CURRENT METHODS OF DIAGNOSING AND TREATING PATIENTS WITH WILSON DISEASE

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Abstract

Wilson disease is a rare genetic disease with autosomal recessive heredity which is characterized by excessive copper deposition in the liver, brain, eyes, kidneys, osseomuscular system as a result of a defect in the biliary excretion of the copper and its incorporation in the ceruloplasmin. The high level of copper in the described structure leads to a different spectrum of pathologic clinical manifestations, which, if not recognized on time can cause a series of neurological, psychological, gastroenterological complications, including death. If diagnosed early, Wilson disease is treatable and most patients could live a normal life.

The aim of the paper is to present the main methods of diagnosing Wilson disease and then to analyze current treatment methods.

Key words: *Wilson disease, copper metabolism, ceruloplasmin, Kayser-Fleischer rings, sunflower cataract*

1. WILSON DISEASE SPECIFICITIES

Wilson disease is a rare genetic disease with autosomal recessive heredity which is characterized by excessive copper deposition in the liver, brain, eyes, kidneys, osseomuscular system as a result of a defect in the biliary excretion of the copper and its incorporation in the ceruloplasmin. The high level of copper in the described structure leads to a different spectrum of pathologic clinical manifestations, which, if not recognized on time, can cause a series of neurological, psychological, gastroenterological complications, including death. If diagnosed early, Wilson disease is treatable and most patients could live a normal life.

The disease appears most often within the age range between 5 and 40 but patients have been described at the age of 2 and 80. The average age of starting the liver syndromes is 11 y.o. (from 6 to 16) and the average age when neurological symptoms start manifesting themselves, is 30-35 y.o.

Wilson disease (W.D.) is more often found in South Italians and Eastern Europeans. In many populations it is considered to be 1 in 40000 people on the basis of investigations and autopsy [1]. But as there are a large number of over 500 mutations in the gene causing W.D., it is not possible to determine the exact disease gene frequency by mutation analysis of the population. That is why maybe the actual frequency is 1 in 55000 [1]. The carrier is 1 in 100 which means that, if a child receives the gene from both patients, it may very probably develop W.D. W.D. is autosomal recessive disease, i.e. it occurs equally in men and women.

At least 1 in 30000 people of all known races and nationalities has the disease. All brothers, sisters and children of the patient with W.D. must be investigated for W.D., as should be other relatives who have had symptoms or have undergone laboratory tests, showing liver or neurological diseases. One in 100 individuals in the population is a carrier of one abnormal copy of the W.D. gene. Carriers have one normal and one pathological gene. All children of those affected with W.D. receive at least 1 pathological copy of the W.D. gene. One half of a carrier's children take at least one abnormal copy of the W.D. gene.

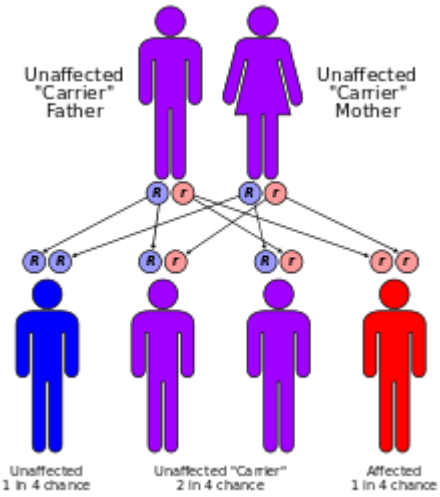


Fig. 1. Wilson disease autosomal recessive pattern of inheritance

Statistics show that each 1 person in 10000 in Australia, each 1 in 12000 in Europe, and each 1 in 30000 in Japan takes at least one abnormal copy of this gene [3]. Worldwide the number of cases is between 10 000 000 and 30 000 000 [4]. The increased frequency is a result of higher rates of consanguinity, which is four times more common in females. In order for the child to inherit this gene, both parents must carry the ATP 7B gene (located on chromosome arm 13q). The defect influences upon the copper-transporting adenosine triphosphatase (ATP) gene**,** which is active predominantly in the liver, kidney and placenta**,** and there is in not so much quantity in the heart, brain, lungs, muscles and pancreas**.**

A genetic diagnosis is possible, if the precise mutation is found. This can help us detect symptoms in relatives, so that they may be treated before they fall ill [3].

