Strategies for the identification and prevention of coeliac disease

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Screening for unrecognised coeliac disease in subfertile couples

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Abstract

Objective: Subfertility has been reported as a long-term complication of unrecognised and/or untreated coeliac disease (CD); however the results from studies on this topic are ambiguous. We aimed to determine the prevalence of unrecognised CD in subfertile male-female couples visiting a fertility clinic compared to the general population.

Methods: Subjects included 1038 male-female couples (n=2076) who visited the fertility clinic of the Leiden University Medical Center in the Netherlands, between 2003-2009. All consecutive patients were routinely, serologically screened, and those with positive test results for antibodies against IgA anti-tissue transglutaminase type 2 and IgA endomysial antibodies were considered to have unrecognised CD. Clinical data on gender, age, height, weight, diagnosis of subfertility and previously diagnosed CD were collected from the clinical files. Subsequently, after serological screening all patients were anonymised. The prevalence of unrecognised CD was compared to the one in the general adult population in the Netherlands (0.35%).

Results: The prevalence of unrecognised CD in subfertile male-female couples was 0.48% (10/2076; 6 females and 4 males) and was not significantly more frequent compared to the general population. Compared to the control group, similar CD prevalences were found within the different subfertility categories separately - unexplained subfertility, anovulation, tuba pathology and male factor (p = NS).

Conclusion: In our large study cohort of subfertile male-female couples, the prevalence of unrecognised CD is comparable to the general population in the Netherlands. No association was observed between CD and subfertility in the different subfertility categories and genders.

Introduction

Coeliac disease is a condition that is provoked by the ingestion of dietary gluten that leads to a T-cell driven inflammatory response directed to the small bowel mucosa. (1) In the general population, the prevalence of CD is nearly 0.2-1.0% in adults.(2-4) CD is often unrecognised; in a study performed in The Netherlands, for every clinically diagnosed CD case, 21 subjects were undetected.(2)

Serological screening for CD is reliable through detection of IgA class anti-tissue transglutaminase type 2 (anti-TG2) and IgA anti-endomysial (EMA) antibodies, which have a 90-100% sensitivity and nearly 100% specificity for subtotal villous atrophy, which is characteristic for the diagnosis of CD.(5) CD has a variable clinical presentation ranging from frank malabsorption, such as diarrhoea, distended abdomen, anorexia and weight loss, to mono-symptomatic presentations like fatigue, osteoporosis, iron-deficiency anaemia, and reproductive disorders.(1,6)

Disorders associated with reproduction, such as recurrent abortions, low-birth-weight or preterm deliveries, impotence, and hypogonadism, has all been described as related to untreated/unrecognised CD.(7-12) Subfertility (a failure to conceive after one year of unprotected regular sexual intercourse (13)) has also been suggested in the literature as a complication of unrecognised/untreated CD, however, on closer scrutiny, the literature is contradictive about this association (9,10,14-18) In subfertile females, the prevalence of unrecognised CD has been reported ranging from equal to, to slightly increased compared to a control group or the general population. (9,10,15,16,19-22) One study group investigated 99 subfertile male-female couples, and in the subfertile males the prevalence of unrecognised CD was comparable to the control group.(9) Most previously performed studies on this topic were based on small cohorts of subfertile females and males.(9,10,15,16,19-22)

Due to the contradictive data of an association between subfertility and unrecognised CD, we performed a screening study in a large cohort of subfertile individuals, by determining the prevalence of unrecognised CD in a cohort of subfertile male-female couples visiting the fertility university clinic. Controls were individuals of general adult population in the Netherlands.

Methods

Patients

In this retrospective cohort study, subjects were consecutive male-female couples who attended the fertility clinic of the Leiden University Medical Center (LUMC), the Netherlands, between 2003-2009. We identified the subjects through the Hospital Information System. These couples were examined for their fertility disorder by a standardised diagnostic work-up programme. As part of this programme, blood samples were collected for the detection of sexually transmitted diseases. These samples were stored in the department of Microbiology at -20° degrees for at least 10 years. Couples were included in this study if samples of both partners were obtained within a 6-month time window around the first visit to the fertility clinic. This time-frame was chosen to ensure that CD would be detected during the presence of subfertility. Couples that did not meet this criterion were excluded. We also excluded female-female couples and single females who required donor insemination because screening for CD would not be possible in the (anonymous) semen donor.

