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DOI: 10.17235/reed.2020.7444/2020
Link: PubMed (Epub ahead of print)

Please cite this article as:
Roseira Joana, Lopes Rita, Silva Mário Jorge, Vieira Ana Margarida, Sampaio Margarida , Calinas Filipe. Gynecological history up to diagnosis and pregnancy outcomes in diagnosed Wilson's disease under therapy – a bicentric matched control cohort study. Rev Esp Enferm Dig 2020. doi: 10.17235/reed.2020.7444/2020.

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OR 7444

Gynecological history up to diagnosis and pregnancy outcomes in diagnosed Wilson's disease under therapy – a bicentric matched control cohort study

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ABSTRACT

Introduction

Most studies narrowly focus on pregnancy outcomes comparisons between Wilson Disease (WD) patients on and off treatment. We aimed to identify menses irregularities in untreated WD and evaluate pregnancy outcomes in treated WD compared with matched controls (with and without liver disease).

Methods

Women with WD, women with Hepatitis C (liver disease controls), and women with other gastrointestinal conditions (controls without liver disease), were identified from two tertiary hospital gastroenterology departments. Gynecological and obstetric data was retrospectively collected. Comparison of gynecological and obstetric outcomes between groups was performed, and regression models were used to further assess obstetric outcomes.

Results

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We identified 18 women with WD, comprising 19 pregnancies under treatment in 11 patients, and 20 women for each control group. Age and liver disease stage between groups was adjusted. The incidence of menses irregularities was higher for WD (late menarche, 83% vs. 10% vs. 10%, p<0.01; irregular cycles, 100% vs. 20% vs. 20%, p<0.01; amenorrhea, 67% vs. 10% vs. 5%, p<0.01). Logistic regression models identified WD as a predictor of miscarriage and low birth weight (OR 6.0; IC 1.1-33.3; p<0.05), but not of birth defects. Neither therapies (D-Pencillamine 300mg or zinc acetate 150mg) nor disease presentation (hepatic or/and neurological) were associated with obstetric complications in WD.

Conclusion

There was a higher incidence of menses irregularities in untreated women with WD. Additionally, our data suggests that treated WD still carries a higher risk of spontaneous abortion and low birth weight, compared to matched control groups with and without liver disease.

Keywords

Hepatolenticular disease; spontaneous abortion; low birth weight; menstruation disturbances.

Abbreviation list

Wilson Disease (WD)

INTRODUCTION

Wilson Disease (WD) is an autosomal-recessive disorder caused by *ATP7B* copper-binding protein malfunction, leading to a decreased hepatocellular excretion of copper and consequently liver cytotoxicity. Excess copper is released in the bloodstream and deposited in multiple organs, namely the central nervous system. Although copper accumulation may lead to injury in many tissues, most individuals present with liver disease and/or neuropsychiatric symptoms (1–3). In females we may observe distinctive manifestations, such as amenorrhea, impaired conception or recurrent

miscarriages after a successful conception (4). Proposed pathophysiological explanations are hormonal imbalance due to liver dysfunction and placental copper deposition (5,6). The mainstay of WD remains lifelong anticopper treatment, with D-Penicillamine, Trientine or Zinc, to halt disease progression and avoid tissue damage. This recommendation extends to pregnancy, to protect the health of both mother and baby (7,8). In fact, there are numerous reports of successful pregnancies in WD patients under therapy (9–11). However, the theoretical drug teratogenicity (10,12,13), complications of treatment-induced maternal copper deficiency (14), and obstetric complications due to cirrhosis (15), remain apprehensions shared by mothers and clinicians. Recently, Pfeiffenberger *et al* (4*),* reported the largest collection of data on pregnancies in WD. The authors confirmed that patients under therapy had better chances of successful pregnancies than untreated patients. Still, the reported abortion rate was 18% for patients under therapy. Similarly, Rabiee *et al* (16*),* issued a letter on this subject reporting better outcomes for treated patients, though describing an abortion rate ranging from 10-27%. These, and most studies, focus on pregnancy outcomes between WD patients with and without treatment, or perform general comparisons with population-based studies.

This study aimed to: 1) identify menses irregularities in WD before diagnosis; 2) evaluate pregnancy outcomes, namely spontaneous abortion, low birth weight and birth defects, in WD patients under anticopper treatment; both compared with two matched control groups, with and without liver disease.