Family screening needs to take place following the below described steps, as described by the Wilson Disease Organization (WDO):

- First of all, biochemical testing must be done on the following two groups of people:
  - Children of patients with Wilson disease. Screening should start at the age of two, if asymptomatic, and repeated once in five years, unless there are any reasons to pursue further);
  - Siblings of patients.
- Secondly, physical examination and brief history of any liver or neurological symptoms needs to be performed.
- Next, liver function tests should be done which include the following: ALAT, ASAT, Albumin, bilirubin, ceruloplasmin and serum copper, 24 hours urine copper, slit lamp exam of the eyes for Kayser-Fleischer rings.
- If there is no Kayser-Fleischer ring, abnormal liver functions tests and low ceruloplasmin, liver biopsy should be done. All siblings and children of W.D. patients should be tested for W.D. Other relatives who have had symptoms or laboratory tests that indicate liver or neurological disease should also be tested for W.D.

Testing methods available at hand to date are the following:

- Linkage analysis (Haplotype analysis). Molecular genetic testing is used to identify a set of closely related segments of DNA (a marker or set of markers), comparing the markers of family members to those of an affected patient. It is useful for screening siblings of an identified patient.

- Gene sequencing (mutation screening of the entire ATP 7B gene to detect and identify disease mutations. An individual with confirmed W.D. needs to be tested first. If both mutations are identified, other family members can then be offered testing. Gene sequencing will identify both mutations in most but not all cases of W.D. It is useful for confirming the diagnosis in suspected patients, it is also good for family members to learn, if they could be affected (although having no symptoms to date), as well as to learn, if they are carriers, or to allow for prenatal testing for confirmed carriers.
- Targeted mutation analysis - analysis of a specific location in the ATP 7B gene for a known particular mutation. It is useful for specific populations of patients where the common mutations are known. It is also useful for screening siblings of patients with two identified mutations. Genetic testing is best coordinated through a genetic counselor who can carefully discuss the best method of testing to be performed and the benefits, limitations, and implications of each method [5].

2. COPPER METABOLISM IN WILSON DISEASE

Copper is an essential element for the normal growth and development of human fetuses, infants and children. It participates into a variety of proteins and metalloenzymes which perform vital metabolic functions. It is necessary for the correct growth, development and maintenance of bone connective tissue, brain, heart and many other organs. Copper takes part in the formation of red blood cells, absorption and utilization of iron, the metabolism of different life-sustaining proteins and enzymes. These enzymes deliver energy to the body cells and regulate nerve transmission, blood clotting and oxygenation. Copper is important for the stimulation of the immune system, repairing injured tissues and promoting healing. The importance of copper is seen in the earliest stages of our life, when during the third trimester of pregnancy the human fetus accumulates copper rapidly in its liver and at birth the infant has four times more concentration of copper than a full-grown adult. Neonate's liver stores fall rapidly after birth, supplying copper to the fast-growing body cells during the breast-feeding period. Human milk does not contain much copper. Infants have special biochemical mechanisms for adequately managing copper in their bodies while permanent mechanisms develop and mature lifelong.

