

Endometriosis- A Systemic Disease?

A Biochemical Investigation.

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**A dissertation submitted in partial fulfilment of the requirement for
the award of M.Sc. in Biomedical Sciences to:**

Virtual School of Biomedical Sciences,

University of Ulster at Coleraine

Coleraine

Northern Ireland

Submitted: November 2001

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ACKNOWLEDGEMENTS

I would like to acknowledge and sincerely thank the following people for their help and support in this study:

Mr Chris Lyons (Hospital Manager) and the Ethics Committee on behalf of Letterkenny General Hospital for granting permission for this study.

Participants in this study, for completing questionnaires and donating blood samples.

The phlebotomy staff and family doctors for assisting in recruitment and drawing blood samples.

The staff of the haematology and biochemistry laboratories in Letterkenny General Hospital for their assistance and support.

Ms Veronica McElhinney (supervisor), Mr William Kinnear (assistance with ELISA testing), Mr Cyril Ward (Chief Technologist).

Dr Eddie Aboud, Consultant Gynaecologist, for reviewing results from this study.

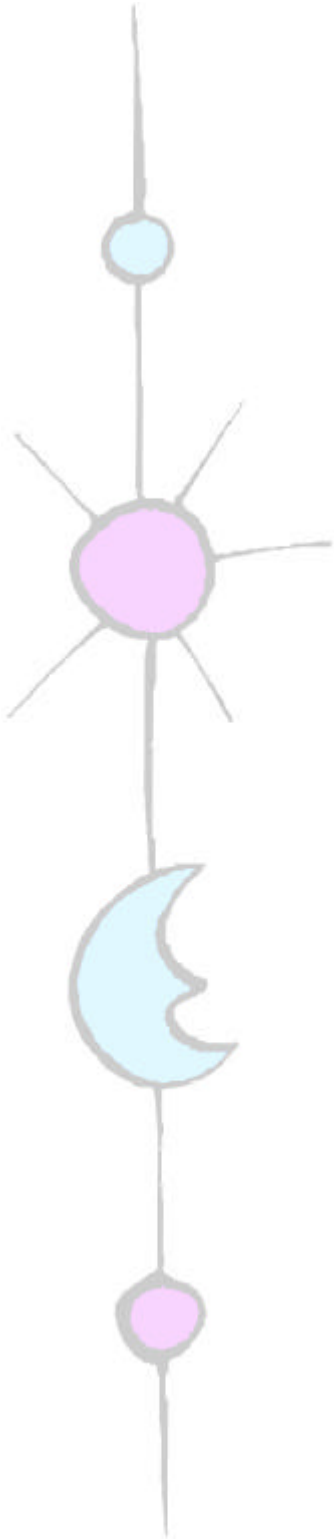
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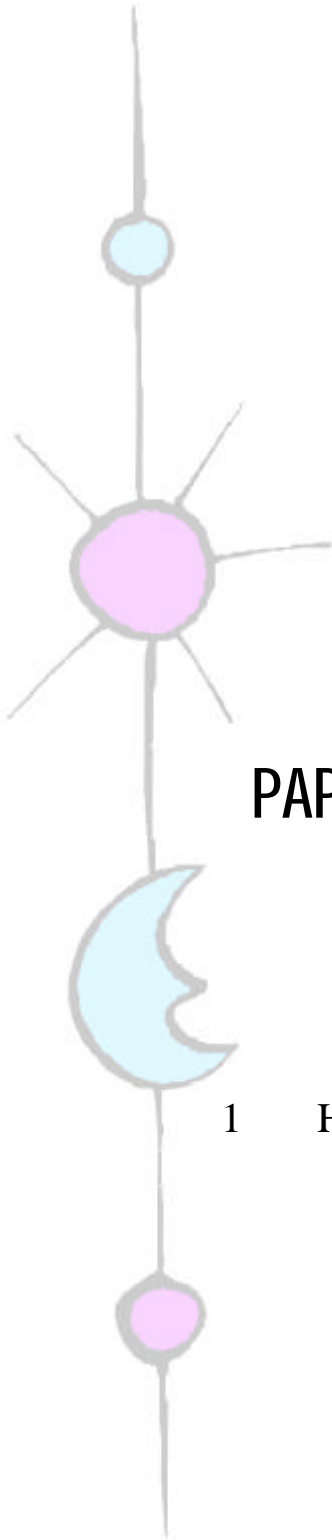
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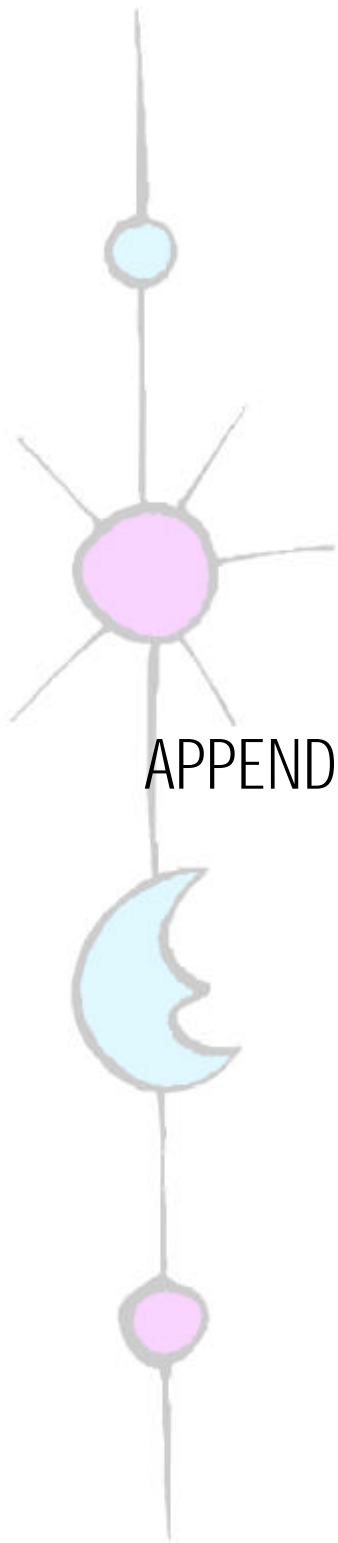


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1 INTRODUCTION

Endometriosis is a condition where the cells that are normally found lining the uterus are also found in other areas of the body: usually within the pelvis. Each month this ectopic tissue, under normal hormonal control, proliferates and is shed in the same way as the endometrium. This internal bleeding into the pelvis, unlike a period, has no way of leaving the body. This leads to inflammation, pain and the formation of scar tissue. Endometriosis is a major cause of infertility and chronic pain. Women with endometriosis may have chronic immune activation due to the presence of endometrial deposits. The immunologic alterations in patients with endometriosis are associated with an exaggerated B-cell response, which can be measured as elevated serum levels of autoantibodies and soluble CD23. Women with endometriosis show a defect in natural killer activity resulting in a decreased cytotoxicity to autologous endometrium. This immune defect in women with endometriosis may account for the incidence of the disease occurring in some women rather than all who experience retrograde menstruation; it may also contribute to symptoms and conditions that women with endometriosis frequently report. In this study it will be important to determine whether women with endometriosis display evidence of an altered immune system. From this it may be evident that endometriosis is indeed a systemic disease, with associated symptoms that are not necessarily gynaecological.

2 THE ENIGMA ENTITLED ENDOMETRIOSIS

The term endometriosis is derived from the ancient Greek:

end means inside

metra means womb

osis means disease, problem or abnormality

The incidence of endometriosis in the general population has been variably assessed with the main consensus being 1/100 women (Rawson, 1991) (Gleicher, 1995). In infertility population the incidence is higher with endometriosis accounting for the single most frequent cause of infertility. Endometriosis also represents a debilitating chronic condition. Pelvic symptoms, chronic pain and associated conditions can lead to absenteeism from work and school. The health care costs for endometriosis encompass not only infertility assessment and treatment but also repeated surgical and medical treatments for the condition itself and its associated symptoms. (Wellbery, 1999)

2.1 Endometriosis

Endometriosis is a condition characterised by the presence of endometrial tissue in ectopic foci outside the uterus. Although not life threatening, endometriosis is a crippling disease that severely compromises patient health and leads to infertility and to the repeated necessity of surgical intervention. Little progress has been made in relation to endometriosis diagnosis or treatment – despite its importance as an increasing worldwide health care problem.