MATERIALS AND METHODS

Study design

This was a retrospective, bicentric, case-control design study. All female patients with an established diagnosis of WD, as defined by WD Working Party (17), were eligible. Data collection was carried out considering gastroenterology clinics from two tertiary hospital centers in order to signal the maximum number of female patients with WD, which was predicted to be the sample limiting factor. For this group, only gynecological data before diagnosis was analysed, and all pregnancies under uninterrupted anticopper treatment were considered for obstetric outcome analysis. In case of

uncertainties about data registry, presential interviews were performed. For the control group with liver disease, women with untreated Hepatitis C, who had once been pregnant, were consecutively identified from the clinics. For the control group with no liver disease, women who had once been pregnant were consecutively identified from the general gastroenterology clinics. The entire hospital chart was reviewed when necessary, in order to fully address the controls' gynecological and obstetric history. Patients with incomplete data registries, older age (≥ 45 years old) pregnancies, patients with known comorbidities (endocrinopathies, autoimmune and metabolic diseases), and with drug use or alcohol consumption during pregnancy, were not considered for the control groups.

Patient characteristics

Concerning the WD group, clinical variables were collected: disease presentation, diagnosis method and time, copper load, therapy during pregnancy.

For all groups, demographics, clinical data, gynecological and obstetric data were collected: age, gender, liver disease stage or gastrointestinal diagnosis other than liver disease, late menarche, irregular menstrual cycles, periods of amenorrhea, spontaneous abortion, low birth weight and birth defects. Liver disease stage was categorized according to liver stiffness by transient elastography using FibroScan® (Echosens, Paris, France). All included patients with hepatitis C (controls with liver disease) and Wilson disease (regardless of having had a biopsy at diagnosis) were evaluated with FibroScan® during their follow-up. We used METAVIR fibrosis stage for easier categorization and comprehensibility. For the control group with liver disease (untreated Hepatitis C), liver disease stage was categorized as "absent/minimal fibrosis" (F0-F1; ≤7.3 kPa), "significant fibrosis" (F2-F3; ≥7.4 kPa <12.5) or "cirrhosis" (F4; ≥12.5 kPa) (18). For the WD group, liver disease stage was categorized as "absent/minimal fibrosis" (F0-F1; ≤5 kPa), "significant fibrosis" (F2-F3; ≥7 kPa <10.1) or "cirrhosis" (F4; ≥10.1 kPa) (19). According to available data and for comparison purposes, liver disease stage for the control group with liver disease was considered during follow-up before pregnancy, and liver disease stage for the WD group was considered during uninterrupted anticopper treatment before pregnancy.

Outcome measures

Gynecological and obstetric outcomes were dichotomously categorized as absent or present according to patients' history. As for consensus definitions: late menarche was defined as failing to have the first menstrual cycle before 14 years of age; irregular menstrual cycles referred to menstrual cycles abnormal length; amenorrhea was defined as the absence of menses for three months or more (20,21). Regarding obstetric outcomes, spontaneous abortion was defined as a non-viable pregnancy at any gestational age; low birth weight was defined as 2.499g or less; birth defect was defined as any anomaly detected at birth (22–24).

Statistical considerations

Statistical analysis was performed using the Statistical Package for Social Sciences version 25.0, and level of significance was established at 5%. According to numerical variables distribution, descriptive data was presented as mean and standard deviation for continuous variables. Categorical variables were summarised using absolute (n) and relative frequencies (%).

For the sample size estimation, a power of 80% was used. Based on menses alterations and adverse pregnancy outcomes historically reported for WD and the general population, we estimated that a difference of 50% to 10% could be found (4,22,25). This assumption led to the calculation that groups of 17 patients were necessary (26).

Chi-square and One-way ANOVA tests were used to compare different groups of subjects. Binominal logistic regression models were used to assess the impact of variables on obstetric outcomes, using the enter method. Independent variables were introduced based on clinical relevance: age, diagnosis of WD, liver disease.

Ethical statement

The study protocol conforms to the ethical guidelines of the Declaration of Helsinki. The authors retrospectively analysed data, thus waiver of consent was approved. All efforts were made to ensure confidentiality of the data. This manuscript follows the standards outlined in the STROBE statement.