The power calculation was based on a two-sided Fisher's Exact test with a significance level of 5% and 90% power to detect a difference of 1% CD risk in a general population (3) versus 4% CD risk as described in subfertile females.(9) According to this power calculation a minimum of 632 subfertile male-female couples visiting the fertility clinic needed to be studied.

As a control group we used data from a screening study on the prevalence of unrecognised CD in the general population of the Netherlands.(2) That screening study was performed by our study group of the LUMC with a comparable serological detection method (see below) for unrecognised CD. In this control group, unrecognised CD was diagnosed in 5 of the 1432 healthy adults (0.35%), including 2 (0.28%) of 716 females and 3 (0.42%) of 716 males, aged 20-59 years.

Methods

Screening for CD was performed through the detection of IgA anti-TG2 in serum using the ELIA™ Celikey® assay at the Immunocap®250 system; Phadia GmbH, Freiburg, Germany (7-10 U/mL is the equivocal area recommended by the manufacturer; >10

U/ml is considered as positive).(23) This system uses human recombinant tissue transglutaminase as an antigen. Samples tested for IgA anti-TG2 values of ≥7 U/mL were subsequently analysed for IgA EMA using monkey's oesophagus as substrate (dilution 1:10), performed according to instructions of the manufacturer (Scimedx). We defined unrecognised CD if test results for both IgA anti-TG2 (>10 U/ml) and IgA EMA were positive in one subject. If both antibodies are positive in one subject, this accurately predicts the presence of subtotal villous atrophy consistent with CD.(5,24,25) This serological screening strategy for the diagnosis of CD has been used by others in subfertile subjects (16,21) but also in other screening studies.(25-27) Small bowel biopsies were not offered to the subjects in case of positive screening tests because only anonymised use of blood samples was allowed by the medical ethics committee of the LUMC and for this reason the results could not be traced back to the individual patients. In the control group (2) a similar serological detection method was used, although for IgA anti-TG2 a guinea pig substrate (in house developed ELISA) was used. Clinical data on gender, age, height, weight, previously diagnosed CD, aetiology of subfertility (primary or secondary), and diagnosis of subfertility (ovulation disorder, tubal pathology, male factor and unexplained (28)) were collected from the patient files.

Statistical Analysis

Continuous data were expressed as mean ± standard deviation or as median and range; discrete data as numbers and percentages. The prevalence of unrecognised CD was expressed as a percentage. Body mass index (BMI) was calculated as weight (kg) / height $(m)^2$. Differences in the prevalence of CD between subjects and controls were quantified as Odds Ratio (OR) with 95% confidence interval (CI). To calculate differences between the groups we used the Chi-square test for discrete values (corrected with the Fisher's Exact test for small sizes of groups), and the student T-test for continuous values. A p-value <0.05 was considered significant. All analyses were done with SPSS 16.0.

Ethics

The study was approved by the Medical Ethics Committee of the LUMC (P08.058). All data and serum samples were available for anonymised research; therefore anonimisation took place by assigning random study numbers after serum samples and clinical data had been retrieved and merged before the samples were tested.

Results

We identified 1180 subfertile male-female couples that were eligible for the study. We included 1038 male-female couples (88%) and excluded the remaining 142 male-female couples (12%) due to unavailability of serum to test. In the included group of females, median age was 32.3 years (range 20-45) and in males 35.4 years (range 20-64). In 789 (76%) of the 1038 females the median BMI was calculated on 23.3 kg/m^2 (range 16-49), and in 590 (57%) of the 1038 males on 25.4 kg/m^2 (range 18-48). From the included couples, 69% of the subjects were examined for primary subfertility and 31% for secondary subfertility. The subfertility diagnoses are listed in table I, categorised by gender and CD diagnosis.

In the medical files, none of the subfertile 2076 subjects was documented with previously diagnosed CD. In the sera of the subjects, we found 12 samples with positive IgA anti-TG2 levels of >10 U/mL (0.6%; median level 60 U/ml, range 13-137). IgA EMA was positive in 10 of the 12 positive IgA anti-TG2 samples (83%), with median IgA anti-TG2 levels of 74 U/mL (range 27-137). Therefore, we detected unrecognised CD in 10 of 2076 subjects (0.48%): 6 females and 4 males. In none of the couples both partners had unrecognised CD. In the remaining 2 samples with positive IgA anti-TG2 levels and negative IgA EMA results, the median IgA anti-TG2 level was marginally elevated (13.5 U/mL; range 13-14).The median IgA anti-TG2 levels of the serum samples tested positive for IgA EMA were significantly higher than in the samples that were tested negative for IgA EMA ($p<0.05$).