Endometriosis is generally seen as an anatomical lesion. This deposit is defined by visual inspection during laparoscopy. However endometriosis is frequently microscopic in nature therefore making it undiagnosable to the naked eye. Atypical endometriotic lesions are often overlooked; additionally if the laparoscopic surgeon is inexperienced a false negative rate of up to 40% of cases is seen (Gleicher, 1995).

Endometriosis has been seen as an endocrinological condition as its continuous growth can be interrupted through hormonal manipulation – in particular with oestrogen withdrawal (Brosens, 1997). Evidence does not support this concept – as endometriosis can neither be induced nor cured through hormonal manipulation (Gleicher, 1992).

Historically endometriosis has also been seen as a surgical condition. Surgical treatment of endometriosis is indicated in many cases, and may improve symptoms. A disease that may be misdiagnosed in up to 40% of surgical interventions and which only rarely can be cured through surgical intervention should not be considered a surgically treatable condition (Gleicher, 1995).

Endometriosis gives rise to two patient populations. Women with considerable organic disease that results in adhesions and pain differ from women who have minimal disease, yet who are still infertile (Brosens, 1997). Endometriosis related infertility cannot be considered purely mechanical in nature (Martinez-Roman *et al.*, 1997). Endometriosis reduces fecundity in other ways – the method of which remains locked inside the puzzle.

2.2 Theories About The Cause Of Endometriosis

Endometriosis is one of the most frequently encountered gynaecological diseases, second only to fibroids. However, for such a frequently recognised condition – the exact cause and pathogenesis remain unclear. The pathogenesis of endometriosis has been debated and most theories fall into two broad divisions. (1) Development in situ by metaplasia or (2) development as a consequence of the dissemination of endometrium (Oral and Arici, 1997). The first widely considered theory of histogenesis was coelomic metaplasia; ovarian and Müllerian ducts are derived from coelomic mesothelium and it is proposed that the germinal epithelium of the ovary is responsible for endometriosis in this site (Matsuura *et al.*, 1999). This accounts for ovarian endometriosis. Endometriosis in the pelvis and peritoneum are considered to have developed from in situ metaplasia of the serosal mesothelium. However flaws in this theory exist, endometriosis has developed in women without endometrium (congenital absence of the uterus), also if coelomic metaplasia occurs in the peritoneum endometriosis would be found in men. Finally endometriosis should only then occur in sites with coelomic membranes – endometriosis has been found in every site in the body with exception of the spleen.

Sampson (1927) initiated the theory of retrograde menstruation in 1927, in which he proposed that menstrual effluent contained viable endometrial cells that could be transplanted to ectopic sites. Retrograde menstruation is an event seen commonly in women (Vinatier *et al.*, 1996) – Novak (1931) questioned why a physiological event should frequently give rise to pathology – and yet there has been no satisfactory explanation. Keetel and Stein (1951) proved that menstrual effluent did contain viable endometrial cells by culturing tissue fragments of endometrium. In support of this theory viable endometrial cells have been found in menstrual effluent and in the

peritoneal fluid, endometrium can be implanted experimentally and grown within the peritoneal cavity and thirdly the fact that all women have some degree of retrograde menstruation. Dissemination of endometrial cells through lymphatic or vascular channels may account for the finding of endometriosis at sites distant from the pelvis. Cases of endometriosis have been documented in episiotomy and laparotomy scars following gynaecological procedures and caesarean section. Such observations suggest that ectopic endometrium can be induced iatrogenically by mechanical transplantation (Kale *et al.* 1971).

A familial probability of developing endometriosis is suspected – endometriosis is found more commonly in patients with familial history of the disease. It is thought to occur through a maternal inheritance pattern (Mathur, 2000).

Immune mechanisms are believed to be involved in the development of endometriosis. This is discussed in more detail in a later chapter.

2.3 Diagnosing Endometriosis

Endometriosis should be considered in any woman of reproductive age who has pelvic pain. The most common symptoms are dysmenorrhoea, dyspareunia and low back pain that are worse around menses. Other symptoms include pain with urination or bowel movements. Endometriosis should also be considered in women who develop cyclical pain after years of pain free cycles. Infertility is also a presenting complaint; infertility patients may have no painful symptoms and their disease is only uncovered in the course of the diagnostic work-up for infertility. Physical examination may reveal masses in the pelvic area, nodules on the uterosacral ligaments or a fixed or retroverted uterus. However most women with endometriosis have normal pelvic

findings on examination. Presently, the definitive tool for diagnosis is laparoscopy (Brosens and Brosens, 2000). No single laboratory test has shown reliable unique predictability; it is thought that a combination of biochemical markers and clinical assessment will decrease the need for surgical confirmation. Imaging techniques such as ultrasonography, computed tomography and magnetic resonance imaging may be useful in identifying individual lesions – but their role is limited in diagnosis and evaluation of the extent of the disease. (Guarnaccia and Olive, 1997) (Mathur, 2000)

Endometriosis is staged using the American Fertility Society's (American Society for Reproductive Medicine, 1996) classifications based on location, diameter and depth of lesions. Correlation between stage and extent of the disease remains controversial – staging of the disease by anatomical means only, does not correlate with the clinically important characteristics of endometriosis. Women with Stage I or II disease (mild to moderate) may have more painful symptoms than a woman with stage III or IV. There is some evidence that mild to moderate endometriosis may be more active forms of the disease with implants being more biochemically active – hence causing more pain.

2.4 A Systemic Overview

Endometriosis appears to be a gynaecological manifestation of a syndrome. It is emerging that it may be the tip of a much larger invisible iceberg – one that represents a whole range of health problems that have underlying hormonal and/or immune dysregulation. Women with endometriosis are presenting with a whole range of symptoms in addition to those traditionally associated with the disease. Along with dysmenorrhea, dyspareunia and infertility – high incidences of allergies, chemical sensitivities, tendency to infections, mitral valve prolapse and *Candida albicans*

related problems are being noted in women with endometriosis. Chronic Fatigue Syndrome, Chronic Immune Dysfunction Syndrome and an increased risk for autoimmune disorders is also observed. A large study recently completed by The Endometriosis Association has provided strong evidence that not only does endometriosis have a hereditary and familial tendency, certain other diseases are more likely to occur in women and relatives of women with endometriosis. (Ballweg, 1995)

The Endometriosis Association study showed that there is an increased risk of breast cancer, ovarian cancer, and melanoma in women and in the relatives of women who have endometriosis. The studies have also shown that the families of women with endometriosis have a higher risk of non-Hodgkin's lymphoma (Duczman and Ballweg, 1999). Women with endometriosis have a higher incidence of thyroid disease including an underactive thyroid (Hypothyroidism), an overactive thyroid (Hyperthyroidism or Graves' Disease), and Hashimoto's Thyroiditis. (Ballweg, 1995)

In addition, other autoimmune diseases that are seen somewhat more frequently in women with endometriosis and in their immediate families include Rheumatoid Arthritis, Lupus, Multiple Sclerosis, and Meniere's disease. If endometriosis is neither an endocrinological or surgical disease – could it be an autoimmune disease?

3 IMMUNOLOGICAL ASPECTS OF ENDOMETRIOSIS

Autoimmune diseases are now widely believed to occur based on a genetic predisposition that may be triggered by environmental cofactors. Normal women do not allow survival of ectopic endometrium. Endometriosis may be considered analogous to tumour cells and immune system surveillance of tumour cells. A normally functioning immune system will not allow survival anywhere but in the endometrial cavity. A normally controlled immune system does not permit survival of newly arising neoplastic cells and similarly ectopic endometrial cells should also be successfully destroyed. In women with endometriosis a dysfunctional immune system may permit the continuous growth of ectopic endometrium. Retrograde menstruation is thought to occur in greater than 90% of women (Vinatier *et al.*, 1996) – in some women refluxing endometrial cells are not destroyed, either due to genetic predisposition not to respond to endometrial antigens or because the menstrual effluent is so abundant that the scavenging capacity of the peritoneal immune cells is overloaded. These ectopic cells may be protected due to abnormal adherence to the mesothelium. Undestroyed, these ectopic endometrial cells may cause inflammation with activation of macrophages (Senturk and Arici, 1999) (Vinatier *et al.*, 2000).