RESULTS

Patient characteristics

Eighteen female patients with an established diagnosis of WD were eligible, comprising a total of 19 pregnancies in 11 patients under anticopper therapy. Most patients were under treatment with D-Penicillamine (78.95%), and four patients were under Zinc therapy during pregnancy (21.05%). **Table 1** presents clinical characteristics of the WD group. Twenty females for each control group (with and without liver disease) were consecutively selected. Age between groups was comparable. Liver disease stage categorization proportions were similar between the WD group and the control group with liver disease. **Table 2** presents demographics of the three groups and additional characteristics of the controls, namely the diagnosis that justified a general gastroenterology follow-up for the patients in the control group without liver disease. Most of these diagnoses were functional gastrointestinal disorders (27).

Outcome assessments

Table 3 presents the gynecological and obstetric outcomes.

The gynecological outcome analysis comprised the 18 WD females, and referred to disease before diagnosis and therapy. Overall, late menarche, irregular menstrual cycles and amenorrhea were significantly more frequent in the WD group, compared with both controls.

The obstetric outcome analysis comprised 19 pregnancies for the 11 women in the WD group that had an history of pregnancy under anticopper therapy. Spontaneous abortion and low birth weight were more frequent in the WD group. Concerning birth defects, three cases were reported in the WD group (two cases of atrial septal defect, and one case of cleft lip), and two cases were reported in the control group without liver disease (one case of esophageal atresia and pulmonary valve atresia). Overall, there was no association between obstetric complications with WD therapy or with clinical presentation. However, low birth weight was significantly more frequent in WD patients with more advanced liver disease (**Figure 1**). Finally, logistic regression models built to assess the impact of each study group, age and liver disease stage on obstetric outcomes are displayed in **Figure 2.** Only spontaneous abortion and low birth weight

were significantly associated with the WD group. A diagnosis of WD, even under treatment, was associated with a 6-time odd increase of spontaneous abortion and low birth weight.

DISCUSSION

This study aimed to identify menses irregularities in WD before diagnosis, and further evaluate pregnancy outcomes, namely spontaneous abortion, low birth weight and birth defects, in WD patients under anticopper treatment, compared with two matched control groups with and without liver disease. To the authors' knowledge, this is the first study comparing obstetric outcomes between WD patients under anticopper therapy with age and liver disease stage matched controls.

Unsurprisingly, our data showed that menses irregularities, such as late menarche, irregular menstrual cycles and amenorrhea were significantly more frequent in the WD group compared to both controls. These results are consistent with previous studies which report menstrual abnormalities and amenorrhea in almost all untreated women with WD (6,28,29). In our cohort, these alterations were present before the diagnosis was made, but in none of the cases led to WD diagnosis. Clinicians should acknowledge that WD is part of the differential diagnosis of unexplained amenorrhea. Liver disease may compromise oestrogen normal metabolism and testosterone breakdown leading to follicular maturation arrest. Additionally, interference on the aromatase enzyme system by copper intoxication may decrease oestradiol production and ovulation. As so, WD-related amenorrhea will be mostly associated with normal dynamic tests, namely hypothalamic, pituitary, thyroid, and adrenal function, low levels of follicular stimulating hormone, luteinizing hormone, oestradiol, and normal levels of testosterone and androstenedione (6,29).

To date, guidelines (1,2) recognise that significant morbidity and mortality are prevented by anticopper treatment, and that failure to comply with lifelong therapy leads to symptom recurrence and liver failure. Similarly, treatment must be maintained throughout pregnancy. The dosage of zinc salts is maintained without change, and chelating agents dosage should be lowered to a minimal dose to avoid insufficient copper supply for the fetus. However, almost all studies and case series

