Study on clinical forms, diagnostics and treatment of Wilson's disease in Hungary

Doctoral thesis

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INTRODUCTION

Though S.A.K. Wilson has already published his paper in 1912, there are many unexplained questions about it nowadays as well.
However the various symptoms make the diagnosis challenging in many cases, new generation sequencing may be a giant leap. We have new result with this method even in international relations for the first time. In some cases we used it to strengthen the uncertain diagnosis.

It is unclear what factors may affect the development of the clinical symptoms. We examined the potential role of environmental and genetic factors by analysing the data of all 232 Wilson's disease patients registered at the Department of Internal Medicine and Oncology of Semmelweis University. We examined the PRNP gene as well. Interestingly we also found consecutive generations among our patients.

We examined the aspects of the therapy and the role of compliance in the treatment of the patients. However, many patients may be well with chelating agents, some patients have to undergo liver transplantation. After the hardness of the first years, now we have better post-transplantation results, which may be the consequence of the accession to the Eurotransplant program partly.

In some cases the compliance was unsatisfying leading to the death of the patient or to the need for liver transplantation. Unfortunately these patients had insufficient compliance later too.

AIMS

I have supplemented and analyzed the data of the database of the Department of Internal Medicine and Oncology of Semmelweis University. Beside the epidemiological observations I have also examined the clinical forms of Wilson's disease and the role of a new mutation. We also recorded the data of patients admitted into the Department of Transplantation and Surgery and the Intensive Care Unit of Péterfy Sándor street

Hospital and Casualty Centre. Sequencing of the whole ATP7B gene was performed at the PentaCore Laboratory.

The aims of the study were:

1. Epidemiological characteristics of the patients:
    a. Does the Hungarian characteristics equal to the international data?
    b. What is the distribution of the clinical forms of the Hungarian Wilson's disease patients? Is there any difference between the acute liver failure and the other patients? What is the frequency of the haematological disturbances? How many hepatobiliary malignancies presented in the patients?

2. Examination of the genetic background of the disease:
    a. What is the occurrence of the ATP7B gene mutations in Hungary? Can we diagnose Wilson's disease without genetic background?
    b. Shall we use next-generation sequencing in practice? Is there any clinical significance of it?
    c. Is there any role of the mutations of the PRNP gene in the clinical manifestation?
    d. What kind of other factors may affect the clinical symptoms?

3. Investigation of the potential therapies of the disease:
    a. How many patients had to be transplanted for Wilson's disease? What are the causes of liver transplantation? What was the long-time outcome?
    b. May the compliance affect the effectiveness of the treatment and the outcome of the disease?

METHODS

I analyzed the data of the 231 patients registered at the Department of Internal Medicine and Oncology of Semmelweis University since 1999. I reviewed the data available in the medical records and the MedSol system.

The high number of patients of this otherwise rare disease is due to the fact that patients and those at risk for Wilson's disease are admitted from the whole country into this hepatology centre.
We included only patients with confirmed Wilson's disease. The diagnosis was based on the international Leipzig score system, and the score of each patient was at least 4. The diagnosis of acute liver failure was established by the King's College criteria in each case.

I have examined the electronic documentations and the outpatient reports, findings and other documentation of the patients registered at the Department of Internal Medicine and Oncology of the Semmelweis University with ICD code E8300. I have also analyzed the medical records of the transplanted patients at the Department of Transplantation and Surgery. Two acute liver failure patients were treated at the Péterfy Sándor street Hospital and Casualty Centre. Since we could not find all examined parameters of all patients, the patient number may be different at the examination of the different parameters.

RESULTS

Epidemiological data

Among the 231 patients 126 (55%) were male and 105 (45%) were female, which corresponds to the expected distribution in autosomal recessive inheritance. The mean age of the patients was 20.90±10.9 years at the development of the first symptom, and the diagnosis was established at a mean age of 23.26±11.25 years. In some cases more than ten years have passed until the ultimate diagnosis. Interestingly, in four families Wilson's disease has developed in two consecutive generations. The late siblings of three patients likely to had Wilson's disease.