3.1 A disease of cell mediated immune response

Several observations in women and in female monkeys have lead to the suggestion of a defective T cell response in endometriosis. Non-specific immune responses are identical however for both women with and without endometriosis (Vinatier *et al.*,

1996). The incidence of endometriosis in Belgium is one of the highest in the world; this is considered linked to the high levels of dioxin pollution (Koninckx *et al.*, 1991). Dioxin pollution is known to suppress cell-mediated immunity. In women with endometriosis it is possible that alterations in T cell immunity facilitate implantation of endometrial cells in ectopic locations, this may be by direct interference with T cell cytotoxicity or indirectly by altering cooperation with other immune cells such as macrophages, natural killer cells and B cells (Braun and Dmowski, 1998) (Senturk and Arici, 1999). Cunningham *et al.* (1992) demonstrated that in women with mild endometriosis there is a preservation of T-helper function with a decrease of T-suppressor activity. In women with severe endometriosis, there is a reduction in T-suppressor activity yet an increase in T-helper activity.

3.2 A disease of humoral mediated immune response

Immunofluorescence techniques demonstrate the presence of immunoglobulin A (IgA) antibodies and C3 complement fraction on the uterine endometrium (Dmowski, 1995) (Lebovic *et al.*, 2001). This evidence when considered with a decrease in total complement proteins may be an indication of an autoimmune response with local activation and consumption of complement factors by the antigen – antibody complexes. Varying results have been obtained in studies comparing results of the concentrations of non-specific factors. Serum and peritoneal immunoglobulin levels have been reported as decreased (Meek *et al.*, 1988), no difference (Gilmore *et al.*, 1992) and in some reports increased (Gleicher *et al.*, 1987). Measurements of complement factors also differ. The relationship between atypical autoantibodies and endometriosis has also been studied (Kaider *et al.*, 1999) (Levent and Arici, 1999)

(Iborra *et al.*, 2000). It is noted that the majority of endometriosis patients present with autoimmune abnormalities. Antibodies to phospholipids, histones and carbonic anhydrase (Kiechle *et al.*, 1994) show large degrees of positivity in women with endometriosis. High levels of antibodies to carbonic anhydrase are associated to endometriosis related inflammation (D’Cruz *et al.* 1996). Evidence of activated B cells in endometriosis has been detected by high serum concentrations of soluble CD23 produced by activated B cells from their membranes (Dmowski, 1995). Serum concentrations of soluble CD23 are documented to decrease significantly on treatment with danazol. (Odukoya *et al.*, 1995) (Matalliotakis *et al.*, 2000)

There is no apparent correlation between the severity of endometriosis and the autoantibody titre – this finding is contrary to most autoimmune diseases. This may imply that the production of autoantibodies is a secondary event. (Gleicher, 1994) (Vinatier *et al.*, 1996)

3.3 A disease of the endometrium

Several features differ ectopic endometrium from native uterine cells. This suggests an abnormality of the endometrium in endometriosis patients. Ectopic endometrium has steroid specific receptors and also expresses epidermal growth factor and its receptors (Braun and Dmowski, 1998) (Vinatier *et al.*, 2000). Circulating autoantibodies to endometrial antigens have been demonstrated in all women, while in women suffering from endometriosis an additional autoantibody has been found (Mathur *et al.*, 1995). Again, no correlation between the severity of the disease and the concentration of antibodies has been found. Women with endometriosis do not possess a particular (HLA) human leukocyte antigen profile (Simpson *et al.*, 1984) (Steele *et al.*, 1984).

Endometrial cells of endometriotic patients seem to be recognised by the immune system. Endometrial cells stimulate the proliferation of lymphocytes, mediated by cytokines (Harada *et al.*, 1999). Interleukin 6 (IL-6), produced by normal endometrium may be a lymphocyte stimulator. In patients with endometriosis, autologous endometrial cells are incapable of secreting cytokines or of obtaining necessary stimulation or by producing inhibitors of proliferation. Endometriotic lesions can produce immunosuppressive factors (Harada *et al.*, 1996). Ectopic endometrium appears to have independence; it is capable of producing complement C3 (Vinatier *et al.*, 1996) (Vintanier *et al.*, 2000). Endometriotic tissue locally secretes complement factors, which are chemotactic for lymphocytes and macrophages. Chemotactic substances (Murphy *et al.*, 1998) for macrophages are produced in a cyclical pattern by the endometrial stroma with a peak during luteal phase. The endometrium of women with endometriosis secretes larger amounts of these chemotactic factors with a loss of the cyclical pattern. Endometriotic tissues also differ from normal endometrium by the presence of lymphocytes. Scattered lymphocytes and cells containing γ -interferon (IFN γ) are more numerous in endometriosis (Senturk and Arici, 1999). The presence of IFN γ receptors in endometriotic lesions may suggest their possible role in the regulation of the proliferation of endometriotic lesions.

3.4 A disease of the macrophage

Macrophages constitute 85% of all the cells in the peritoneal fluid, with the remaining 15% consisting of shed cells and lymphocytes. The number of macrophages varies with the menstrual cycle – peaking in the postmenstrual phase. In women with endometriosis macrophages are at a higher level of activation (Dmowski, 1995)

(Koninckx *et al.*, 1999). This increased activation is measurable by increased size of the macrophages, release of substantial amounts of complement factors C3 and C4 and an increase of the secretion of lysosomal phospholipase (Halme *et al.*, 1988) (Vintanier *et al.*, 2000). This phospholipase is capable of acting on membranous phospholipids – thus releasing a source of arachidonic acid used to synthesise prostaglandins. This may be the cause of raised peritoneal prostaglandins in women with endometriosis – this raised level is thought to account for some of the painful symptoms encountered (Zriek and Olive, 1997). Macrophages may contribute to the initiation, development and growth of endometriosis. Macrophages could be involved in the adherence of endometrial cells on the peritoneum, by production of fibronectin (involved in the adhesion of endometrial cells), fibronectin also acts as a complement factor permitting the conversion of endometrial cells from stage G₀ to stage G₁ of the cell cycle – thus rendering them sensitive to growth factors. Oestrogen may be one of these growth factors. An increased activation of peritoneal macrophages combined with a relative hyperoestrogenaemia could permit proliferation of retrograde endometrial cells (Vintanier *et al.*, 1996).

Activated macrophages secrete tumour necrosis factor α (TNF α). TNF α is involved in adherence (Sharpe-Timms, 1997). Generalised symptoms (malaise, body aches and pains) experienced by women with endometriosis may be due to systemic effects secondary to an increase in production of TNF α . Women with endometriosis have elevated levels of TNF α , these elevated levels correlate with the stage of disease (Hong-Nerng *et al.*, 1996) (Lebovic *et al.*, 2001). Transforming growth factor β (TGF β) is produced by peritoneal macrophages; it is another cytokine whose concentration is raised in women with endometriosis. TGF β is normally involved in

tissue repair, an excess of TGF β leads to defective scars and adherences, it also leads to proliferation of stromal cells of the endometrium (Senturk and Arici, 1999) (Vinatier *et al.*, 2000). TGF β stimulates angiogenesis; endometriosis can only develop and proliferate if angiogenesis takes place. Blocking angiogenesis is a possible therapeutic approach for endometriosis.

3.5 A disease of natural killer cells

Natural killer (NK) cells are directly cytotoxic; their aggressiveness is moderated by several cytokines. Circulating NK cells efficiently destroy endometrial cells. In endometriosis patients the cytotoxic power of circulating NK cells against endometrium is diminished (Hill *et al.*, 1992) (Dmowski, 1995). The sera of these patients contain an inhibiting factor against NK cells. In endometriosis the endometrium becomes resistant to the cytotoxicity of NK cells. Steroid hormones may have a role in the alteration of NK function; the reduction of NK activity could be correlated with serum concentrations of oestradiol (whose immunodepressive properties are known) (Garzetti *et al.*, 1993) this may suggest that oestradiol is involved in the evolution of endometriosis rather than its onset (Vinatier *et al.*, 1996).

