominal or pelvic pain can be suggestive of endometriosis. Vaginal ultrasound is an adequate diagnostic method to detect ovarian endometriotic cysts and deeply infiltrative endometriotic nodules, but does not rule out peritoneal endometriosis or endometriosis-associated adhesions ***(Mihalyi et al., 2010).***

MRI has a high sensitivity in detecting endometrial cysts but poor diagnostic accuracy for endometriosis in general. Empiric diagnosis and treatment of endometriosis is reasonable, based on clinical suspicion and presentation. Patients with persistent symptoms after empiric treatment should be referred for laparoscopy ***(Mounsey et al., 2006).***

The gold standard for the diagnosis of endometriosis is laparoscopic inspection, ideally with histological confirmation ***(Mihalyi et al., 2010).*** It is the most sensitive examination, because only laparoscopy can identify superficial peritoneal implants. Laparoscopy, however, is an invasive technique and should be performed only after imaging techniques prove insufficient for confident diagnosis ***(Kocakoc et al., 2008).***

Lack of a non-invasive diagnostic test contributes to the long delay between onset of symptoms and diagnosis of endometriosis ***(Mihalyi et al., 2010).*** Additional tools are needed for non-invasive classifications in order to reduce the number of unnecessary laparoscopies without adversely affecting outcomes. Finding specific and more sensitive biomarkers in endometriosis is critical, because endometriosis is usually diagnosed only in advanced stages, and there is a high rate of morbidity for this disease ***(Zachariah et al., 2009).***

Various theories have been advanced regarding the etiopathogenesis of endometriosis, but its etiology is still enigmatic ***(Lermann et al., 2010).*** An increasing body of evidence links endometriosis with local and systemic abnormalities in immune response with plenty of data suggesting that altered immune responsiveness can be of particular importance in the pathogenesis of endometriosis. A study suggested that peritoneal fluid of endometriosis patients contains an increased number of activated macrophages that secrete local products with important angiogenic properties,

including vascular endothelial growth factor (VEGF) and tumor necrosis factor alpha (TNF-α) ***(Xavier et al., 2006)***.

Compared to healthy women, endometriosis patients have an endometrium with higher potential to implant and develop outside the uterus. The generation of new capillary blood vessels is probably required for the implant to grow larger than 2–3 mm, favoring an angiogenesis-dependent mechanism in endometriosis ***(Xavier et al., 2006).***

Interleukin (IL)-6, a multifunctional cytokine involved in numerous immunological and proliferative processes, has been found at high concentrations in the peritoneal fluid of endometriosis patients ***(Akoum et al., 1996)*** and the values of IL-6 have been correlated with the degree of severity of the disease ***(Cheong et al., 2002; Khan et al., 2002).***

Interleukin-8 (IL-8), a chemokine, is a potent angiogenic, pro-inflammatory, growth promoting factor ***(Koch et al., 1992)***. It is also a chemo-attractant for neutrophils and induces expression of several cell adhesion molecules ***(Koch et al., 1992)***. It can also lead to neutrophil activation ***(Peveri et al., 1988)*** and hence might contribute to the pathogenesis of endometriosis. The presence of inflammation and neovascularization observed in and around ectopic endometrial implants, and the presence of inflammatory neutrophils in these lesions ***(Khorram et al., 1993)***, is compatible with the biological actions of IL-8 ***(Van Deuren et al., 1992)***.

The discovery of circulating cell-free ccf DNA in circulation has opened up the possibilities of non-invasive diagnosis and monitoring of a wide variety of malignant diseases. Increased concentrations of ccf DNA have also been found in inflammatory conditions, such as systemic lupus erythematosus and rheumatoid arthritis ***(Zhong et al., 2007).***

Since endometriosis is related to a chronic inflammatory reaction, the possibility of circulating ccf DNA serving as a reliable apoptotic and necrotic marker for the detection of pathological processes has been raised. Elevated concentrations of ccf DNA in cancers and in inflammatory diseases have been suggested for developing non-invasive diagnosis ***(Zachariah et al., 2009).***

AIM OF THE WORK

The aim of the current study is to assess the validity of serum and peritoneal interleukin 6, interleukin 8 and plasma cell-free nuclear DNA (ccf nDNA) as biomarkers in diagnosis of stage I and stage II of pelvic endometriosis.

