

Serological testing for celiac disease in women undergoing assisted reproduction techniques

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BACKGROUND: The assertion of a causal relationship between celiac disease and infertility is suggested by several lines of research. Nevertheless, robust evidence has not yet been provided. The present study evaluated, for the first time, the prevalence of celiac disease in women undergoing assisted reproduction techniques (ART). **METHODS:** Serum samples from 200 Italian women undergoing ART were evaluated for celiac disease by endomysium antibody (EMA) and transglutaminase antibody (t-TGA)—two highly sensitive and specific serological markers. Two hundred women not reporting reproductive problems and having delivered at least one child served as controls. In cases of positive serology, the diagnosis was confirmed by jejunal biopsy. **RESULTS:** Five (2.5%) women from the study group and two (1.0%) from the control group were found to have celiac disease ($P = 0.44$). The main indications for ART in women found to have celiac disease were tubal factor in two cases and male infertility in three cases. None of these women reported major gastrointestinal complaints. Extra intestinal signs linked to celiac disease were noted in four out of five patients. **CONCLUSION:** This study raises the issue of celiac disease screening in ART programmes. Given the available evidence in the literature combined with our observations from this study, the value of serological testing for celiac disease in infertile women remains uncertain. Further studies to address this issue are required.

Key words: assisted reproduction techniques/celiac disease/infertility/serologic screening

Introduction

Celiac disease is a gluten-sensitive enteropathy induced by dietary wheat gliadin and related proteins in genetically susceptible individuals (Green and Jabri, 2003; Thompson, 2005). The clinical spectrum of the disease is extremely variable ranging from the classical mode of presentation, characterized by diarrhoea with or without malabsorption, to the clinically silent form (Green and Jabri, 2003; Thompson, 2005). With the introduction of highly sensitive and specific serological markers, it has become apparent that most sufferers have clinically occult or inapparent forms. Overall, it has been estimated that 0.5–1.0% of adults suffer from the disease (Green and Jabri, 2003; Thompson, 2005).

Celiac disease has been associated to several extraintestinal manifestations/ complications, one of which is an adverse reproductive outcome (Rostami *et al.*, 2001; Collin *et al.*, 2002). The likelihood for a causal relationship between celiac disease and reproductive problems including infertility, recurrent abortions and intrauterine growth restriction has received support in a number of reports. In a study examining the effect of celiac disease on reproduction, patients on a normal diet were found to be at increased risk for infertility in comparison to patients on a gluten-free diet (Ferguson *et al.*, 1982). In a case-control study,

women with celiac disease had fewer children, more miscarriages and delayed menarche and early menopause than controls (Sher and Mayberry, 1994). Collin *et al.* (1996) investigated the prevalence of sub-clinical celiac disease in women with infertility or recurrent miscarriages by using serological screening tests. In their series, no cases of celiac disease were identified in the control group of 150 fertile women. On the other hand, 2.7% (four out of 150) of infertile women were found to have sub-clinical celiac disease. In the Mediterranean island of Sardegna, celiac disease prevalence is known to be particularly high (~10.6 per 1000) (Meloni *et al.*, 1999b). When infertile couples from this endemic geographic area were evaluated for sub-clinical celiac disease, an increased prevalence of the disease was found in infertile women (three out of 99; 3%) compared with the general population (17 cases out of 1607 women; 1.06%) (Meloni *et al.*, 1999a). Celiac disease has been also found to be more prevalent among the Arab population. When a group of 192 Arab women suffering from unexplained infertility was tested for serologic markers for celiac disease, 2.65% (five out of 192) were found to be affected. This figure was five times higher than the control level of 0.5% (one out of 210) (Shamaly *et al.*, 2004).

There is no unanimous consensus on the concept that celiac disease represents a risk factor for infertility. Kolho *et al.* (1999)

used serology to screen infertile women for undiagnosed celiac disease. The prevalence of the disease was found to be comparable among the study groups, with 1.6% of women with recurrent miscarriage, 2.1% of women with unexplained infertility and 2.0% of control women having celiac disease, respectively. In addition, a recent large general population-based cohort study found that women with diagnosed celiac disease have fertility similar to that of the general population, though they tended to have their babies at an older age (Tata *et al.*, 2005).