4 ORGANOCHLORIDES AND ENDOMETRIOSIS

Environmental contaminants are defined as unwanted by-products of synthetic chemicals that have no commercial use but have become ubiquitous in the environment. Chemical pollutants are now recognised as a major threat to the health and well being of all living organisms. These toxins usually affect specific organs or systems. Among environmental toxins known to affect immune and reproductive systems are numerous organochlorides, including 1,1,1-trichloro-2,2-bis(4-chlorophenyl)ethane (DDT), polychlorinated biphenyls (PCBs) and 2,3,7,8-tetrachloro-dibenzo-*p*-dioxin (TCDD or dioxin). Of the numerous environmental chemicals with biological action, dioxin and dioxin like compounds are classic examples of endocrine and immune disruptors in human and wildlife populations. (Osteen and Sierra-Rivera, 1997) (Koninckx, 1999)

4.1 Hormonal and Immunological Effects

In humans dioxins are known to alter gene expression and to affect genes involved in inflammation and differentiation. Dioxins have a major effect on the immune system; dioxins inhibit T lymphocytes and cytokine production and decrease natural killer cell activity. Dioxin can act as an anti oestrogen or as a weak oestrogen. Dioxins are metabolically stable and they accumulate in the environment and in animal tissues. Animals and humans consume dioxins when eating fish and other animals that were exposed to the pollutants. Dioxins are excreted in breast milk. (Osteen and Sierra-Rivera, 1997) (Koninckx, 1999)

4.2 Dioxins and endometriosis

The possible relationship between endometriosis and industrialisation is worrying. The reported prevalence of endometriosis has increased in industrialised countries. This trend may be subject to a recognition bias. Microscopic, deep or clear lesions have been identified and increased recognition among gynaecologists may account in part for this trend. There is some evidence to support the claim that dioxin exposure is related to the development or worsening of endometriosis. In a long term controlled study of 24 rhesus monkeys by Rier *et al.* (1995), groups of monkeys were exposed to either no dioxin or 5 or 25 parts per trillion dioxin for 4 years. At the end of the study (10 years after termination of dioxin exposure) the incidence of endometriosis was directly correlated with dioxin exposure, and the severity of the endometriosis was dose related. Studies of dioxin exposure in women with endometriosis have found a possible link between endometriosis and dioxin. Mayani *et al.* (1997) found in their study that among endometriosis patients 18% were found positive for dioxin as compared to 3% in the controls. They did not however find a correlation between dioxin concentrations in the blood and the severity of endometriosis, however they speculate that the degree of sensitivity to dioxin might vary among different individuals.

Osteen and Sierra-Rivera (1997) demonstrated that matrix metalloproteinase (MMP) expression in endometriotic lesions appeared to be altered relative to normal endometrium. MMP expression is required for extracellular matrix breakdown and tissue invasion. MMP's are found to contribute to the establishment of endometriosis in an experimental model (Nothnick, 2001). (Bruner *et al.* 1997) In addition expression of local growth factors and cytokines may contribute to MMP

misexpression at these ectopic sites. They suggest that some of the deleterious effects of dioxin exposure on reproductive function in addition to endometriosis may relate to MMP expression and action. Dioxin exposure may block suppression of an endometrial MMP that is normally highly sensitive to progesterone regulation. The ability of dioxin to interfere with progesterone mediated MMP suppression could partially explain why dioxin exposure leads to an increased incidence of endometriosis. Dioxin exposure may also increase the production of cytokines by immune or somatic cells within endometrial tissue, which can also lead to an increase in MMP expression.

Koninckx *et al.* (1994) in a report on dioxin pollution and endometriosis in Belgium suggest further investigation on this matter. The incidence of endometriosis in Belgium at the time of the report was 60-80% of women with infertility and/or pain; one of the highest reported incidences in the world. Koninckx and Martin (1992) reported the first and largest series of deeply infiltrating endometriosis – also originating in Belgium. Dioxin concentrations in breast milk in Belgium are among the highest in the world.

4.3 Tampons, dioxins and endometriosis

Taking into consideration that retrograde menstruation is one of the theories concerning the cause of endometriosis; the possibility that tampon use might increase incidence of this event has been suggested. Women with endometriosis do not appear to use tampons more than the general population of women (Maloney, 1999) (Scialli, 2001). Additionally retrograde menstruation is believed to occur in a large percentage

of women regardless of tampon use. Another consideration is that tampons may contain dioxins as a result of the manufacturing process (Scialli, 2001).

Many tampons contain rayon; some are made from cotton/rayon blend while some are all cotton. Rayon is a cellulose fibre made from wood pulp – which is purified before manufacture. Chlorine dioxide is used in this bleaching process – this results in dioxin levels in final pulp fibres below 0.1 parts per trillion. Due to widespread background environmental contamination with dioxins, all tampons may contain extremely low levels of dioxins. (Maloney, 1999)

An article entitled “The Truth About Tampons” (Holmes, 1990) the author cites an 1981 FDA study that found boron, aluminium, waxes, surfactants, alcohols, acids, nitrogen compounds and hydrocarbons occurring in tampons. These chemicals may be a health hazard to chemically sensitive women; many women with endometriosis are believed to be chemically sensitive (Ballweg, 1997).

Scialli (2001) concludes that there is no credible evidence that tampons, whether rayon or cotton, bleached or unbleached, contribute to endometriosis. His findings state that on a wide array of products – the analytical data would be indistinguishable. Nor did he find any credible evidence that exposure to the potential low levels of dioxins in tampons would contribute to human exposure.

5 LABORATORY EVALUATION

Chronic inflammation is considered where a prolonged duration, in which active inflammation, tissue destruction and attempts at repair are proceeding simultaneously. Chronic inflammation includes some of the most common and disabling human diseases, such as rheumatoid arthritis, atherosclerosis, tuberculosis and chronic lung diseases. Endometriosis deposits may invoke a chronic inflammatory state.

5.1 Erythrocyte Sedimentation Rate

The erythrocyte sedimentation rate (ESR) determination is a simple and inexpensive laboratory test that is frequently ordered in clinical medicine. The test measures the distance that erythrocytes have fallen after one hour in a vertical column of anticoagulated blood under the influence of gravity. Although there is an enormous body of literature concerning the ESR, an elevated value remains a non-specific finding. Women tend to have higher ESR values; the female normal range is 5-15 mm/hour.

5.2 C Reactive Protein

C-reactive protein (CRP) is a glycoprotein produced during acute inflammation or tissue destruction. Major activation functions of CRP are the ability to activate the classic complement pathway after interaction with many of its biologic ligands and the ability to bind to and modulate the behaviour of phagocytic cells in proinflammatory

and anti-inflammatory ways, suggesting that CRP may play many pathophysiologic roles during the course of the inflammatory process. There are few studies on endometriosis and CRP (Abrão *et al.*, 1997); the association of CRP with Interlukin 1 and Interlukin 6 (Harada *et al.*, 1996) (Lebovic *et al.*, 2001) and tumour necrosis factor (Iwabe *et al.*, 1999) also involved in endometriosis, indicate its potential use in the diagnosis of the disease.

5.3 Proteins, Ferritin and Cortisol.

Studies comparing serum and peritoneal concentrations of immunoglobulins are variable (Odukoya *et al.*, 1995) (Meek *et al.*, 1988) (Gleicher *et al.*, 1987). Serum albumin and globulin are also markers of inflammation.

Cortisol influences the immune system and many other body systems. Cortisol suppresses inflammation and cellular immune activation, and reduced levels might relax constraints on inflammatory processes and immune cell activation.