2.1. Pathophysiology

Copper is an essential element for every living creature. It takes part in many enzymes, responsible for vital functions in the human body like neurotransmission (dopamine B monooxidase), electronic transfer in the respiratory chain (cytochrome oxidase), conjunctive tissue formation (lysyl oxidase), pigment production (thyrosinase), oxidative stress destruction (superoxide dismutase). A human takes between 1.5 and 3.0mg. copper daily with food. One half of this quantity is being absorbed and carried into the jejunal cells by specific proteins (copper membrane transporter 1, CMT 1). In the enterocytes one part of the copper is incorporated in metallothioneins and other one is excreted to the portal vein depending on the quantity of incoming copper. This process is regulated by ATP7A enzyme, situated in the trans-Golgi network. Within the portal vein system the copper is bound with albumin and in such a way reaches the liver, where, using similar to jejunal CMT, enters the hepatocytes. Here the copper, first using another transporting protein (ATP7B), is incorporated in a protein called apoceruloplasmin, then it connects to the Antioxidant protein 1 (ATOX1) the way it does in the enterocytes, and this has a very important role for preventing the oxidative stress cellar destruction and for making intracellular distribution of the copper into the different cellular organelles and secretory vesicles. Excessive quantity of copper is being excreted from the liver cells into the bile using again copper-transporting protein ATP7B. In patient with Wilson disease as a result of over 280 mutations of the ATP 7B gene, the production of copper-transporting ATPhase is disturbed. Therefore, the hepatocellular excretion of the copper in the bile is reduced significantly and the normal incorporation of the copper in the apoceruloplasmin is impeded. This is the reason for the low level of serum ceruloplasmin it these patients. Copper excretion is reduced over 10 times, while intracellular copper

accumulation is raised over 100 times. This induces strong oxidative stress as a result of which the liver undergoes pathological processes like steatosis, steatohepatitis, steatofibrosis and at last cirrhosis (process known like Fenton chemistry). As a result of hepatocellular destruction, the level of copper, not bound to ceruloplasmin in the blood, rises significantly and with the bloodstream it is deposited into organs and systems in the human body like central nervous system (basal ganglia putamen, globus pallidus), excretory system (kidneys), eyes, causing a spectrum of different clinical manifestations.

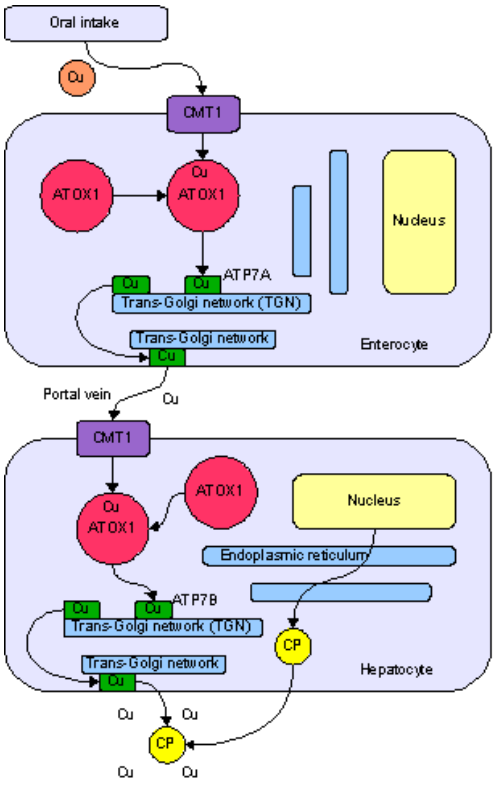


Fig. 2. Normal absorption and distribution of copper

3. CLINICAL MANIFESTATIONS

As W.D. is a multisystemic disease and it has a large spectrum of clinical manifestations which begin soon after birth and progress during life. Patients may have no symptoms for many years. The clinical picture of the disease is extremely variable. It can be acute or chronic. Most often the disease starts at the age of 16 (younger than 35y.o.) with different hepatic disorders (which is the case of more than half of all patients) which can vary from elevated liver enzymes, acute hepatitis, chronic active hepatitis, cirrhosis to fulminant hepatic failure [4]. That's why each patient with fulminant hepatic failure, Coombs-negative intravascular hemolysis, moderately elevated serum transaminases, low alkaline phosphatase, ratio of alkaline phosphatase to serum bilirubin less than 2 (especially in individuals younger than 40), is suspicious for Wilson disease. Patients with hepatic disorders usually have neurological disturbances; however, atypical cases of patients with liver cirrhosis at the age over forty without neurological symptoms have also been described.