A rationale approach to assisted reproduction techniques (ART) should be based on the clear identification of all possible factors underlying infertility. In this context, one unanswered issue is whether serologic screening for celiac disease should be part of the diagnostic work up of patient undergoing ART. The present study was undertaken to evaluate the prevalence of celiac disease in a cohort of Italian women undergoing ART. This was achieved by means of a serological screening based on immunoglobulin A endomysium antibody (IgA EMA) and IgA transglutaminase antibody (t-TGA) against human tissue transglutaminase, which offered a sensitivity of 85–100% and 95–100%, and a specificity of 95–100% and 94–100%, respectively (Collin, 2005). The inclusion of both serological tests in our screening strategy is rationalized by the notion that reliance on EMA or t-TGA as a single test can lead to underestimation of celiac disease prevalence (Green and Jabri, 2003).

Subjects and methods

The study involved 200 consecutive women undergoing ART (IVF or ICSI) in our infertility unit. The control group consisted of 200 apparently healthy females not reporting reproductive problems with at least one child delivered and attending our hospital for routine screening visits. All the women were Caucasian from Italy. All the couples referred for ART had undergone a clinical assessment to identify the cause of infertility (The study was approved by the internal Institutional Review Board and patients gave their informed consent).

The presence of ovulation was determined by measurements of serum progesterone in the mid-luteal phase. Evaluation of female endocrine status also included determinations of FSH, LH, prolactin, androstenedione, testosterone and thyroid hormones. The tubal status was investigated by hysterosalpingography. Hysteroscopy and laparoscopy were performed in selected cases. All male partners underwent semen analysis according to World Health Organization recommendations (WHO, 1993). To maximize collection of information on the potential factors underlying infertility, investigation for chromosomal anomalies was performed by cytogenetic analysis of both partners of each infertile couple. Serological screening was conducted on venous blood samples collected during the initial days of controlled ovarian stimulation. Following separation by centrifugation, the sera were stored at -80°C until analysis. All serological determinations were performed without knowledge of the patient status.

The human t-TGA assays for IgA was carried out using a commercial immunoenzymatic assay (Immuno Pharmacology Research S.p.A., Catania, Italy), where the antibodies, if any, are recognized by an anti human IgA antiserum conjugated with peroxides, which in the presence of a substrate produce a colorimetric reaction measured using a spectrophotometer at 450 nm. According to the manufacturer's indications, the result was considered positive when a value higher than 3.0 U/ml was recorded.

The EMA assays for IgA was carried out using a commercial indirect immunofluorescence assay on monkey oesophagus sections

(Immuno Pharmacology Research S.p.A.). The patient's serum was incubated on a slide with adherent sections of the distal third of the monkey oesophagus. The IgA anti-endomysium, if present, fix themselves on the muscularis mucosae and the external muscularis tonaca. Those antibodies are evidenced by means of an anti human IgA antibody conjugated with fluorescein isothiocyanate.

Between-group differences in celiac disease prevalence were assessed by the χ^2 test. Continuous variables were analysed using Student's *t*-test. A *P* value <0.05 was considered statistically significant.

Results

The indications for ART were tubal factor (23.0%), ovulatory dysfunction (5.0%), endometriosis (3.0%), male factors (26.5%), unexplained infertility (26.5%) and mixed causes (16%). The mean duration of infertility was 5.17 ± 3.16 years. For 67% of women, the current cycle represented the first ART attempt. The women reporting two, three or more than three ART attempts were 17.5%, 8.5% and 7.0%, respectively. In all instances, infertility was primary.

The results obtained by serological screening, together with age and body mass index (BMI) parameters, are reported in Table I. Clinical specifics of the women of the study group (referred for ART) testing positive to serology are illustrated in Table II. The mean age of the study group was significantly higher in comparison to the control group (35.1 ± 5.1 versus 31.4 ± 5.2 ; $P < 0.001$). No significant between-group differences with respect to the BMI parameter were noted (control group: $22.6 \text{ kg/m}^2 \pm 3.1$; study group: $22.3 \text{ kg/m}^2 \pm 3.0$; $P > 0.05$).