Ferritin is a high molecular weight iron containing protein that functions in the body as an iron storage compound. Ferritin levels are also raised in acute or chronic inflammation; the increase is seen as disproportionate in relation to iron stores. (Jacobs, Worwood 1975)

5.4 Autoantibodies

A number of autoimmune diseases have been correlated with the presence of antinuclear antibodies. Reproductive autoimmune failure syndrome (RAFS) is

included among autoimmune diseases. RAFS is the association of recurrent pregnancy loss, unexplained infertility or endometriosis with the presence of circulating autoantibodies, including antinuclear antibodies (Kaider *et al.*, 1999). Antiphospholipid antibodies are a group of organ non-specific antibodies that bind to phospholipids. Their presence has been associated with reproductive failure characterised clinically as unexplained infertility, recurrent pregnancy loss and endometriosis (Roussev *et al.* 1996) (Lucena and Cubillos, 1999). The presence of antiphospholipid and antinuclear antibodies is detected using an ELISA (Enzyme Linked Immunosorbent Assay) method (Pierangeli *et al.*, 2001).

5.5 sCD23

Soluble CD23 (sCD23) is a fragment produced by the proteolytic cleavage of the transmembrane glycoprotein CD23. Elevation of serum soluble CD23 concentrations reflects activation of B cells (Gordon, 1992). Polyclonal activation of B cells has been demonstrated among patients with endometriosis. Serum soluble CD23 concentrations are significantly elevated among patients with endometriosis when compared with controls (Odukoya *et al.*, 1996). This is assumed due to an increase in activity of T cells mediated through Interlukin 4. Interlukin 4 is produced by cultures of endometrial and stromal cells from women with endometriosis. No apparent correlations between soluble CD23 concentration and extent of disease have been observed. Danazol (but not gonadotropin releasing hormone agonists (GnRHA)) treatment results in a significant fall in sCD23 concentrations. (Odukoya *et al.*, 1995) (Matalliotakis *et al.*, 2000) The findings suggest that immunomodulating therapy should reduce the recurrence rate of endometriosis.

5.6 Thyroid Function Tests

Women with Endometriosis have a higher incidence of thyroid disease including hypothyroidism, hyperthyroidism, Graves' disease and Hashimoto's Thyroiditis. Thyroxine (T4) circulates in the blood as an equilibrium mixture of free and serum bound hormone. Free T4 values provide a good indication of thyroid dysfunction, as free T4 is less sensitive to changes in serum binding proteins. Human thyroid stimulating hormone (TSH) stimulates the production and secretion of the metabolically active thyroid hormones (T4 and Triiodothyronine (T3)). T3 and T4 are responsible for regulating diverse biochemical processes throughout the body. Primary hypothyroidism is associated with low T3 and T4 levels and elevated TSH levels. Primary hyperthyroidism is associated with high levels of T3 and T4 and low or undetectable levels of TSH. T4 and TSH are measured using immunoassay techniques (Chemiluminescent Microparticle Immunoassay). (Burger *et al.*, 1972) The incidence of diabetes in the general population is approximately 6%. However, in the families of women with endometriosis, the incidence is 42%. (Ballweg, 1995)

**Incidence of disease in women with endometriosis,
their families, and the general population.**

Disease	Women With Endometriosis	Their Families	General Population
Hypothyroidism	7%	15%	2%
Hyperthyroidism	1.5%	6%	1%
Hashimoto's	2%	2%	0.01%
Rheumatoid Arthritis	2%	17%	0.8%
Lupus	0.8%	6%	0.05%
Multiple Sclerosis	0.6%	6%	0.1%
Meniere's Disease	0.9%	3%	0.2%

(Adapted from Ballweg 1995)

5.7 CA 125

CA 125 is a mucin like molecule that is produced by mesothelial cells of the peritoneum and endometrium. CA 125 is a serum marker for monitoring patients with epithelial ovarian cancer (Bast *et al.*, 1998). Elevated CA 125 levels have been associated with a number of benign conditions, and CA 125 has been detected in several normal tissues including the endometrium and the lung. CA 125 has been evaluated for management of benign gynaecologic diseases such as endometriosis (Fang-Ping *et al* 1998). Mol *et al.* (1998) conclude that the performance of CA 125 measurement in the detection of endometriosis was low, but was better for the detection of severe endometriosis. Serum levels of CA 125 are elevated in women

with endometriosis, with the more marked increases shown in women with stage III and IV, compared with Stage I and II. The value of CA 125 as a tool for monitoring treatment or disease progression has also been noted. (Abrão *et al.*, 1997) (Chen *et al.* 1998) (Imai *et al.*, 1998) (Abrão *et al.*, 1999) (Vinatier *et al.*, 2000)

6 AIMS OF STUDY

- To demonstrate that endometriosis is a systemic disease, defined by a collection of symptoms, not just a gynaecological manifestation.
- To determine evidence of the immune system involvement by measuring key factors, and use this evidence as a supplemental diagnostic indicator for women with suspected endometriosis.
- To assess a group of women with endometriosis versus women without any gynaecological / health problems, to determine any differences in areas that may indicate that endometriosis does have a characteristic biochemical pattern.

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Endometriosis – A Systemic Disease?

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ABSTRACT

Background

Endometriosis is a major cause of infertility and chronic pain. Women with endometriosis may have chronic immune activation due to the presence of endometrial deposits. The objective of this study was to determine whether women with endometriosis display evidence of an altered immune system and to seek evidence for a diagnostic biochemical profile. From this it may be evident that endometriosis is indeed a systemic disease, with associated symptoms that are not necessarily gynaecological.

Methods

Thirty-four women living in the North Western Health Board area were recruited to take part in the study. Endometriosis group (13) control group (21). All women completed a questionnaire about menstrual history and lifestyle factors. Blood was collected for analysis of antinuclear antibodies, anticardiolipin antibodies, CA 125 and other markers of inflammation.

Results

With exception to CA125 and basophil levels, no marked statistically significant differences were found between the blood profiles of both study groups. Questionnaire data correlated with previous literature findings on endometriosis.

Conclusions

This study should be carried out with multi regional participation to ensure a larger sample number. The diagnosis rate of endometriosis in the North Western region appears to be lower than the estimated 10% of the female population.

Key Words: biochemical / endometriosis / immune / inflammation / systemic

INTRODUCTION

Endometriosis is a condition characterised by the presence of endometrial tissue in ectopic foci outside the uterus. Although not life threatening, endometriosis is a crippling disease that severely compromises patient health and leads to infertility and to the repeated necessity of surgical intervention. Endometriosis is a major cause of infertility and chronic pain. Women with endometriosis may have chronic immune activation due to the presence of endometrial deposits. The immunologic alterations in patients with endometriosis are associated with an exaggerated B-cell response, which can be measured as elevated serum levels of autoantibodies and soluble CD23. Women with endometriosis show a defect in natural killer activity resulting in a decreased cytotoxicity to autologous endometrium. This immune defect in women with endometriosis may account for the incidence of the disease occurring in some women rather than all who experience retrograde menstruation; it may also contribute to symptoms and conditions that women with endometriosis frequently report. The incidence of endometriosis in the general population has been variably assessed with the main consensus being 1/100 women (Rawson, 1991) (Gleicher, 1995). In infertility populations the incidence is higher with endometriosis accounting for the single most frequent cause of infertility. Little progress has been made in relation to endometriosis diagnosis or treatment – despite its importance as an increasing worldwide health care problem.

Endometriosis is generally seen as an anatomical lesion. This deposit is defined by visual inspection during laparoscopy. However endometriosis is frequently microscopic in nature therefore making it undiagnosable to the naked eye. Atypical

endometriotic lesions are often overlooked; additionally if the laparoscopic surgeon is inexperienced a false negative rate of up to 40% of cases is seen (Gleicher, 1995).

Endometriosis has been seen as an endocrinological condition as its continuous growth can be interrupted through hormonal manipulation – in particular with oestrogen withdrawal (Brosens, 1997). Evidence does not support this concept – as endometriosis can neither be induced nor cured through hormonal manipulation (Gleicher, 1992).

Historically endometriosis has also been seen as a surgical condition. Surgical treatment of endometriosis is indicated in many cases, and may improve symptoms. A disease that may be misdiagnosed in up to 40% of surgical interventions and which only rarely can be cured through surgical intervention should not be considered a surgically treatable condition (Gleicher, 1995).

Endometriosis gives rise to two patient populations. Women with considerable organic disease that results in adhesions and pain differ from women who have minimal disease, yet who are still infertile (Brosens, 1997). Endometriosis related infertility cannot be considered purely mechanical in nature (Martinez-Roman *et al.*, 1997). Endometriosis reduces fecundity in other ways – the method of which remains locked inside the puzzle.