that assign the evidence to these orientations focus on pregnancy outcomes between WD patients on and off treatment. Definitely, WD patients under anticopper treatment throughout pregnancy have better results than undiagnosed, untreated or WD patients who interrupt treatment (4,9–11,16). But what about when patients ask if maintaining treatment will make it all fine during pregnancy? We do not have all evidence to comprehensively deal with this question. In this study, we aimed to assess obstetric outcomes for patients with WD under anticopper treatment, and patients, with comparable age, with and without liver disease. Patients' copper status was optimized before pregnancy, no therapeutic switches were performed after pregnancy confirmation, and treatment with D-Penicillamine was reduced to 300 mg, as this is all part of the routine practice in our institutions. Still, spontaneous abortion and low birth weight were more frequent in the WD group compared to controls. We found no association between these complications, therapies (D-Penicillamine or zinc) or disease presentation (hepatic or/and neurological), but did identify an association with liver fibrosis stage: low birth weight was significantly more frequent in WD patients with more advanced liver disease. Finally, to account for potential multicollinearity, logistic regression models were separately built to assess the impact of the study groups, age and liver disease stage on obstetric outcomes. Spontaneous abortion and low birth weight were only significantly associated with the WD group. A diagnosis of WD, even maintaining treatment, was associated with a 6-time odd increase of spontaneous abortion and low birth weight. To the authors' knowledge there is only one study comparing WD pregnancy outcomes with age matched controls, instead of literature controls (30). However, in that study, the number of cases significantly outweighed the controls, 90% of WD females interrupted anticopper treatment during pregnancy, and liver disease stage was not considered. Liver stiffness by ultrasound based transient elastography is an excellent surrogate marker of fibrosis and cirrhosis. Liver stiffness cut-off values are mainly defined for viral hepatitis, but have also been assessed for other diseases including WD. In our study, for comparison purposes, we categorized WD patients' liver disease according to liver stiffness cut-offs that previously showed to correlate with biopsy proven fibrosis, and to outscore other noninvasive approaches to categorize liver disease stage (19, 31). We believe our study adds to the literature as

it suggests that treated WD still carries a higher risk of spontaneous abortion and low birth weight, compared to matched control groups with and without liver disease. In this cohort, birth defects were not associated with WD diagnosis, therapies, clinical picture or liver disease. Birth defects are common, affecting one out of 33 babies in the United States per year. Minor cardiovascular defects, cleft lip and gastrointestinal defects are among the most frequent (32). One may elaborate that ensuring an adequate copper balance before conception as well as reducing chelation during pregnancy may reduce the risk of teratogenicity and avoid insufficient copper supply to the fetus. Similar results have been reported in larger samples (4,16).

Nevertheless, some limitations should be noted. This study has and important sample size frailty, and its retrospective design does not allow to exclude information or sample bias. Still, the patient consecutive selection from two tertiary hospital centers, and the powered sample size calculation may prevent over significant bias. Additionally, it matters to say that performing trials in pregnant patients raises serious ethical questions and that WD patients are sufficiently rare to preclude large cohort studies. Therefore, evaluations with alternative methodologies are probably not feasible. This study is an honest attempt to answer an unresolved question, concerning WD pregnancy complications among patients maintaining anticopper treatment as recommended, and avoiding the simpler solution that would be to compare our results with historical unmatched literature controls.

In conclusion, expectably there was a higher incidence of menses irregularities in untreated women with WD compared to controls. Additionally, our data suggests that treated WD still carries a higher risk of spontaneous abortion and low birth weight, compared to matched control groups with and without liver disease.

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Table 1. Wilson disease patients' clinical characteristics.

Characteristics	
Disease presentation, n (%)	11 (61.11)
Hepatic	7 (38.89)
Neurological	
Diagnostic method	12 (66.67)
Clinical	3 (16.67)
Histological	3 (16.67)
Genetic	17.06 ±
Time of diagnosis; years, mean ± SD	9.83
Laboratory values before pregnancy was known	
Platelets (10^5/dL)	
Aspartate aminotransferase (UI/dL)	229.89 ±
Alanine aminotransferase (UI/dL)	86.79
Prothrombin time international normalised ratio	27.44 ± 9.36
Liver stiffness by ultrasound based transient elastography; kilopascal, mean ± SD	30.89 ± 7.66 0.90 ± 0.16
Urinary copper excretion per 24 hours before conception; µmol, mean ± SD	12.47 ± 22.15 7.36 ± 2.11
Daily treatment during pregnancy, n (%)	
D-Penicillamine 300 mg	15 (78.95)
Zinc 150 mg	4 (21.05)

SD: standard deviation

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ENFERMEDADES DIGESTIVAS
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Table 2. Demographics of the study groups and additional characteristics of the controls.