Positive serology was identified in five (2.5%) of the 200 women in the study group. While cases number 1 and 2 tested positive for t-TGA but not for EMA, cases 3–5 tested positive for both t-TGA and EMA. Following evidence of positivity to the screening test, case 5 reported being aware of being affected by celiac disease (confirmed by jejunal biopsy) for a few months and to be on gluten-free diet. The diagnosis of celiac disease in cases 1–4 was confirmed by jejunal biopsy. Gastrointestinal symptoms were reported only by two patients (cases 4 and 5), and consisted of mild and undefined abdominal discomfort. Signs consistent with extra-intestinal features of celiac disease were identified in four patients. These signs included elevated liver enzymes (cases 1 and 5), hyperprolactinemia (cases 2 and 4), autoimmune hypothyroidism (case 4) and iron deficiency anaemia (case 5).

Table I. Serological features of the women screened for celiac disease

	Control group	Study group*
Number of women screened	200	200
Age (mean \pm SD)	31.4 ± 5.2	35.1 ± 5.1 †
Body mass index (kg/m^2 ; mean \pm SD)	22.6 ± 3.1	22.3 ± 3.0
Number of positive tests to t-TGA IgA	1	2
Number of positive tests to EMA IgA	0	0
Number of positive tests to EMA IgA + t-TGA IgA	1	3
Total number of positive tests**	2 (1.0%)	5 (2.5%)‡

EMA = endomysium antibody; t-TGA = transglutaminase antibody. *Women undergoing assisted reproduction techniques (IVF/ICSI). **In all instances, celiac disease was confirmed by jejunal biopsy. †Student's *t*-test $P < 0.001$; ‡ χ^2 test: $P = 0.44$.

Table II. Specifics of women with celiac disease undergoing assisted reproduction techniques (ART).

Case	Age (years)	Duration of infertility (years)	Gastrointestinal symptoms	Extra-intestinal features	Indication(s) to ART	t-TGA	IgA	EMA	IgA	Karyotype	ART technique applied	ART outcome
1	35	9	Absent	Elevated γ -glutamyl transferase	Tubal (one salpinx patent) Ovulatory dysfunction (oligoamenorrhea)	+	-	-	-	46,XX	IVF	Not pregnant
2	29	3	Absent	Hyperprolactinemia (in therapy with cabergoline)	Male (oligoasthenozoospermia)	+	-	-	-	47,XX, +Mar*	ICSI	Not pregnant
3	40	6	Absent	-	Tubal (one salpinx patent)	+	+	+	+	46,XX	IVF	Not pregnant
4	43	4	Mild	Hyperprolactinemia (in therapy with bromocriptine) Autoimmune hypothyroidism (in therapy with L-thyroxine) Elevation of transaminases Iron deficiency anaemia	Male (oligoasthenozoospermia)	+	+	+	+	46,XX	Cycle cancelled due to poor response to ovarian stimulation	-
5	35	5	Mild	-	Male (severe oligoasthenozoospermia)	+	+	+	+	46, XX,9qh+	ICSI	Miscarriage

EMA = endomysium antibody; t-TGA = transglutaminase antibody. *MultiFISH analysis revealed the marker chromosome to be a chromosome 15. This finding was confirmed by a painting probe and a centromeric probe for the chromosome 15.

The karyotype of case 2 showed the presence of a marker chromosome (47, XX, +Mar). MultiFISH analysis revealed the marker chromosome to be chromosome 15. This finding was confirmed by a painting probe and a centromeric probe for the chromosome 15. The karyotype of case 5 revealed the presence of a chromosomal polymorphism (46, XX,9qh+).

Major indications to ART were tubal factor in cases 1 and 3, and male factor in cases 2, 3 and 5. ART resulted only in one pregnancy (in case 5), which ended in miscarriage at 6 weeks gestation. In the control group, positive results to serological screening were recorded in two of the 200 (1.0%) women studied. In both cases, one testing positive for both TGA and EMA and the other testing positive only for TGA, the diagnosis was confirmed by jejunal biopsy. Statistical analysis revealed the prevalence rates of positive serology in the study group (five out of 200) versus the control group (two out of 200) as not significant ($P = 0.44$).