Clinical forms

Wilson's disease has very diverse symptoms. Two third of the patients (73.7%) had liver disease, and half of them (50.2%) had neurological symptoms. 17 asymptomatic patients (7.8%) were diagnosed as siblings. Most patients with liver disease had chronic hepatitis; however 22 patients (9.5%) had acute liver failure.

Most acute liver failure patients were female (F/M=18/4), and their mean age was similar to all patients' (18.4±4.5 years vs. 20.90±10.9 years, p=0.27). 8 of the 22 patients have died prior to the liver transplantation, 10 patients underwent liver transplantation and four patients recovered with conservative treatment. One patient ameliorated during MARS treatment.

The deRitis quotient was higher in the acute liver failure group than found in all patients (2.26 vs 0.90, $p<0.05$), while the ALP was lower (74 IU/l vs 317 IU/l, $p<0.05$). The ALP/bilirubin ratio considered to be specific was 0.13 in the acute liver failure patients compared with 15.8 for all patients ($p<0.05$).

In case of some patient intercurrent infection, antibiotic treatment and copper sulphate supposed to be associated with acute liver failure.

Coombs negative haemolysis is a well-known symptom of Wilson's disease; we found it in 36 patients (15.6%), mostly in females (N/F=25/11). In the background of the haemolysis the elevation of free copper concentration was supposed to be, however in our patients we could not prove it.

Hepatocellular carcinoma has been reported less frequent among Wilson's disease patients than in cirrhosis of other origin. Two of our patients have developed hepatocellular carcinoma and one patient had cholangiocarcinoma. Lack of compliance emerged as a potential trigger in one hepatocellular carcinoma patient.

Causes of death

46 of the examined patients have died, 24 for decompensation of chronic liver disease. In three patients lack of compliance has clearly played a role in the decompensation, however this number is likely much higher in reality. The second most frequent cause was acute liver failure (12/46). Three patients has died of oncological disease, all of

them had hepatobiliary malignancies; two hepatocellular carcinoma and one cholangiocarcinoma (see above). The cause of death were advanced neurological symptoms in two patients: movement disorder and Parkinson syndrome may played a role. One patient died of an accident, ethylene glycol intoxication.

Genetic distribution of the patients

H1069Q, the most frequent mutation in Hungary could be detected in 146 out of 231 patients (63.2%), 60 patients (26%) were homozygous and 86 patients (37.2%) were heterozygous for this mutation. 10 patients (4.3%) had K844K-fs mutation, one was homozygous and nine were heterozygous. A total of 39 different mutations were detectable in our patients. The presence of H1069Q mutation did not have an effect on the phenotype. For other mutations the small number of patients made impossible the examination of the genotype-phenotype association.

In one third of the patients the polymerase chain reaction was insufficient to detect the mutation, in these cases new-generation sequencing would be an option. We sequenced the ATP7B gene in six, H1069Q negative or heterozygous patients by IonTorrent machine. Each patients were identified as compound heterozygous, and we detected a formerly not known mutation (A1270I) for the first time. On the other hand we supposed A1063V might be a disease-causing variant instead of variant of unknown significance described formerly.

Examining the genetic factors may influence the symptoms of the disease we analyzed the potential role of the PRNP (prion-related protein) gene, but no mutation could be detected in any patients.

Treatment of the patients with Wilson's disease

Most patients were treated with chelating agents, 152 patients get D-penicillamine and eight patients get trientine. The most frequent side effect of D-penicillamine treatment was thrombocytopenia (in 4 cases), one patient had leucopenia as well. Two patients had penicillin allergy; and proteinuria, hematuria, and myasthenia appeared in one

patient. These side effects ameliorated as the treatment was withdrawn. Patients treated with trientine developed no side effects.

44 patients were treated with zinc salts, in 15 of them as a first line therapy. We investigated the efficacy of a new, magistrally prepared, prolonged-release zinc acetate dihydrate tablet together with Mikszáth Pharmacy. 17 patients were treated with this treatment. 10 patients were changed from D-penicillamine because of side effects (thrombocytopenia, deteriorating renal function, progression of the underlying disease and lack of compliance). Six patients get it as first line therapy; 5 patients until the authorization of chelating therapy while one asymptomatic patient get it permanently.