Table 1. Prevalence of unrecognised coeliac disease divided by causes of subfertility in 1038 male-female couples visiting a fertility clinic in the Netherlands.

	Study Group (n=2076)		**Unrecognised CD‡ (n=10)**	
Subfertility diagnosis	**Female (n=1038)** n (%)	**Male (n=1038)** n (%)	**Female (n=6)** n (%)	**Male (n=4)** n (%)
Ovulation disorder*	203 (20)	-	3 (1.48)	-
Tubal factor*	100 (10)	-	0	-
Male factor*	-	464 (45)	-	1 (0.22)
Partners of subject with particular subfertility diagnosis**	384 (37)	223 (22)	1 (0.26)	1 (0.45)
Unexplained	351 (34)	351 (34)	2 (0.57)	2 (0.57)

‡ *Diagnosis for CD is defined when both anti-TG2 (>10 U/mL) and EMA were positive; *Including male-female couples both having a subfertility diagnosis: 51 with male factor and ovulation disorder, and 29 with male factor and tubal factor; **Including partners with; 384 male factor, 152 ovulation* disorder*, and 71 tubal factor*

The mean age of subjects with unrecognised CD was 29 years (SD±5.3) in females, and 36 years (SD±3.1) in males. The mean BMI could be calculated in 4 out of 6 females with CD and was 25.4 kg/m^2 (SD±2.9), and in 2 (50%) of 4 males with CD and was 24.5 kg/m^2 (SD±0). There were no significant differences concerning age or BMI between subfertile males and females with and without CD. Primary subfertility was diagnosed in 5 (83%) of the 6 females and 2 (50%) of the 4 males with CD.

The prevalence of unrecognised CD in all subjects (10/2076; 0.48%) was somewhat higher, but not significantly different compared to the prevalence of unrecognised CD in the general population (5/1432, 0.35%; OR 1.38, 95% CI 0.471- 4.05; Fisher's Exact test).

When analysed separately by gender, the prevalence of unrecognised CD in female subjects (6/1038, 0.58%) and in male subjects (4/1038, 0.39%) was not significantly different compared to the prevalence of unrecognised CD in females (2/716, 0.28%; OR 2.08, CI95% 0.42-10.31; Fisher's Exact test) and males (3/716, 0.42%; OR 0.92, CI95% 0.21- 4.12; Fisher's Exact test) in the general population.

The prevalence of unrecognised CD in the unexplained subfertility category for females (2/351, 0.57%; OR 2.05, CI95% 0.29-14.58; Fisher's Exact test) and males (2/351, 0.57%; OR 1.35, CI95% 0.23-8.19; Fisher's Exact test) were both not significantly different compared to the general population. The prevalence of

unrecognised CD was almost 5 times higher in females with an ovulation disorder (3/203, 1.48%) compared to females in the general population (OR 5.36, CI95% 0.89-32.27, Fisher's Exact test), but this difference did not reach statistical significance (p>0.05). None of the 100 women with a tubal pathology had CD. One of 464 men (0.22%) with subfertility due to male factor was diagnosed for CD and that was not significantly more frequent as compared to males in the general population (OR 0.51, CI95% 0.05-4.95; Fisher's Exact test).

Discussion

In our study, the prevalence of unrecognised CD in subfertile males and females individuals was comparable to the general population (2); 0.48% versus 0.35% (p=0.6). Moreover, no statistically significant difference was found concerning the prevalence of unrecognised CD within the different subfertility diagnostic categories in males and females compared to the control group. Based on these results, we conclude that in our study cohort there is no association between subfertility and unrecognised CD.

In literature there is a discrepancy between the different prevalences of unrecognised CD in subfertile individuals. In females, this association has been extensively described, however, some investigators found comparable prevalences (19-22), others found a significantly higher number of unrecognised CD in subfertile cases compared to the control group.(9,10,15,16) The discrepancies could be explained by methodological issues. Some studies have based their prevalence on underpowered cohorts which could result in selection bias.(9,10,15,22) In one study on the occurrence of unrecognised CD in women with subfertility (10), the authors did not find any CD case in the control group of fertile females which makes the comparison difficult between the two groups. Other studies only determined the prevalence of unrecognised CD in females with *unexplained* subfertility (9,10,15), instead of including *any* subfertility category, such as anovulation disorder and tubal pathology. In our opinion, all causes of subfertility should be taken into account because the underlying pathophysiological mechanism of the association is not known. When

assessing the prevalence of CD in just one subfertility subgroup this could result in an overestimation.