PATHOPHYSIOLOGY OF ENDOMETRIOSIS

Endometriosis is defined as the presence of endometrial glands and stroma outside the endometrial cavity and uterine musculature. These ectopic endometrial implants are usually located in the pelvis, but can occur nearly anywhere in the body ***(De Nardi and Ferrari, 2011).***

Endometriosis is one of the most common benign gynecologic disorders. This disease is present in about 10% of all reproductive-aged women, and its prevalence rises to 20%-50% in infertile women ***(Cho et al., 2012).***

Endometriosis exhibits a great degree of variability in not only the symptomatic presentation, but also in terms of the age of onset, progression of the disease, response to treatment and rate of recurrence ***(Berbic and Fraser, 2011).***

The presence of endometrial tissue within the uterine musculature is termed adenomyosis, referred to as endometriosis interna in older reports. Adenomyosis occurs when the normal relationship between the basal endometrial layer and the myometrium is disrupted; this results in pockets of endometrial glands and stroma within the myometrium ***(Templeman et al., 2008).***

Prevalence of Endometriosis:

The prevalence of endometriosis in specific categories of patients has been reported, but the prevalence in the general population is not known ***(De Nardi and Ferrari, 2011)***.

Estimates of prevalence vary widely (depending on the study population), but up to 15% of women may be affected. There is a 10-fold increase in prevalence in women who have an affected first-degree relative ***(Kocakoc et al., 2008).***

Widely varying figures for the prevalence of endometriosis have been published. Roughly, 3- 10% of women in the reproductive age group and 25-35% of infertile women have endometriosis. It is clear to all gynecologists that its incidence is increasing which may be due to increased clinical awareness, better education in gynecologic pathology, and the availability of better visualization using video-assisted laparoscopy. It is also probable, however, that the disease is actually increasing in frequency ***(Darwish et al., 2006).***

Gross and Microscopic Pathology:

The most frequent sites of implantation are the pelvic viscera and the peritoneum. Endometriosis can be divided into intra- and extra-peritoneal sites (Fig. 1). In decreasing order of frequency, the intra-peritoneal locations are ovaries (30%), uterosacral and cardinal ligaments (18%-24%), fallopian tubes (20%), pelvic peritoneum, pouch of Douglas, and gastrointestinal (GI) tract. Extra-peritoneal locations include

cervical portio (0.5%), vagina and rectovaginal septum, round ligament and inguinal hernia sac (0.3%-0.6%), navel (1%), abdominal scars after gynecological surgery (1.5%) and caesarian section (0.5%). Endometriosis rarely affects extra-abdominal organs such as the lungs, urinary system, skin and the central nervous system ***(De Ceglie et al., 2008).***

