Serological testing for celiac disease in women with endometriosis. A pilot study

F.M. Aguiar[1], *M.D.*; S.B. Castanheira Melo[2], *M.D., Ph.D.*; L. Carvalho Galvão[2], *M.D., Ph.D.*; J.C. Rosa-e-Silva[1], *M.D., Ph.D.*; R.M. dos Reis[1], *M.D., Ph.D.*; R.A. Ferriani[1], *M.D., Ph.D.*

[1]Sector of Human Reproduction, Department of Gynecology and Obstetrics, [2]Department of Puericulture and Pediatrics
Faculty of Medicine of Ribeirão Preto, University of São Paulo, SP (Brazil)

Summary

Purpose of investigation: Celiac disease (CD) involves immunologically mediated intestinal damage with consequent micronutrient malabsorption and varied clinical manifestations, and there is a controversial association with infertility. The objective of the present study was to determine the presence of CD in a population of infertile women with endometriosis. *Methods:* A total of 120 women with a diagnosis of endometriosis confirmed by laparoscopy (study group) and 1,500 healthy female donors aged 18 to 45 years were tested for CD by the determination of IgA-transglutaminase antibody against human tissue transglutaminase (t-TGA) and anti-endomysium (anti-EMA) antibodies. *Results:* Nine of the 120 women in the study group were anti-tTGA positive and five of them were also anti-EMA positive. Four of these five patients were submitted to intestinal biopsy which revealed CD in three cases (2.5% prevalence). The overall CD prevalence among the population control group was 1:136 women (0.66%). *Conclusion:* This is the first study reporting the prevalence of CD among women with endometriosis, showing that CD is common in this population group (2.5%) and may be clinically relevant.

Key words: Celiac disease; Endometriosis; Infertility; Serologic screening; Pelvic pain.

Introduction

Celiac disease (CD) is characterized by chronic intolerance to gluten ingestion in genetically susceptible individuals, leading to immunologically mediated intestinal tissue damage [1, 2]. The disease may occur in different forms, with patients being asymptomatic or having the classical clinical forms characterized by diarrhea with or without intestinal malabsorption. This broad spectrum of manifestations often impairs the diagnosis of this disease. CD has been described in several world regions including Europe, North America, South America, Africa, India, and New Zealand, and is rare among African blacks and Asians such as Chinese and Japanese subjects. The disease predominates among white individuals and the female to male ratio is 2:1.

Some clinical conditions are related to CD, such as diabetes mellitus, selective IgA deficiency, autoimmune thyroiditis, primary biliary cirrhosis, neuropsychic changes, herpetiform dermatitis, autoimmune disease, osteoporosis, and reproductive disorders [1]. Several studies have indicated a higher prevalence of reproductive disorders among women with CD, such as spontaneous abortion, late menarche, early menopause, amenorrhea, and infertility [3-5].

One of the major causes of infertility is endometriosis, whose incidence in infertile patients ranges from 10-25%, with a prevalence increasing to 60-70% in cases of chronic pelvic pain, and it may be present in 1-20% of asymptomatic women. Although the pathogenesis of endometriosis is not completely understood, endometriosis is associated with changes in both cell-mediated and humoral components of innate and acquired immunity with an increase of auto-antibodies and circulating immunocomplexes [6]. Celiac disease is also an immunologically mediated disease with production of auto-antibodies and association with a series of other autoimmune diseases such as IgA deficiency, diabetes mellitus, autoimmune thyroiditis, and herpetiform dermatitis [2]. Although there are many reports of CD among infertile patients, there are no reports specifically evaluating a group of women with endometriosis. The present study was undertaken to evaluate the prevalence of celiac disease in a cohort of Brazilian women with endometriosis. This was achieved by means of serological screening based on anti-t-TGA, confirmed by anti-endomysial antibody (EMA) IgA, which showed a sensitivity of 95-100% and 85-100%, and a specificity of 94-100% and 95-100%, respectively, and final confirmation was obtained with a jejunal biopsy and by patient follow-up.