Table 1. Wilson Disease Systemic Disorders

Wilson Disease Systemic Disorders	Clinical Manifestations
Hepatic damage	Asymptomatic hepatomegalia
	Elevated liver enzymes
	Hepatic steatosis
	Splenomegalia
	Acute hepatitis
	Fulminant hepatic failure
Neurologic signs	Tremor, parkinsonism, ataxia, rigidity, dystonia
	Dysartrie, dysphagia, pseudobulbar paresis
	Migraine, headache, vigilance
Ocular manifestations	Kayser-fleisher ring, bilateral sunflower cataracts
Psychiatric problems	Behavioral disturbances, neuroses, depressions, schizophrenic-like, affective and cognitive disturbances,
Musculoskeletal symptoms	Degenerative arthropaty, osteoporosis, osteochondritis dissecans, chondromalacia patellae, chondrocalcinosis.
Renal symptoms	Urolithiasis, haematuria, nephrocalcinosis, fanconi-like syndrome
Hematologic symptoms	Coombs-negative acute intravascular hemolysis, thrombocytopenic purpura
Other symptoms	Pancreatitis, cardiomyopathy,hypoparathyroidism, secondary amenorrhea, spontaneous abortions.

When the disease starts with neurological symptoms, most of the patients have already had a different stage of hepatic disorders (cirrhosis). Approximately half of the patients with Wilson disease have neurological symptoms, most common of which is the asymmetrical tremor. It is variable and may be resting, postural, kinetic, may engage the four extremities and / or the head. Patients may have Parkison-like tremor, tremor of the head like in patients with Multiple sclerosis, may have abnormal limb movements as a result of hypertonic dystonia or may have choreoathetoid abnormal movements. The tremor, dysartrie, excessive salivation, ataxia, handwriting disorders, clumsiness and behavior disturbances are the most common early neurological symptoms. If the disease is not recognized and treated on time, patients may develop extrapyramidal disorders (12 years after the beginning of the disease) - dystonia, spasticity, grand mal seizures, rigidity and flexion contractures. If a patient with neurological Wilson disease is not treated, Kayser-Fleischer rings may be seen with 98% probability. In 10 to 20% of the patients the disease may start with psychiatric features like emotional lability, impulsiveness, self-harm, reduced mental capacity, etc. Patients with Wilson disease have also osseomuscular disturbances like osteopenia, degenerative arthropathy, osteoarthritis, Osteochondritis dissecans, chondromalacia patellae, chondrocalcinosis. When a symptomatic joint disease occurs (which happens in 20-50% of the patients), Wilson disease usually progresses for approximately 20 years.

Ocular signs typical for Wilson disease are the sunflower cataract and the Kayser-Fleischer ring. Siemerling and Oloff described the sunflower cataract in 1922, finding copper deposition in the anterior capsule. It is not absolutely specific for Wilson disease, because it has been described also in patients with intraocular copper bodies (chalcosis) primary biliary cirrhosis and Hereditary hyperferritinemia cataract syndrome (HHCS-autosomal-dominant disease characterized by early-onset cataracts and increased serum L-ferritin concentration without iron overload.). The exact mechanism

of forming the cataract is not known completely, but it is characterized by the formation of copper central disc-like deposition with radiating folds at the periphery in the anterior or posterior lens capsule. As proposed by Duke-Elder, the radiating folds are thought to be a result of the impression of the posterior surface of the iris on the anterior lens capsule and may regress partially or completely after penicillamine therapy**.** In 1902 Bernhard Kayser and in 1903 Bruno Fleischer described copper deposition in the descemet membrane of the cornea at the limbus. 95% of the patients with neurological manifestations and 65% with liver dysfunction have KF ring.