The underlying Wilson's disease was stable in each patient, and all side effects of D-penicillamine decreased, however the platelet level remained lower in two patients. (It is worth to note that both patients had liver cirrhosis.)

As side effect, two patients had abdominal complaints, one patient had abdominal discomfort and the other had nausea. These complaints regressed as the patients took the tablet during meals.

We also examined the data of the patients underwent liver transplantation in Hungary. 24 out of 231 patients (10.4%) had liver transplantation, which was the 2.9% of all liver transplantations (24/853 at the time of the study); it is in concordance with the literature data. The mean age was 26±12 years, and more female were among the transplanted patients (F/M=13/11). Nearly half of the patients were transplanted because of acute liver failure (10 patients), and 15 were transplanted for chronic liver disease. One patient needed retransplantation. Acute liver failure patients were 12 years younger than the chronic liver disease patients, and more female were aming them (F/M=7/3 vs 6/9 respectively). The liver transplantation became possible via Eurotransplant in three acute liver failure patients.

8 out of the 24 transplanted patients have died (4 with acute and 4 with chronic liver disease). Three acute liver failure patients have died for acute respiratory distress syndrome and disseminated intravascular coagulation and one for sepsis after retransplantation needed for chronic rejection. Two patients with chronic liver disease have been deceased for sepsis, one for heart failure and one for chronic rejection due to

incompliance. Most patients (6/8) has died within two months after the transplantation, and only two patients has deceased after 43 and 44 months later.

The overall five-year survival of the transplanted patients was 66%. The fact whether the patient was transplanted because of acute liver failure or chronic liver disease had no impact on the survival: the 5-year survival was 60% in acute liver failure and 71% in chronic liver disease patients (log-rank test, p=0.93, see Figure 1.).

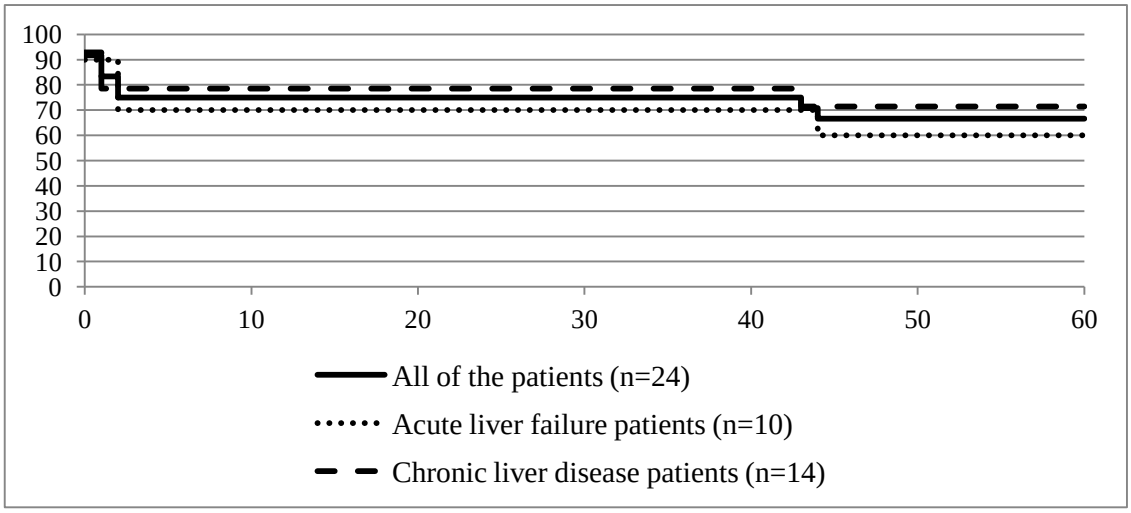

Figure 1. Kaplan-Meier curve of the survival of the transplanted patients. There is no difference between the acute and chronic liver disease patients.