Characteristics	Wilson Disease (n=18)	Controls with liver disease (n=20)	Controls without liver disease (n=20)	p-value
Age (years), mean ± SD	32.78 ± 6.99	35.65 ± 5.69	34.35 ± 5.89	0.33*
Liver disease stage, n (%)‡				0.65†
F0-F1	7 (38.89)	10 (50.00)	-	
F2-F3	3 (16.67)	4 (20.00)	-	
F 4	8 (44.44%)	6 (30.00)	-	
Diagnosis, n (%)				
Irritable bowel	-	-	6 (30.00)	
syndrome	-	-	5 (20.00)	
Functional dyspepsia	-	-	5 (20.00)	
Helicobacter pylori	-	-	2 (10.00)	
gastritis	-	-	2 (10.00)	
Colorectal diminute polyps				
Gastroesophageal reflux				

SD: standard deviation

*p-value based on One-way ANOVA test; †p-value based on chi-square test comparison between Wilson disease group and control group with liver disease

‡ Liver disease stage was categorized according to liver stiffness by transient elastography using FibroScan® (Echosens, Paris, France). According to METAVIR fibrosis stage: F0-F1 or "absent/minimal fibrosis"; F2-F3 or "significant fibrosis"; F4 or "cirrhosis"

Table 3. Gynecological and Obstetric outcomes of the study population.

	Wilson Disease	Controls with liver disease	Controls without liver disease	p-value
Late menarche, n (%)	15/18* (83.33)	2/20 (10.00)	2/20 (10.00)	**0.00**
Irregular menstrual cycles, n (%)	18/18* (100.00)	4/20 (20.00)	4/20 (20.00)	**0.00**
Amenorrhea, n (%)	12/18* (66.67)	2/20 (10.00)	1/20 (5.00)	**0.00**
Spontaneous abortion, n (%)	7/19† (36.84)	3/20 (15.00)	2/20 (10.00)	**0.04**
Low birth weight, n (%)	7/12† (58.33)	1/17 (5.88)	2/18 (11.11)	**0.02**
Birth defects, n (%)	3/12† (25.00)	0/17 (0)	2/18 (11.11)	0.12

*for the gynecological outcome analysis, the 18 patients with a diagnosis of WD were considered

†for the obstetrical outcome analysis, the 19 pregnancies accounted in the WD group were considered. Low birth weight and birth defects were only considered among live births.

p-value based on chi-square tests

	Penicillamine	Zinc	p-value	Hepatic	Neurological	p-value	F0-F1	F2-F3	F4	p-value
Spontaneous abortion, n	5/15	2/4	0.65	4/11	3/8	0.85	4/9	1/4	2/6	0.39
Low birth weight, n	6/15	1/4	0.64	4/11	3/8	0.85	1/9	2/4	4/6	**0.03**
Birth defects, n	2/15	1/4	0.53	2/11	1/8	0.79	1/9	1/4	1/6	0.89

Figure 1. * Liver disease stage was categorized according to liver stiffness by transient elastography using FibroScan® (Echosens, Paris, France). According to METAVIR fibrosis stage: F0-F1 or "absent/minimal fibrosis"; F2-F3 or "significant fibrosis"; F4 or "cirrhosis"

†p-values based on chi-square test

	Spontaneous abortion			Low birth weight			Birth defects		
	OR	95% CI	p-value	OR	95% CI	p-value	OR	95% CI	p-value
Diagnosis									
Controls without liver disease	Ref.								
Wilson Disease	6.00	1.08-33.27	**0.04**	6.00	1.08-33.27	**0.04**	1.69	0.25-11.42	0.59
Controls with liver disease	1.59	0.24-10.70	0.64	0.48	0.04-5.69	0.56	0.00	-	0.99
Age	0.96	0.81-1.14	0.63	0.95	0.79-1.15	0.59	0.89	0.69-1.15	0.39
Liver disease stage									
F0-F1	Ref.								
F2-F3	1.30	0.23-7.32	0.77	-	-	0.99	2.57	0.14-47.02	0.52
F4	0.43	0.07-2.62	0.36	-	-	0.99	1.64	0.09-28.90	0.74

Figure 2. OR: Odds ratio; CI: Confidence interval; Ref: Reference

*** Liver disease stage was categorized according to liver stiffness by transient elastography using FibroScan® (Echosens, Paris, France). According to METAVIR fibrosis stage: F0-F1 or "absent/minimal fibrosis"; F2-F3 or "significant fibrosis"; F4 or "cirrhosis"**