KF rings can also, like the sunflower cataract, occur in intraocular copper bodies (chalcosis) and primary biliary cirrhosis and may regress completely or partially following penicillamine therapy and after liver transplantation. KF rings may remain visible all life long, even after chelatic treatment. It can be seen usually by slit lamp examination, but can also be visible to the naked eye. It does not obstruct vision. In patients with acute hepatic failure, the hepatic cytolysis leads to increased level of copper and intravascular hemolysis. So, the coombs-negative acute hemolytic anaemia is a typical, but rare (in 10-15% of the cases) complication of the disease. As the gene of Wilson disease is expressed in kidneys, patients may have primary or secondary renal dysfunctions. Fanconi-like syndrome may be found as a result of defective renal acidification and excess renal losses of amino acids, glucose, fructose, galactose, phosphate, uric acid and calcium. That is why more than 16% of the patients with Wilson disease may have urolithiasis. Haematuria, proteinuria, peptiduria and nephrocalcinosis are also possible features, but can be seen as side effects of D-penicillamine treatment. This is the reason why strict observation of the renal function is absolutely necessary before starting treatment with chelating agents. Fulminant Wilson disease is a part of the clinical presentation of the disease. It is more often found in children, and it is characterized by coma, acute hemolysis, renal failure, low serum transaminases, low serum alkaline phosphatase. Neurologic symptoms are missing.

4. DIAGNOSTIC METHODS

Early diagnosis is vitally important, but it is often missed initially. The delays are between 1 month and 18 years and Wilson disease is often mistaken with Neurosis, Neurasthenia, Hysteria, Myasthenia gravis, Parkinson disease, Schizophrenia [10]**.** In patients with neurologic symptoms differential diagnoses are disseminated sclerosis, Parkinson disease, Postencephalitic status [9]. In patients with hepatic clinical presentation, differential diagnosis should be made with all hepatic diseases including even viral hepatitis. Patients with NASH and autoimmune hepatitis, non-responding to treatment have high probability of having Wilson disease [12].

The diagnosis of Wilson disease is not easy and it must be put after a combination of clinical, laboratory and instrumental (noninvasive and invasive) tests.

4.1. Clinical features

Each patient at the age between 3 and 45 year with obscure hepatic damage is suspicious for Wilson disease. These patients must be investigated from ophthalmologist for Kayser-Fleischer rings (slit lamp examination). The absence of KF ring does not reject the diagnosis even in patients with leading neurologic symptomatic. KF ring is common in 95-100% of the patients with neurologic symptoms, 50-85% in patients with hepatic disorder and in 10-50% in asymptomatic patients [11]. Tremor is the most common (in 2/3 of the patients) neurological symptom. It is usually light, asymmetrical at first but in the progress of the disease it engages the four limbs and the head and does not disappear after distracting the attention or applying placebo. Together with dysarthria, especially when combined with Parkinson-like symptoms, depression or behavioral disturbances, the diagnosis of Wilson disease is very possible.

4.2. Laboratory tests

1) Ceruloplasmin must be examined in each patient considered for Wilson disease, especially in young patients with hepatic, neurologic or psychiatric manifestations. If ceruloplasmin concentration in the blood is less than 0.2 g/L., it is certain that the patient has Wilson disease, but the normal level of this protein does not mean that such a diagnose is not possible (10% of all patients with neurologic form of Wilson disease have normal values of ceruloplasmin and 40% of all patients with hepatic form of Wilson disease have normal values of ceruloplasmin). Ceruloplasmin blood level may be less than normal in patients with malnutrition, malabsorption and nephrotic syndrome, all forms of chronic liver disease (especially PBC). Low serum copper may be found by an increase of over 30% in inflammatory states.
2) Copper in the serum and 24 hours urine copper excretion. In patients with Wilson disease the serum copper level is usually diminished, but normal values do not reject the diagnosis. This index is not specific for the disease. It could be higher in patients with non-Wilson disease induced hemolysis, but when blood level is higher in patients with Wilson disease, it means that the disease has progressed and the hepatic capacity for copper-storing has been depleted. Usually serum level of the copper in patients with Wilson disease is < 12 mmol/L). Basal 24 hour copper excretion is higher than 1.25 mcmol/24 hours, but when this value is about or over 0.6mcmol, additional tests are necessary.
3) D-Penicillamine provocation test in children. If the 24 hours urine copper concentration rises over 1600mcg (>25mcmol / 24 hours) after taking 500mg D-Penicillamine and 12 hours later, the diagnosis is confirmed. This test is not recommended for adults. If equal results are obtained from the penicillamine test, the next step is to make a liver biopsy so that the tissue copper content is measured [2].
4) Copper concentration in the liver tissue. Some authors think that the investigation of copper content in the liver is more reliable for the diagnosis, but is difficult to take appropriate reference material and adequate patient samples. Even if perfect liver sample for copper concentration evaluation is taken, additional tests are needed. In case the copper content in the liver biopsy sample is above 250 mcg in 1 g dry liver substance, WD is highly probable. If the patients have not been treated, the diagnosis is rejected in availability of less than 40-50 mcg copper in 1 g dry liver substance.