Discussion

The possible causal link between celiac disease and infertility has been the subject of a number of investigations (Sher and Mayberry, 1994; Collin *et al.*, 1996; Kolho *et al.*, 1999; Meloni *et al.*, 1999; Shamaly *et al.*, 2004; Tata *et al.*, 2005). As a novel element, the present study focussed on women undergoing ART for various infertility factors. The results obtained showed that five out of 200 (2.5%) of the women referred to ART were affected by celiac disease compared with a prevalence of two out of 200 (1.0%) in the control group.

In all instances, the definitive diagnosis of celiac disease was established by intestinal biopsy. Although it may be tempting to extrapolate this finding to suggest that celiac disease screening might be worthwhile for women referred to ART, there was no statistically significant difference between study and control groups—implying that our findings can only be regarded as indicative of a trend. The lack of statistical significance is likely to reflect an insufficient size of sample. This is supported by the evidence that the prevalence of celiac disease observed in our control group mirrors the prevalence rates found by others in the Italian population. Indeed, a recent mass screening conducted using a TGA assay identified a prevalence of celiac disease in Italian schoolchildren as high as 1:96 (Tommasini *et al.*, 2004). Another recent study carried out in the Italian region of Campania on pregnant women by t-TGA and EMA assays identified a prevalence of 1:70 (Martinelli *et al.*, 2000). In line with these figures is also the prevalence of celiac disease (10.6 per 1000) reported for the Italian island of Sardegna (Meloni *et al.*, 1999b).

In our study, the mean age of the study group was significantly higher compared with the control group. It is difficult to estimate whether this represents a confounding factor. An intriguing relationship between the age and fertility of women with celiac disease has been recently observed by Tata *et al.* (2005). These authors found that, compared with the general population, women affected by celiac disease have lower fertility when younger but recover at older ages.

With the introduction in clinical practice of serological tests for celiac disease, it has become apparent that, in the majority

of cases, the disease remains clinically silent or symptoms emerge outside the gastrointestinal tract. In the present investigation, none of the celiac women referred to ART reported major gastrointestinal complaints. Two reported non-specific and mild abdominal discomfort. Extra intestinal signs linked to celiac disease were noted in four out of five patients. These included autoimmune hypothyroidism, hyperprolactinemia, unexplained elevation of liver enzymes and iron deficiency.

Collin *et al.* (1996) detected a high frequency of celiac disease (4.1%) in women with unexplained infertility. Evidence supporting unexplained infertility factor as the type of infertility more commonly associated to celiac disease was also provided by Meloni *et al.* (1999a). In contrast with the results offered by these studies, none of the women found to be celiac in our study was from couples with unexplained infertility. Indeed, major indications to ART were male infertility in three cases and tubal factor in the remaining two cases. There is no easy explanation for this discrepancy. On theoretical grounds, it might be possible that when infertility is caused by celiac disease (and regarded as an unexplained form because of the missed diagnosis of the disease) first level infertility treatments such as superovulation in combination with insemination or timed intercourse are sufficient to achieve conception. In contrast, when infertility has a multifactorial aetiology with celiac disease being involved, first levels of interventions fail and patients are referred to ART.

If it is assumed that a causal link between celiac disease and infertility exists, then a crucial question to address is whether gluten-free diet can minimize infertility. Initial research is in agreement with this possibility (Ciacci *et al.*, 1996; American Gastroenterology Association, 2001; Hin and Ford, 2002). The potential of a gluten-free diet to exert a positive effect on reproduction is rationalized by the possibility that nutritional imbalance—especially malabsorption of selective nutrients including zinc, selenium, iron and folate—may underlie celiac disease-mediated reproductive disorders (Rostami *et al.*, 2001). Nevertheless, reproductive problems cannot be completely explained by malabsorption of nutrients (Collin *et al.*, 2002). In agreement with this concept, a recent study found that children conceived by celiac men had a lower birth weight (Ludvigsson and Ludvigsson, 2001).

Infertility is currently regarded as a condition in which celiac disease should be borne in mind (Rostami *et al.*, 2001; Collin *et al.*, 2002; Green and Jabry, 2003; Collin, 2005). Given the absence of a significant difference in the incidence of celiac disease between the study and control group, the

present study is far from offering a conclusion. It suggests a need for further studies to address the issue of celiac disease screening in ART programmes.

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