Material and Methods

The present cohort consisted of 120 consecutively selected women with endometriosis attending a tertiary university service due to pelvic pain and/or infertility between 2000 and 2003. Inclusion criteria were age between 18 and 45 years and a laparoscopic and histological diagnosis of endometriosis made up to six months before blood collection. As a population control, blood samples were collected from 1,500 apparently healthy female blood donors aged 18 to 45 years at the Blood Center of Ribeirão Preto, State of São Paulo, Brazil, from September 2001 to October 2002 (same city for all patients). Data about these individuals have been published previously [7]. The

Revised manuscript accepted for publication June 2, 2008

studies were approved by the internal Institutional Review Board and patients gave written informed consent to participate.

Serological screening was applied to blood samples collected during the early follicular phase from the study group. After separation, serum was stored at -70°C and the samples were tested in duplicate in the same assay. The blood samples were tested for the presence of t-TGA antibodies by ELISA and when a sample was positive for this antibody, it was tested for EMA-IgA by indirect immunofluorescence. The INOVA commercial kit (San Diego, CA, USA) was used for the detection of t-TGA. Serum samples were diluted 1:100 and ELISA readings were obtained at 450 nm wavelength. A value of less than 20 IU was considered to be negative, a value of 20 to 30 IU was considered to be weakly positive, and a value of more than 30 IU was considered to be moderately/strongly positive. The sensitivity and specificity of this test were 95-98% and 94-95%, respectively. Another INOVA kit was used for the detection of EMA-IgA by indirect immunofluorescence using slides with monkey esophagus sections as substrate. Sera were diluted 1:5, 1:10 and 1:20 with phosphate buffered saline (PBS), pH 7.2. A result was considered to be positive when immunofluorescence was observed starting from the 1:10 dilution. The sensitivity and specificity of this test were 90-100% and 97-100%, respectively. An intestinal biopsy was proposed when both tests were positive. The biopsy was obtained by jejunal aspiration capsule and/or by upper digestive endoscopy and analyzed histologically according to Marsh criteria [8]. Subjects diagnosed as having CD underwent a standard clinical assessment aimed at detecting symptoms or signs of digestive tract disease or manifestations suggestive of extra-intestinal involvement.

Data were analyzed statistically using the GraphPad Prism® 2.01 32 Bit Executable program (GraphPad Software Inc., San Diego, CA, USA). Paired variables with normal distribution were analyzed by the Student's *t*-test and analysis of variance was carried out using the F test for comparison of the prevalence of CD, with the level of significance set at $p < 0.05$. The chi-square test was used to compare the prevalence of CD among the three groups studied (endometriosis x population control x endometriosis control), and the Fisher exact test was used for 2 x 2 comparisons.

Results

Mean age was 29.2 ± 5.6 years for the endometriosis group and 27.9 ± 4.5 years for the population control ($p = 0.10$ for the endometriosis group). The symptoms reported by the 120 women with pelvic endometriosis were infertility (81.0%), dysmenorrhea (91.0%), and dyspareunia (57.1%). The patients had Stage I/II (56.2%) or III/IV (43%) endometriosis as defined by the American Society of Reproductive Medicine (ASRM). Of the 120 patients with endometriosis, nine were positive for t-TGA at levels ranging from 25.54 to 190.7 IU (Table 1), with a prevalence of 7.5%. When these nine patients were tested for EMA-IgA, five were found to be positive at 1/10 and 1/20 dilution (5/120 = 4.16%). One patient refused the jejunal biopsy (patient no. 8, Table 1) and three of the four patients submitted to the biopsy (prevalence of confirmed cases: 3/120, 2.5%) fulfilled the histopathological criteria for CD Marsh grade III (destructive pattern) (nos. 1, 4 and 5, Table 1). In a later analysis, the three patients with confirmed CD presented mild gastrointestinal symptoms, with undefined abdominal discomfort but without persistent diarrhea. The other two EMA-IgA-positive patients without confirmation by the biopsy (nos. 6 and 8, Table 1) had no gastrointestinal symptoms. There were no signs or symptoms of extra-intestinal disease in any of the nine t-TGA-positive patients.