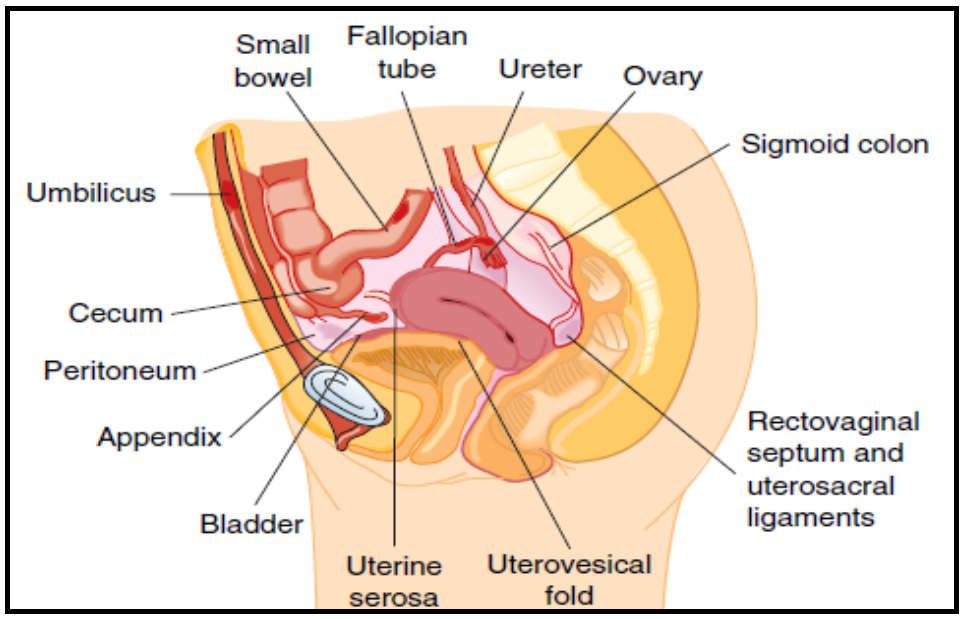

Figure (1): Common locations of endometriosis within the abdomen and pelvis ***(Quoted from Schorge et al., 2008).***

The appearance and size of the implants are quite variable (Fig. 2). Areas of peritoneal endometriosis appear as raised flame-like patches, whitish opacifications, yellow-brown discoloration, translucent blebs, or reddish or reddish blue irregularly shaped islands. The peritoneal surface may be scarred or puckered ***(De Nardi and Ferrari, 2011).***

These may be superficial or described as deep if they extend more than 5 mm beneath the peritoneal surface. Overall appearance may vary from the barely visible through to the ‘‘frozen pelvis’’ ***(Barton-Smith et al., 2006).***

Endometriosis of the ovary may present as superficial implants, or as pelvic masses comprising cyst-like structures (endometriomas) that contain blood, fluid, and menstrual debris ***(De Nardi and Ferrari, 2011).***