Autoimmune diseases are now widely believed to occur based on a genetic predisposition that may be triggered by environmental cofactors. Normal women do not allow survival of ectopic endometrium. Endometriosis may be considered analogous to tumour cells and immune system surveillance of tumour cells. A normally controlled immune system does not permit survival of newly arising neoplastic cells and similarly ectopic endometrial cells should also be successfully

destroyed. In women with endometriosis a dysfunctional immune system may permit the continuous growth of ectopic endometrium.

Evidence of immune system alteration can be measured in the laboratory by inflammatory and autoimmune markers. A number of autoimmune diseases have been correlated with the presence of antinuclear antibodies. Antiphospholipid antibodies are a group of organ non-specific antibodies that bind to phospholipids. Their presence has been associated with reproductive failure characterised clinically as unexplained infertility, recurrent pregnancy loss and endometriosis. Reproductive autoimmune failure syndrome (RAFS) is included among autoimmune diseases. RAFS is the association of recurrent pregnancy loss, unexplained infertility or endometriosis with the presence of circulating autoantibodies, including antinuclear antibodies. There is no apparent correlation between the severity of endometriosis and the autoantibody titre – this finding is contrary to most autoimmune diseases. This may imply that the production of autoantibodies is a secondary event. (Gleicher, 1994) (Vinatier *et al.*, 1996)

Erythrocyte sedimentation rate (ESR), a non-specific test, has three major uses: as an aid in detection and diagnosis of inflammatory conditions; as a means of following the activity, clinical course, or therapy of diseases with an inflammatory component, and to demonstrate or confirm the presence of occult organic disease, either when the patient has symptoms but no definite physical or laboratory evidence of organic disease or when the patient is completely asymptomatic. Infectious diseases, neoplasia, non-infectious inflammatory conditions and chronic renal disease cause marked elevation of ESR. C reactive protein, (CRP) concentrations are significantly increased in women with polycystic ovarian syndrome (PCOS) relative to those in

healthy women with normal menstrual rhythm and normal androgen levels. CRP is a marker of low-grade chronic inflammation. Ferritin levels are also raised in acute or chronic inflammation. Serum albumin and globulin are also markers of inflammation. Cortisol influences the immune system and many other body systems. Cortisol suppresses inflammation and cellular immune activation, and reduced levels might relax constraints on inflammatory processes and immune cell activation. CA 125 is a mucin like molecule that is produced by mesothelial cells of the peritoneum and endometrium. Serum levels of CA 125 are elevated in women with endometriosis, with the more marked increases shown in women with stage III and IV, compared with Stage I and II.

Alone these indices cannot determine the presence of endometriosis, however when tested together as a profile along with other indices that indicate chronic inflammation and immune activation, they may assist in diagnosis.

This study will estimate the usefulness of these serum indices as a diagnostic tool to be used alongside medical history and symptoms. It also demonstrates that endometriosis has systemic effects, measurable by biochemical markers.

MATERIALS AND METHODS

Thirty-four women were recruited to take part in the study. Thirteen women were diagnosed with endometriosis previously, either by laparoscopy or laparotomy. The remaining twenty-one women were described as the control group and had not received a diagnosis of endometriosis. All women were asked to complete a questionnaire about menstrual history and general lifestyle factors. Women with endometriosis completed an additional questionnaire regarding endometriosis symptoms and treatments. Blood samples were collected from these women, through the hospital phlebotomists or the patient's family doctor. Blood was collected in to serum tubes with clot activator and gel for serum separation (Becton-Dickinson Vacutainer Systems, NJ, USA) for analysis of antinuclear antibodies (ANA), anti-cardiolipin antibodies (ACA), immunoglobulins (IgA, IgM, IgG), free thyroxine (T4), human thyroid stimulating hormone (TSH), cortisol, ferritin, CA 125, C reactive protein (CRP) and proteins (albumin, globulin). For determination of whole blood parameters, (White Cell Count (WCC) Red Cell Count (RCC) and Haemoglobin (Hb)) a whole blood tube with 7.2mg K₂EDTA (Becton-Dickinson Vacutainer systems, NJ, USA). For measurement of the erythrocyte sedimentation rate (ESR) blood was collected into a Becton-Dickinson Seditainer tube with 105M buffered Sodium Citrate solution (Becton-Dickinson Seditainer System, NJ, USA).

The erythrocyte sedimentation rate was measured using the Westergren method (Gambino, 1965). WCC, RCC, Hb, and white cell differential cell measurements were completed on the Abbott Cell Dyn 4000 automated haematology analyser (Abbott Laboratories, IL, USA) (Bowen *et al*, 1998).

Human thyroid stimulating hormone (TSH), free thyroxine (T4) and ferritin levels were analysed using the Abbott Architect System (Abbott Laboratories, IL, USA). The Architect assays are a two-step immunoassay to determine the presence of TSH/T4/Ferritin in human serum using chemiluminescent microparticle immunoassay technology with flexible assay protocols, referred to as Chemiflex™ (Abbott Laboratories, IL, USA). In the first step, sample, antibody coated paramagnetic microparticles and assay diluent are combined. TSH/T4/Ferritin present in the sample binds to the microparticles. Conjugates are added in the second step. The resulting chemiluminescent reaction is measured as relative light units. A direct relationship exists between the amount of TSH / Ferritin in the sample and the relative light units detected by the Architect optical system. An inverse relationship exists between the amount of free T4 in the sample and the relative light units detected by the Architect optical system.

Measurements of CA 125 levels were obtained using the Abbott AxSYM system (Abbott Laboratories, IL, USA). The AxSYM CA 125 assay is based on microparticle enzyme immunoassay technology. This technology uses a solution of suspended submicron sized latex particles coated with a capture molecule specific for CA 125. Reagents and sample are combined in a reaction vessel, incubated and transferred to an inert glass fibre matrix, where irreversible binding of the microparticles causes the immune complex to be retained by the glass fibres. A conjugate and fluorescent compound is added; measurement of the fluorescent compound generated on the matrix is proportional to the concentration of CA 125 in the sample.

Cortisol status was measured using serum samples on the Roche Elecsys 1010 system (Roche Diagnostics, Hoffmann-La Roche Ltd, USA). Elecsys cortisol measurement

makes use of a competition test principle using a polyclonal antibody that is cortisol specific. Endogenous cortisol in the sample is liberated from binding protein with danazol; this competes with exogenous cortisol derivative in the test, which has been labelled with a ruthenium complex for binding sites on the biotinylated antibody. The reaction mixture is exposed to a voltage to induce the chemiluminescent emission – which is measured by a photomultiplier. Results are determined via a calibration curve.

Immunoglobulin levels were measured in serum samples using an immunoturbidimetric test principle on the Cobas Integra 400 (Roche Diagnostics, Hoffmann-La Roche Ltd, USA). Human immunoglobulins A, G, M (IgA, IgG, IgM) form a precipitate with a specific antiserum which is determined turbidimetrically at 340nm. The Cobas Integra systems automatically calculate the immunoglobulin concentrations in the sample.

C reactive protein (CRP) response in serum samples is determined using the Cobas Integra 400 by a particle enhanced immunoturbidimetric method. Human CRP agglutinates with latex particles coated with monoclonal anti-CRP antibodies. The precipitate is determined turbidimetrically at 552nm. The Cobas Integra system automatically calculates the CRP concentration of the sample.

Serum protein measurements were made on the Roche Hitachi Modular system (Roche Diagnostics, Hoffmann-La Roche Ltd, USA). Total protein is determined by a colorimetric assay in which divalent copper reacts in an alkaline solution with protein peptide bonds to form a purple coloured biuret complex. The colour intensity is directly proportional to the protein concentration, which can be determined photometrically. Albumin concentration is determined by a colorimetric assay with

endpoint method. Sample is mixed with buffer and substrate, at a pH of 4.1 albumin binds to bromocresol green to form a blue-green complex. The colour intensity is directly proportional to the albumin concentration and is determined photometrically. Roche Hitachi Modular systems calculate the total protein and albumin concentrations in the sample automatically.