In the population control group 20 women showed moderately/strongly positive t-TGA levels and 12 of them were EMA-IgA positive, while the other eight were EMA negative. These individuals who were positive to both serum tests were submitted to an intestinal biopsy. Of these, two were classified as Marsh type 0, and 10 as Marsh ≥ type 1, with a global prevalence of CD among females of 0.66% (10 confirmed cases, 1:136 women) [7]. Statistical analysis revealed that the prevalence rates of positive serology in the study group (5 out of 120) versus the population control group (12 out of 1,500) was significant ($p = 0.001$), odds ratio (OR) = 5.4 with a confidence interval (CI) = 1.8 – 15.5 and a test power = 60%. The prevalence rates of biopsy-confirmed CD were: three out of 120 versus the population control group (10 out of 1,500), with the difference being non significant ($p = 0.065$), OR = 3.8 with CI = 1.03 – 14.08 and a test power = 40%.

Table 1. — *Serologic results for the patients with an initial positive screening for t-TGA.*

Patient	t-TGA(IU)	EMA-IgA	Biopsy
1	138.9	1:20	Type 3
2	81.03	Negative	ND
3	25.54	Negative	ND
4	102.5	1:20	Type 3
5	190.7	1:20	Type 3
6	63.34	1:20	Type 0
7	26.5	Negative	ND
8	86.71	1:20	ND
9	28.01	Negative	ND

ND: not done.

Discussion

Celiac disease is an enteropathy that occurs in genetically susceptible individuals and is characterized by permanent lesion of the intestinal mucosa triggered by the ingestion of gluten, with full recovery occurring when gluten is removed from the diet. Dietrich *et al.* [9] showed that the enzyme tissue transglutaminase is a target of immunological reaction. The deamidation activity of this enzyme seems to generate a negative charge in the prolamine bound to the HLA molecule and this complex is recognized by T helper cells, which activate other lymphocytes in the intestinal mucosa, causing an inflammatory response by the production of interleukins, interferon-gamma and tumor necrosis factor [1].

Clinically, the classical form is characterized by chronic diarrhea (malabsorption syndrome) and malnutrition, and the atypical form by various other symptoms, including manifestations of thyroid disease, epilepsy and infertility [1]. Individuals with the silent form are asymptomatic but have positive serology for CD and alteration

of the intestinal mucosa, and individuals with the latent form have positive serology but do not present histological changes of the intestinal mucosa [10]. CD is probably much underdiagnosed.

Several studies have tried to relate a higher incidence of infertility to the presence of of CD or a higher incidence of CD to the presence of infertility. Collin *et al.* [11] studied women of reproductive age with primary or secondary infertility and women with repeated abortion. Anti-reticulin and anti-gliadin antibodies were used to screen for CD and positive cases were confirmed by an intestinal biopsy. Four of 150 infertile patients (2.7%) and none of the 150 control women had a diagnosis of CD. In the group with sterility of no apparent cause, the incidence of CD was 4.1%. Other studies have reported an incidence of CD ranging from 1.6% to 3.3% among infertile couples, with a higher prevalence (8%) being observed when patients with sterility of no apparent cause are evaluated separately [5, 12]. However, there is no consensus about a systematic survey of this affection in infertile patients.

Since endometriosis is one of the main causes of infertility and since there are no studies specifically evaluating a group of women with endometriosis, we conducted a study of CD in women with proven endometriosis regardless of the type of symptoms presented, although most of them were infertile. Our interest was to determine the prevalence of disease (CD) in a group of patients with clinical manifestations of confirmed endometriosis. As reported in surveys of CD among infertile women, we observed that CD is frequent among women with endometriosis and that this frequency is higher than that for the general population, although we did not detect a statistically significant difference, probably due to the small number of cases, as also reported in other studies on the association of infertility and CD [5]. We used a significant control of the same ethnic composition as the study population since there are race-related differences in the prevalence of CD. Although blood donors cannot be considered to be representative of the general population, we selected them because these are apparently healthy individuals, especially regarding nutrition and the absence of serious diseases. The population of the State of São Paulo presents important miscegenation with Europeans, which was now found to be associated with a higher prevalence of CD than previously recorded. This miscegenation mainly occurred during the first 50 years of the last century (specifically from 1920 to 1930), after the immigration of huge numbers of Italian and Spanish citizens to São Paulo, where these immigrants came to work on coffee plantations and in the incipient Brazilian industry.