In subfertile males, the prevalence of unrecognised CD has only been described by one study that investigated 99 male-female couples and in males no higher prevalence of unrecognised CD was found compared to the control group.(9) Our results are in accordance with these findings.

Recently, a large population-based cohort study was performed on fertility in females and males with recognised/diagnosed CD in Sweden.(29,30) The investigators concluded that women with diagnosed CD had similar fertility rates compared to women in the general population, but fertility decreased in the last 2 years preceding CD diagnosis.(29) In another population-based study in the United Kingdom, the authors also found that females with recognised CD had normal fertility rates, but had their babies at an older age compared to women in the general population.(31) The Swedish investigators also showed that males with recognised CD did not have a lower fertility compared to the control group.(30) In our cohort of 2076 subfertile persons we did not detect previously diagnosed cases with CD. Nevertheless we would expect to have found some subjects with recognised CD, since the prevalence of recognised CD in our country was determined as 0.016% (95%CI 0.008-0.031).(2) If, as suggested in literature, fertility disorders are reversible when patients are treated for CD by a gluten-free diet, it could be reasoned that less patients with recognised CD may visit a fertility clinic.(14,32-34) Another explanation could be that, due to our retrospective study design, the medical staff in the fertility clinic did not specifically document if patients were previously diagnosed for CD or that the patients considered their disorder as unimportantly to mention in this situation. On the other hand, other (autoimmune) gastro-intestinal disorders, such as Crohn's disease and ulcerative colitis were reported in patient's histories.

In several recent large studies on fertility rates in patients with recognised CD, it was shown that recognised CD is not associated with subfertility. Our data now show that the same may hold for unrecognised CD. We therefore think that the assumed association between reproductive disorders as a complication of CD may

be much less strong than previously reported. However, this does not mean that CD should be neglected in subfertile patients.(14,17,32,34,35) In small-case series it was described that after starting a gluten-free diet fertility restored and that the number of other reproductive complications, such as prematurity and miscarriages, decreased.(7,14,32,34-36) The pathophysiological mechanism is not understood, but it suggested that CD may affect reproductive organs. For this reason, intervention studies should be performed on the effect of a gluten-free diet in the restoration of fertility in subfertile females and males with CD. If that hypothesis is correct, 10 of 2076 subfertile individuals with CD in this study may have been saved the burden of diagnostics and therapy, while it is also favourable for the costs.

In our study some limitations can be found. Twelve percent of subfertile male-female couples were excluded due to missing sera of the couples. No reason could be found why sera of this group of couples was not collected in time or stored in the freezers at the Department of Microbiology of the LUMC. This is probably due to coincidence. Another disadvantage of our retrospective cohort study is that we could not confirm the diagnosis for CD by performing small bowel biopsies in (anonymised) patients with simultaneous positive serology for anti-TG2 and EMA. However, it is accepted that both antibodies are highly sensitive and specific for predicting villous atrophy, and our strategy has been used in several screening studies in the recent past.(5,19,24-26) Moreover, in the control group anti-TG2 guinea pig has been evaluated, which is now known to give incidentally false positive results (2), CD was only 'diagnosed' if confirmed by EMA. This serological screening procedure was also used in our cohort of subfertile individuals; therefore we consider the present screening strategy comparable to that in the control group. The same investigators were involved in the screening procedures of the general population (2) as well as in the subfertile cohort of male-female couples. The prevalence of unrecognised CD in the control group (0.35%) was comparable to another mass screening study in Dutch adults by Rostami *et al* (0.2%, p=0.49)(37), and other mass screening studies performed in different countries: 0.2%-1%.(3) Although we do not know the fertility rate in our control group, we detected comparable CD prevalences in the control group if we would have corrected for the effect of age on fertility in females (20-39

years; CD prevalence of 0.26%) and males (20-44 years; CD prevalence of 0.42%). For this reason we have used the control group aged 20-59 years old. In our study, we have found an OR of 1.38 meaning that CD was far from significant more present in subfertile couples compared to the general population (OR 1.38, 95% CI 0.471-4.05). As the confidence interval is quite large, however, we do not have enough power to conclude that the frequency of CD in subfertile couples is really equal to the general population. Further investigation should be performed to warrant a direct comparison between a subfertility group and a matched fertility group of couples.

In conclusion, the prevalence of unrecognised CD in subfertile male-female couples in our country is at the same level as the general population. In combination with the conclusions of the large population studies on recognised CD, we conclude that subfertility in females and males is not associated with CD.

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