Antinuclear antibody (ANA) presence was screened for using the Varelisa ReCombi ANA Screen kit (Varelisa, Pharmacia & Upjohn Diagnostics GmbH, Germany). This kit screened serum samples for eight antinuclear antibodies, anti-dsDNA, RNP, Sm(B,B',D), SS-A/Ro, SS-B/La, Scl-70, centromere and Jo-1 in a single microwell. Varelisa ReCombi ANA screen is an indirect non competitive enzyme immunoassay for the qualitative determination of eight antinuclear antibodies in serum. The wells of a microplate are coated with human recombinant and native purified nuclear antigens and dsDNA. Antibodies specific for the nuclear antigens present in the patient sample bind to these nuclear antigens. An enzyme labelled second antibody binds to the antigen-antibody complex, which leads to the formation of an enzyme labelled antigen-antibody sandwich complex that converts the added substrate to form a coloured solution. The rate of colour formation from the chromogen is a function of the amount of the conjugate complexed with the bound antibody and thus is proportional to the initial concentration of ANA in the patient sample.

Anti-cardiolipin antibodies (ACA) are phospholipid antibodies; their presence was screened for using Varelisa cardiolipin antibodies screen. This is an indirect non-competitive enzyme immunoassay for the quantitative determination of cardiolipin antibodies (IgG, IgA, IgM) in serum. The wells of the microplate are coated with cardiolipin, antibodies specific for cardiolipin present in the patient sample bind to the

antigen. Enzyme labelled second antibody is added, which binds to the antigen-antibody complex. The enzyme labelled antigen-antibody complex converts the added substrate to form a coloured solution. The rate of colour formation is a function of the amount of enzyme conjugate complexed with the bound antibody and is proportional to the initial concentration of cardiolipin antibodies in the sample. Results for ANA and ACA were determined by the following ratio: optical density (sample) / optical density (cut off). Interpretation of the ratios gives a positive (>1.4), negative (<1.0) or equivocal (1.0-1.4) result.

The questionnaire required basic demographic details (age, occupation, health, lifestyle), details of menstrual history (age of menarche, cycle length, duration of menses, symptoms experienced, products used), details of parity (number of children / pregnancies) and for women with endometriosis, details of associated symptoms, diagnosis and treatments. Data from the questionnaire was entered anonymously to an online questionnaire database, to allow efficient retrieval of results. The questionnaire was made available on the internet for women globally, to provide a comparison group to the local study.

Microsoft[®] Excel was used to store entered data and for statistical analyses. Data was examined for normality. T tests were used to compare both groups, where data failed to display normality; a Mann Whitney Rank Sum test was used. The formula used for the between surveys percentage test is:

$$P_1 - P_2 \pm Z * \sqrt{\frac{P_1(100-P_1)}{n_1} + \frac{P_2(100-P_2)}{n_2}}$$

Where: P_1 and P_2 = the two percentages. n_1 and n_2 = the corresponding sample sizes. Z
= Z -score for the desired confidence interval under the standard normal distribution

RESULTS

Women recruited for this study were of menstrual age, the average age of the control group was 34.6 years (SD 8.3) the average age of the endometriosis group was 35.7 years (SD 7.0). The average ages of menarche were found to be 12.36 years (SD 1.12) in the endometriosis group and 13.05 (SD 1.1) in the control group. A T Test was used to evaluate the differences between the groups. The difference in mean values of the two groups is not great enough to reject the possibility that the difference is due to random sampling variability. There was no statistical significance between the two groups ($P=0.109$). When data was analysed from the larger study population of questionnaires completed on the internet ($n = 687$) a significant difference is seen at the 95% confidence interval. The difference in the mean values of the two groups is greater than would be expected by chance, there is a statistical difference between the two groups ($P=0.036$). This data shows that menarche occurs earlier in women with endometriosis.

Taking into consideration that retrograde menstruation is one of the theories concerning the cause of endometriosis; the possibility that tampon use might increase incidence of this event has been suggested. Women with endometriosis do not appear to use tampons more than the general population of women (Maloney, 1999) (Scialli, 2001). Additionally retrograde menstruation is believed to occur in a large percentage of women regardless of tampon use. Another consideration is that tampons may contain dioxins resulting from the manufacturing process (Scialli, 2001). In the local study group, 63.6% of women with endometriosis had used tampons, compared to 80% of the control group. This difference is not statistically significant. When the same analysis is applied to the larger study group ($n=687$), the difference is statistically

significant at the 95% confidence interval level. Women with endometriosis were more likely (81.9% of this group) to have used tampons than women who have not been diagnosed with endometriosis (71.8% of this group).

Symptoms such as pain during or after intercourse, bowel symptoms, painful urination and fatigue were all noted as occurring more frequently in the endometriosis group as compared to the control group. These figures were also reflected in the local study. All differences were significant at the 99% confidence interval (Table 1). This further supports evidence that endometriosis is a major cause of fatigue and low energy due to the chronic pain and symptoms associated with the condition.

Women with endometriosis experienced more pain symptoms throughout the menstrual cycle than those without (Table 2). The percentages were compared and all were significant at the 99% confidence interval. Women without endometriosis are more likely to experience mild period pain (cramps) or have a pain free cycle than those who suffer from endometriosis.

Women with endometriosis also experience more surgical procedures that require general anaesthesia, the mean value of surgical procedures for the endometriosis group is 3.74 (SD 2.9), compared to the mean value for the control group 2.96 (SD 1.9). This difference is significant at the 99% confidence interval. In evaluating claims that women with endometriosis are more susceptible to allergies, chemical sensitivities and frequent yeast infections – the questionnaire data confirms the claim for both chemical sensitivities and frequent yeast infections (significant at 99% confidence interval). However, with allergies the data difference is not as strong, the endometriosis group has a higher percentage of allergies, however this is only significant at the 90% confidence interval.

From the questionnaire data it appears that women with endometriosis have a higher incidence of irritable bowel syndrome (significant difference at 95% confidence interval). Women with endometriosis reported higher incidences of cervical cancer, cervical dysplasia, skin cancer and ovarian cancer. As the control group is a smaller group, it is not possible to determine if this is a true increase in incidence.

On analysis of the blood parameters, the endometriosis group did not show considerable differences from the control group. Immunoglobulin levels were generally higher in the endometriosis group, with the exception of IgM. Values were compared using a T test, no significant difference was found. CRP levels were scattered within the normal range for both groups, with some of the higher values distributed within the endometriosis group. On comparison, using a Mann Whitney Rank Sum Test, the differences in the median values between the two groups were not great enough to exclude the possibility that the difference is due to random sampling variability ($P=0.804$). There are few studies on endometriosis and CRP (Abrão *et al.*, 1997); the association of CRP with Interlukin 1 and Interlukin 6 (Harada *et al.*, 1996) (Lebovic *et al.*, 2001) and tumour necrosis factor (Iwabe *et al.*, 1999) also involved in endometriosis, indicate its potential use in the diagnosis of the disease. Studies comparing serum and peritoneal concentrations of immunoglobulins are variable (Gleicher *et al.*, 1987) (Meek *et al.*, 1988) (Odukoya *et al.*, 1995).

The albumin globulin ratio was decreased slightly in the endometriosis group. Ferritin and ESR levels in the endometriosis group were slightly higher. These findings are expected with chronic inflammatory states, however due to the small number of samples involved, this difference cannot be considered significant at the 95% confidence interval.

T4 and TSH showed no apparent differences in this study group. The complete blood count also showed that both groups were considered similar with the exception of the percentage basophil count shows a statistically significant difference in the endometriosis group (Mean = 0.975, SD 0.63) (Control Mean = 0.413 SD 0.3). This finding is consistent with the inflammatory process. Secretion of specific pro-inflammatory cytokines (e.g., Interlukin (IL) -3, IL-4, IL-5, IL-13) participate in the recruitment of secondary effector cells (eosinophils, basophils, neutrophils) with development of persistent inflammation and chronic symptoms in a patient with chronic inflammation. Interlukin 4 is produced by cultures of endometrial and stromal cells from women with endometriosis. Serum soluble CD23 concentrations are significantly elevated among patients with endometriosis when compared with controls (Odukoya *et al.*, 1996). This is assumed due to an increase in activity of T cells mediated through Interlukin 4. The increase in basophil levels may also be due to increased production of interlukin 4 in women with endometriosis.