4.3. Noninvasive investigation

Ultrasound investigation of the liver and other abdominal organs is necessary for all patients with Wilson disease, especially in those with hepatic manifestation and laboratory tests for progressed liver failure. In patients with neurologic signs a MRI of the cerebrum is necessary to diagnose pathological findings (accumulations) in the basal ganglia. Slit lamp investigations are obligatory to find out Kayser-Fleisher rings. In specialized laboratory, a haplotype analysis is applied to make a family screening of the disease.

4.4. Invasive tests

Liver biopsy is used as a major invasive test aiming at the identification of Wilson disease.

Wilson Disease Scoring System can be used for diagnosing Wilson disease. It was developed at the 8th International Meeting on Wilson disease in Leipzig 2001 [14].

5. TREATMENT METHODS

Treatment of Wilson disease is a serious problem given the genetic character of the disease, late diagnosing of the disease, the development of different hepatic, neurological, psychiatric, renal and other complication during the progress of the disease, toxicity of some of the medicines on the one side and inadequate therapeutic effect of other medicines on the other side, not keeping an appropriate diet from the patients and so on. Treatment of the disease includes a strict dietary regimen, drug treatment and surgical treatment of the complications.

5.1. Dietary regimen

Diet needs to be applied and kept together with the drug treatment. All alimentary products that contain high level of copper are forbidden: chocolate, cocoa, nuts, beans, lentils, walnuts, oats, mushrooms, spinach, barley, rye made bread, turkey, liver, shrimps, some fish (cod-fish). Grape or other fruits, treated with Copper sulfate or other Copper-containing compounds must be washed very well with brush under running water. Foods which do not contain or which contain only small quantities of copper, are milk, eggs, chicken and cabbage. Absorption of copper increases during plentiful nutrition with animal proteins and decreases when eating cellulose and fiber, sugar, phytin.

Below more details are given on the groups of foods which should be eaten or avoided.

- Milk and milk products. Permitted are defatted cow milk, defatted yoghurt, curds, green cheese, defatted cheese (cow, goat). Restricted are cream, cheese (sheep, buffalo), fatty yellow cheese, melted cheese, smoked cheese).
- Meat and meat containing products. Permitted are non-fatty, free of tendon and membrane meat 100 - 150g per day - beef, chicken (without turkey meat), rabbit. Restricted are defatted pork meat, ham, fillet. Forbidden are fatty meat, sausages, plucks, game meat, tinned meat food.
- Permitted are low fat fresh fish (barbell, Squalius leuciscus, white fish, trout, scad).
- Eggs and egg containing products. Permitted are fresh hen eggs (2-3 per week). Forbidden are duck and goose eggs, mayonnaise.
- Nutritive fats. Permitted are vegetable oils (sunflower and maize oil, olive oil). Forbidden are animal (pork) fat, sheep suet, butter, margarine, bacon.
- Vegetables and vegetable tinned foods. Forbidden are, except for those, pointed above, dock, nettle, onion, sorrel, all kinds of pickled vegetables, fried potatoes (crisps).
- Sugar and sugar products. Permitted are honey, forbidden are fatty cakes, pastry, Turkish syrup-soaked shredded sweets, ice cream.
- Drinks: Permitted are herbal tea, alkaline mineral water. Forbidden are soda, coffee, cocoa, all alcohol beverage.
- Spices. Permitted are parsley, dill, mint, lemon juice, vanilla. Restricted are tomato paste and sweet red pepper. Forbidden are cayenne, vinegar, ketchup, garlic, pimento, pepper, cinnamon, and clove.