To examine the impact of the learning phase on the survival we examined the patients transplanted after 2002. In this setting the 5-year survival was as high as 79%, while the patients transplanted prior to 2002 had 20% 5-year survival. The low number of the patients may explain why no difference could be find (log-rank test, p=0.093). The survival of the patients in regard to the transplantation can be seen in Figure 2.

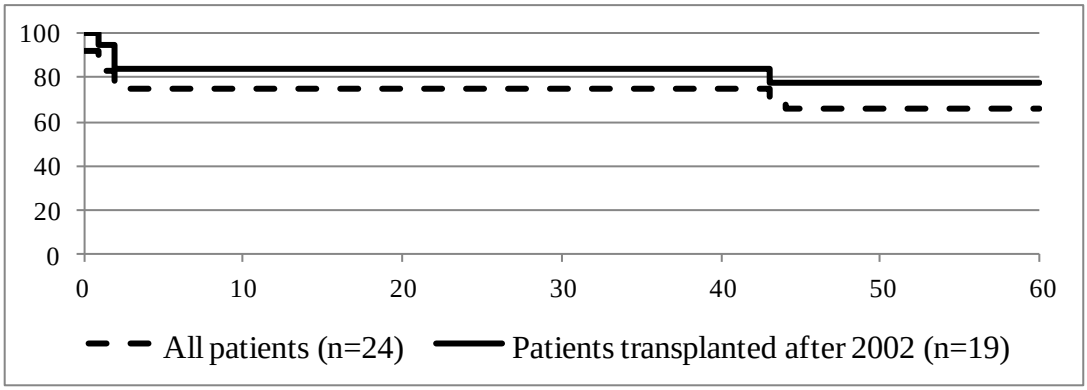

Figure 2. Survival of the transplanted patients. The 5-year survival improved from 66% to 80% if we examine the patients transplanted after 2002.

CONCLUSIONS

Wilson's disease is an inherited disorder of copper metabolism presenting various symptoms from liver disease and neurological symptoms to endocrine disturbances. Diagnosis may be challenging even nowadays, however new genetic methods, such as next-generation sequencing may help us.

Our data confirm that there is no connection between the genetic background and the clinical symptoms. It seems that prion-related protein (PRNP) plays no role in the clinical manifestation of Wilson's disease.

As Wilson's disease is an autosomal recessive inherited disorder, we must emphasize the screening of the first degree relatives, especially the siblings. In case of some families we found that all three siblings are affected, and even in consecutive generations have Wilson's disease. According to our data we call attention to the importance of the family history.

Most deceased patients have died of liver disease even today, which may be connected to the delay in the diagnosis and the lack of compliance. Incompliance may be related to the psychiatric symptoms of the underlying disease and the diagnosis of Wilson's disease in the teenage years in many cases (however more patients are diagnosed in the late fifties and sixties nowadays). My experience also confirms the importance of the

patient-doctor relationship. Convincing asymptomatic and well-treated patients about the importance of the good compliance may be particularly hard.

Acute liver failure and decompensated cirrhosis may be an indication for liver transplantation. After the learning curve of the first years the optimal selection of the recipients and the ideal timing may improve the outcome as well as the joining to the Eurotransplant organisation.

The results of the study show the importance of the prompt diagnosis, the optimal treatment and care and the good relationship between the patient and the doctor.

LIST OF PUBLICATIONS

In connection with theme

Papers

1. **Németh D**, Folhoffer A, László SzB, Kóbori L, Görög D, Fehérvári I, Fazakas J, Gerlei Zs, Máthé Z, Szalay F. (2019) Májátültetés Wilson-kóros betegekben. Orv Hetil, 160(51): 2021-2025. **IF: 0,564***
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1. Horváth E, Balla B, Kósa JP, Lakatos PA, Lazáry Á, **Németh D**, Jozilan H, Somorácz Á, Korompay A, Gyöngyösi B, Borka K, Kiss A, Kupcsulik P, Schaff Zs, Szalay F. (2016) A D-vitamin metabolizmusa humán hepatocellularis carcinomában és az azt körülvevő tumormentes májszövetben. Orv Hetil, 157(48): 1910-1918. **IF: 0,349**

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