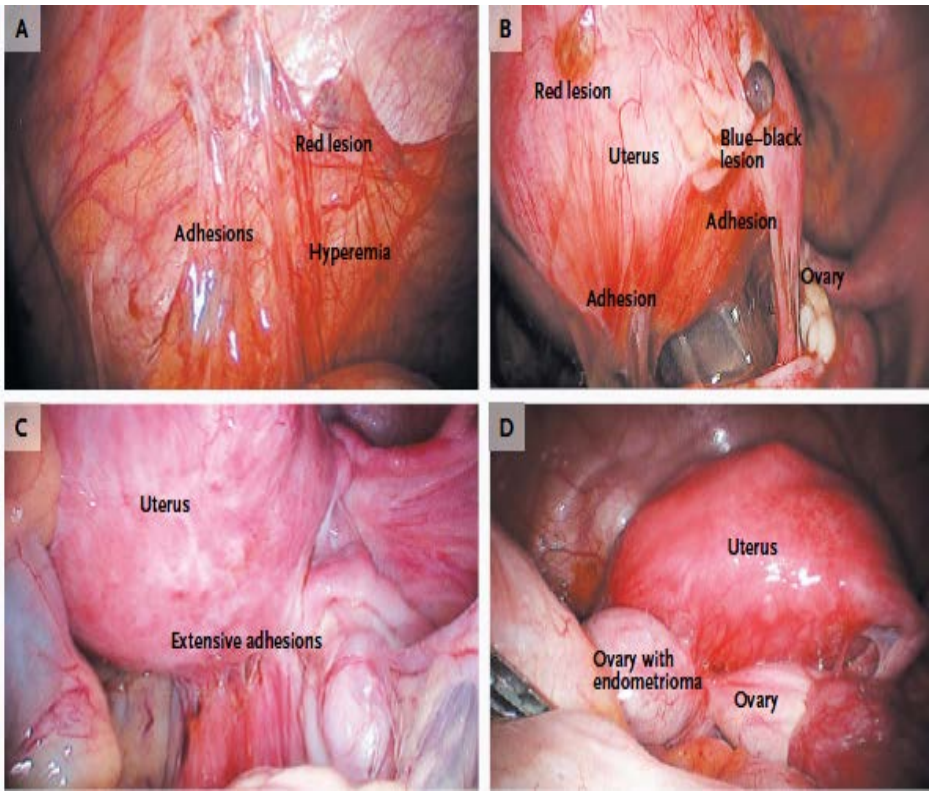

Figure (2): Peritoneal lesions and an ovarian endometrioma due to endometriosis. Panel A shows an endometriotic implant (red lesion), adhesions, and hyperemia in the peritoneum. Panel B shows peritoneal implants, including red and blue-black lesions and adhesions. Panel C shows extensive adhesions distorting the normal pelvic anatomy. Panel D shows an endometrioma adherent to the posterior uterus and distending the ovarian capsule ***(Quoted from Giudice, 2010).***

Superficial Endometriosis:

The term “superficial endometriosis” usually is used synonymously with “peritoneal endometriosis,” although one of the most common superficial locations is the ovarian surface ***(Kocakoc et al., 2008).***

Endometriotic Cysts (Endometriomas):

Endometriomas usually occur within the ovaries and result from repeated cyclic hemorrhage. More than 90% of endometriomas are pseudocysts formed by invagination of the ovarian cortex, which is sealed off by adhesions. Endometriomas may replace normal ovarian tissue completely. Cyst walls usually are thick and fibrotic and frequently have dense fibrous adhesions and areas of discoloration. Cyst content generally is composed of thick, dark, degenerate blood products, an appearance that has been called “chocolate cyst”. Endometriomas are bilateral in approximately 50% of the cases and may be large, although they rarely exceed 15 cm in diameter. Large lesions and lesions with wall nodularity should be considered suspicious and sampled to rule out malignancy. Endometriosis usually regresses substantially after menopause ***(Kocakoc et al., 2008).***

Deep Pelvic Endometriosis:

Deep endometriotic lesions are located most often in the pouch of Douglas, the rectovaginal septum, and the uterosacral

ligaments and more rarely in the vesico-uterine space and are responsible for pelvic pain. The intensity of pelvic pain is proportional to the depth of the lesions. Intestinal involvement occurs in 3% to 37% of the estimated 15% of menstruating women in whom endometriosis develops. Intestinal lesions predominantly affect serosa, muscularis propria, and submucosa; the mucosa is rarely involved ***(Kocakoc et al., 2008).***

Adhesions:

Extensive adhesions can distort the normal pelvic anatomy and obliterate the pouch of Douglas. The diagnosis of kissing ovaries is made when the ovaries are joined behind the uterus in the cul-de-sac and are not separable by pushing the transvaginal probe or by manipulating the uterus transabdominally ***(Kocakoc et al., 2008).***

Extraperitoneal Endometriosis:

Extraperitoneal sites include the lungs, pleura, skin, skeletal muscle, and central nervous system ***(Kocakoc et al., 2008).***

The microscopic appearance of endometriotic tissue is similar to that of endometrium in the uterine cavity; the two major components of both are endometrial glands and stroma (Fig. 3). Unlike endometrium, however, endometriotic implants

often contain fibrous tissue, blood, and cysts ***(De Nardi and Ferrari, 2011).***