CA 125 is a serum marker for monitoring patients with epithelial ovarian cancer (Bast *et al.*, 1998). CA 125 has been evaluated for management of benign gynaecologic diseases such as endometriosis (Fang-Ping *et al* 1998). Mol *et al.* (1998) conclude that the performance of CA 125 measurement in the detection of endometriosis was low, but was better for the detection of severe endometriosis. Serum levels of CA 125 are elevated in women with endometriosis, with the more marked increases shown in women with stage III and IV, compared with Stage I and II. The value of CA 125 as a tool for monitoring treatment or disease progression has also been noted. (Abrão *et al.*, 1997) (Chen *et al.* 1998) (Imai *et al.*, 1998) (Abrão *et al.*, 1999) (Vinatier *et al.*, 2000). The findings in this study show that women with endometriosis have a higher

level of CA 125 (significant at 95% confidence interval). Most values obtained for CA 125 fell within the normal range with the higher levels seen in endometriosis patients.

Cortisol influences the immune system and many other body systems. Cortisol suppresses inflammation and cellular immune activation, and reduced levels might relax constraints on inflammatory processes and immune cell activation. Cortisol analysis revealed marginally higher values in the control group. Due to the circadian rhythm of cortisol levels in serum, sample collection times need to be similar for each participant. Serum cortisol concentrations show a diurnal variation, maximum concentrations are reached early in the morning and then decline during the day to an evening level that is about half of the morning concentration. The majority of samples used in this study were collected in the morning. There were no statistically significant differences between both groups, when analysed by the Mann Whitney Rank Sum test. Differences are assumed to be due to various sampling times and the small sample number.

Gleicher (Gleicher *et al.* 1987) and colleagues first reported elevated levels of ACA in endometriosis, with only a minority of patients showing values above the normal range. Kennedy *et al.* (1989) showed that the levels found in endometriosis is lower than those found in systemic lupus erythematosus. Kilpatrick *et al.* (1991) findings also indicate that cardiolipin antibody levels are slightly skewed in endometriosis. A number of autoimmune diseases have been correlated with the presence of antinuclear antibodies. Reproductive autoimmune failure syndrome (RAFS) is included among autoimmune diseases. RAFS is the association of recurrent pregnancy loss, unexplained infertility or endometriosis with the presence of circulating autoantibodies, including antinuclear antibodies (Kaider *et al.*, 1999). In this study, no

positive values (>1.4) were obtained for ANA or ACA, the endometriosis group does show a higher average ratio for both assays. This variation may be due to the small numbers in this study and may only account for random sampling variation. The difference between the groups is not regarded as statistically significant. This correlates data from Kilpatrick *et al.* (1991)

DISCUSSION

Endometriosis is generally seen as an anatomical lesion. This deposit is defined by visual inspection during laparoscopy. However endometriosis is frequently microscopic in nature therefore making it undiagnosable to the naked eye. A familial probability of developing endometriosis is suspected – endometriosis is found more commonly in patients with familial history of the disease. It is thought to occur through a maternal inheritance pattern (Mathur, 2000). The most common symptoms are dysmenorrhoea, dyspareunia and low back pain that are worse around menses. Results from the questionnaire data (687 respondents) demonstrate that women diagnosed with endometriosis frequently experience dysmenorrhoea, dyspareunia and pain with urination or bowel movements.

Infertility is also a presenting complaint; infertility patients may have no painful symptoms and their disease is only uncovered in the course of the diagnostic work-up for infertility. Presently, the definitive tool for diagnosis is laparoscopy (Brosens and Brosens, 2000). No single laboratory test has shown reliable unique predictability; it is thought that a combination of biochemical markers and clinical assessment will decrease the need for surgical confirmation. The findings from this study cannot be considered conclusive due to the low sample number. This study indicates however, the usefulness of CA 125 as an indicator and monitoring tool for women with endometriosis.

Endometriosis appears to be a gynaecological manifestation of a syndrome. It is emerging that it may be the tip of a much larger invisible iceberg – one that represents a whole range of health problems that have underlying hormonal and/or immune dysregulation. Women with endometriosis are presenting with a whole range of

symptoms in addition to those traditionally associated with the disease. Along with dysmenorrhoea, dyspareunia and infertility – high incidences of allergies, chemical sensitivities and *Candida albicans* related problems are being noted in women with endometriosis and were correlated in this study. A larger study completed by The Endometriosis Association has provided strong evidence that certain other diseases are more likely to occur in women and relatives of women with endometriosis. (Ballweg, 1995)

The Endometriosis Association study showed that there is an increased risk of breast cancer, ovarian cancer and melanoma in women and in the relatives of women who have endometriosis (Duczman and Ballweg, 1999). Women with endometriosis have a higher incidence of thyroid disease including hypothyroidism, hyperthyroidism, Graves' disease and Hashimoto's Thyroiditis. (Ballweg, 1995). In this small group observed, there was no indication of this increase in thyroid problems.

Autoimmune diseases are seen somewhat more frequently in women with endometriosis and in their immediate families including Rheumatoid Arthritis, Lupus, Multiple Sclerosis and Meniere's disease. If endometriosis is neither an endocrinological or surgical disease – could it be an autoimmune disease?

Diagnosing endometriosis presents a number of difficulties for both patient and health care provider. A diagnostic profile, consisting of a questionnaire and blood analysis may assist in the early diagnosis of the condition. The number of participants in this study was small, however there were some indications in the blood analysis that may display further significance in a larger study. It is important for health care providers and patients that this condition is recognised early – to prevent prolonged suffering, fertility problems and repeated surgical procedures. As displayed in the questionnaire

data – women with endometriosis undergo more surgical procedures than their counterparts. Diagnosis rate in the North Western Health Board area appears to be low, all consultant gynaecologists and family doctors in the area were contacted about subject recruitment. Responses were only received from a small number of practices. Statistical data from Letterkenny General hospital indicate that in the years 1998 – 2001 (June) sixty-two women were admitted to the hospital with a diagnosis of endometriosis. The average age of these women was 40 years (SD 3.7) (HIPE, 2001). As endometriosis is known to affect women from menarche, this area needs to be addressed. Further correlation of blood parameters and questionnaire responses will allow family doctors and gynaecologists to screen women more readily for signs of endometriosis. The author recommends that this study be continued on a larger scale, incorporating multiple regions within Ireland.

ACKNOWLEDGEMENTS

I would like to acknowledge and sincerely thank the following people for their help and support in this study:

Mr Chris Lyons (Hospital Manager) and the Ethics Committee on behalf of Letterkenny General Hospital for granting permission for this study.

Participants in this study, for completing questionnaires and donating blood samples.

The phlebotomy staff and family doctors for assisting in recruitment and drawing blood samples.

The staff of the haematology and biochemistry laboratories in Letterkenny General Hospital for their assistance and support.

Ms Veronica McElhinney (supervisor), Mr William Kinnear (assistance with ELISA testing), Mr Cyril Ward (Chief Technologist).

Dr Eddie Aboud, Consultant Gynaecologist, for reviewing results from this study.

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Table 1

**Comparison of symptoms experienced at menses.
Data obtained from questionnaire analysis.**

	Endometriosis Group (n = 563)		Control Group (n = 124)	
	Yes %	No %	Yes %	No%
Pain during or after intercourse	76.6	17.2	21.8	58
Pain with bowel movements, diarrhoea, stomach upsets.	84.4	11.2	47.6	33.9
Fatigue, exhaustion, low energy.	91.3	5.1	53.3	29.8
Painful Urination	38.4	55.8	5.6	75

Table 2

**Comparison of symptoms experienced
during menstrual cycle.
Data obtained from questionnaire analysis.**

	Endometriosis Group (n = 563)	Control Group (n = 124)
	%	%
Pain Free Cycle	2.0	11.3
Mild period pain (cramps)	20.2	54.8
Pain at ovulation	61.6	27.4
Pelvic Pain	78.3	38.7
Back Pain	69.8	44.4
Joint / Limb Pain	40	16.9
Non Cyclical Pelvic Pain	45.1	7.3