This is the first report on CD prevalence in women with endometriosis, demonstrating that CD is common (2.5%) in this patient population and may be of clinical relevance. If the presence of CD had been confirmed in the patient who refused the intestinal biopsy, the prevalence of CD in the endometriosis group would have been 3.3%, a value that would be statistically significant ($p = 0.01$), and that would support the possibility that CD is common among women with endometriosis.

If we assume that there is a causal link between CD and endometriosis, independently of the presence of infertility, we can speculate about a common genetic/immunologic link more than about a possible nutritional deficit. Although the pathogenesis of endometriosis is not completely understood, endometriosis is associated with changes in both the cell-mediated and humoral components of innate and acquired immunity [6]. Celiac disease also is an immunologically mediated disease, with the production of autoantibodies and an association with other autoimmune diseases [2].

The present study is the first to associate CD with endometriosis, although the clinical relevance of this increase may be questioned. As reported in surveys of CD among infertile women, we observed that CD is frequent among women with confirmed endometriosis and that this frequency is higher than that in the general population. Further studies will probably elucidate the clinical relevance of these findings.

References

[1] Farrell R.J., Kelly C.P.: "Celiac Sprue". *New Engl. J. Med.,* 2002, *346,* 180.
[2] Green P.H., Jabri B.: "Celiac disease". *Annu. Rev. Med.*, 2006, *57,* 207.
[3] Sher K.S., Mayberry J.F.: "Female fertility, obstetric and gynaecological history in coeliac disease: a case control study". *Acta Paediatr Suppl.,* 1996, 412, 76.
[4] Sheiner E., Peleg R., Levy A.: "Pregnancy outcome of patients with known celiac disease". *Eur. J. Obstet. Gynecol. Reprod. Biol*., 2006, *129*, 41.
[5] Tiboni G.M., de Vita M.G., Faricelli R., Giampietro F., Liberati M.: "Serological testing for celiac disease in women undergoing assisted reproduction techniques". *Hum. Reprod.,* 2006, *21*, 376.
[6] Seli E., Arici A.: "Endometriosis: Interaction of immune and endocrine systems". *Sem. Reprod. Med.,* 2003, *21*, 135.
[7] Melo S.B., Fernandes M.I., Peres L.C., Troncon L.E., Galvão L.C.: "Prevalence and demographic characteristic of celiac disease among blood donors in Ribeirão Preto, State of São Paulo, Brazil". *Dig. Dis. Sci.,* 2006, *51*, 1020.
[8] Marsh M.N.: "Gluten, major histocompatibility complex and the small intestine. A molecular and immunobiologic approach to the spectrum of sensitivity (celiac sprue)". *Gastroenterology,* 1992, *102*, 330.
[9] Dieterich W., Laag E., Schoopper H., Volta U., Ferguson A., Gillett H. *et al.*: "Autoantibodies to tissue transglutaminase as predictors of celiac disease". *Gastroenterology,* 1998, *115,* 1317.
[10] Fasano A., Catassi C.: "Current approaches to diagnosis and treatment of celiac disease: an evolving spectrum". *Gastroenterology*, 2001, *120*, 636.
[11] Collin P., Vilska S., Heinonen P.K., Hällström O., Pikkarainen P.: "Infertility and coeliac disease". Gut., 1996, *39*, 382.
[12] Shamaly H., Mahameed A., Sharony A., Shamir R.: "Infertility and celiac disease: do we need more than one serological marker?". *Acta Obstet. Gynecol. Scand.,* 2004, *83,* 1184.

Address reprint requests to:
R.A. FERRIANI, M.D., Ph.D.
Setor de Reprodução Humana,
Departamento de Ginecologia e Obstetrícia,
Faculdade de Medicina de Ribeirão Preto,
Universidade de São Paulo,
14049-900 Ribeirão Preto, SP (Brasil)
e-mail: raferria@fmrp.usp.br