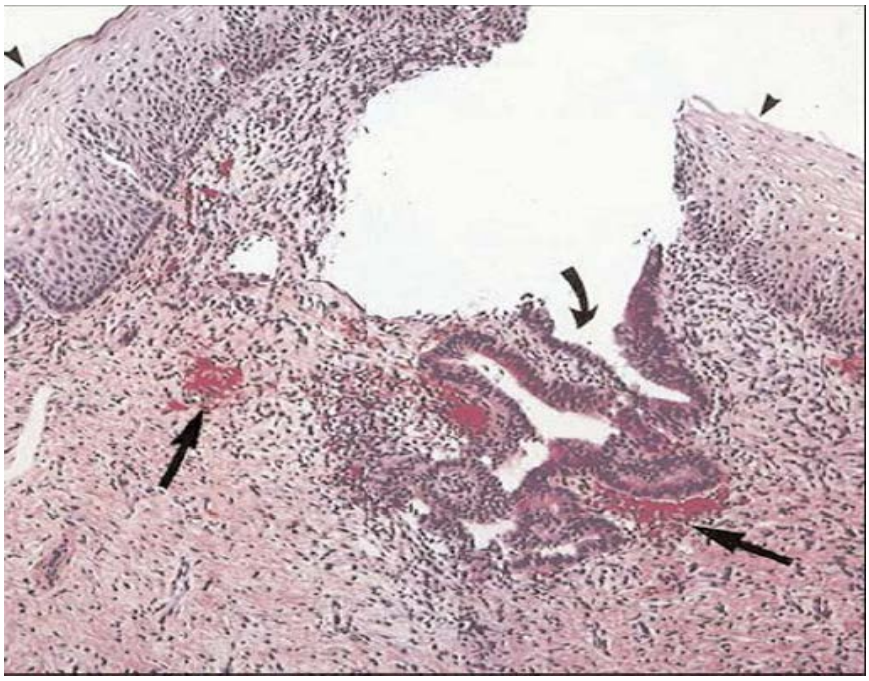

Figure (3): Light micrograph of peritoneal endometriotic implant shows endometrial glandular epithelium (*arrow*) and surrounding stroma ***(Quoted from De Nardi and Ferrari, 2011).***

Classification:

Currently, the most used classification system for endometriosis is The American Fertility Society revised (r-AFS) classification. The original and revised AFS classifications are unique because they provide a standardized form for recording pathologic findings, and because they assign scalar values to disease status in an effort to predict the probability of pregnancy following treatment ***(De Nardi and Ferrari, 2011).***

The scoring system of the r-AFS is directed at the variability in assessing ovarian endometriosis and cul-de-sac obliteration.

To improve the accuracy of the scoring system, ovarian endometriotic cyst should be confirmed by histology or by the presence of the following features:

(1) Cyst diameter <12 cm.

(2) Adhesion to the pelvic sidewall and/or broad ligament.

(3) Endometriosis on surface of ovary.

(4) Tarry, thick, chocolate-colored fluid content.

Cul-de-sac obliteration should be considered partial if endometriosis or adhesions have obliterated part of the cul-de-sac, but some normal peritoneum is visible below the uterosacral ligaments. Complete obliteration of the cul-de sac exists when no peritoneum is visible below the uterosacral ligaments. The morphology of peritoneal and ovarian implants should be categorized as red (red, red-pink, and clear lesions), white (white, yellow-brown, and peritoneal defects), and black (black and blue lesions). The percentage of surface involvement of each implant type should be documented ***(De Nardi and Ferrari, 2011).***

It seems easy to consider deeply infiltrating endometriosis (DIE) as stage IV of the r-AFS classification.

However, this scheme was devised mainly with the object of stratifying patients according to their reproductive prognosis. This is because great value is attributed to ovarian endometriomas in the r-AFS classification. A finding of a bilateral 4 cm ovarian cyst is sufficient to reach a point score indicating severe or fourth-stage endometriosis, according to the patient's reproductive outcome. On the other hand, this apparently advanced stage could be easily improved by a simple surgical approach, whereas a second stage endometriosis could represent a more difficult situation for the surgeon if the total score is obtained by considering the extent of adherences, which often require a laparotomy approach ***(De Nardi and Ferrari, 2011).***

Koninckx and Martin in (1992) were the first to define deep endometriosis. They distinguished posterior cul-de-sac and rectovaginal lesions in three different subgroups:

- Type I, a conically shaped lesion derived from infiltration.
- Type II, a deeply located lesion, surrounded by extensive bowel retraction and adhesions.
- Type III, the most severe form, one or more spherical nodules located in the rectovaginal septum with the largest dimension under the peritoneum, which appears as a small typical lesion or sometimes even a normal peritoneum overlying an indurations.

(De Nardi and Ferrari, 2011)