Way of preparation: Allowed are baking, boiling, steaming, bain-marie, grilling, mashing, fresh fruit and vegetable juices. Forbidden are frying, frying in egg and bread crumbs, stewing, marinating, cooking with smoke and so on.

5.2. Regimen

Feeding should be 4-5 times a day in strictly defined hours. The last meal must be at least 3-4 hours before going to bed. It is good to avoid very cold, ice-cold or hot drinks and dishes. Proteins are in the physiological standard and priority is given to milk proteins in the form of milk, curds, cheese, and

yellow cheese. Restricted are fats, except for vegetable oils. Carbohydrates should be in normal quantities, as sweet carbohydrates are admitted, which are easy to assimilate. Energy input must not be too large to avoid overweight and obesity. The liquids are in physiological norm - to 1.5-2 liters per day.

5.3. Drug treatment

There are two groups of drugs for the treatment of Wilson disease:

1. Chelators - they increase excretion of the copper in the urine. They are D-Penicillamine, Trientinee and Tetrathiomolybdate
2. Metallothioneins, blocking the intestinal absorption of the copper-Zinc.

D-Penicillamine treatment should start with doses of 250mg - 500mg daily with gradually raising the dose with 250mg for 4-7 days up to reaching a daily dose 750-1500mg divided into two intakes. As food suppresses the absorption of the medicines, drugs should be taken 1 hour before or 2 hours after nutrition. About 6-12 months later, treatment should continue with a decreased dose of 750-1000mg. If the 24 hours urine copper excretion is 3-8mcmol after starting the chelating therapy, the therapy is considered to be effective and the concentration of copper non-bounded with ceruloplasmin diminishes. If the patient suffers from decompensated liver disease, the clinical effect could be reported at least 2 months later. The treatment must not be stopped (except in cases when side effects develop), since 5-6 months later a patient could develop acute liver failure or fulminant form of the disease. Side effects are seen in 20-30% of the treated patients. After 1 year successive treatment, the medication with D-penicillamine could be substituted with Zinc as the toxicity and the side effects are less than these of D-Penicillamine. D-Penicillamine has a stronger effect but more often has side effects. There are two types of side effects – early and late ones:

- The early side effects begin 1-3 weeks after starting therapy and include: pyrexia, rushes, lymphadenopathy, proteinuria, thrombocytopenia, neutropenia. If a patient reveals hypersensibilisation, treatment must be stopped.
- The late side effects include ‘’lupus-like’’ syndrome, nephrotoxicity, Goodpasture syndrome, bone-marrow suppression to total aplasia, aphthous stomatitis, lichen planus. If nephrotoxicity develop, the treatment is stopped immediately. In other cases corticosteroids could be used.

D-penicillamine must be taken obligatory together with Pyridoxine in daily dosage 25-100mg.

Trientine (Triethylenetetramine dihydrochloride) also stimulates urinary excretion of copper. It is used in cases with allergic reactions to D-penicillamine, evidence for renal disease, cytopenia (thrombocytopenia), autoimmune signs in patients with decompensated liver disease. When starting therapy with Trientine, instead of D-penicillamine, the neurological symptomatic worsens more rarely. Therapeutic dose is 750-1500mg., divided into 2-3 times per day; the supporting dose is 750-1000mg. The drug must also be taken 1 hour before or 2 hours after nutrition, because the food diminishes absorption. Therapy is considered to be effective and the concentration of copper non-bounded with ceruloplasmin diminishes, if the 24 hour copper urine excretion is 3-8mcmol. Side effects are rare, but as the Trientine is chelator to the iron, too, it could not be applied to medicines used for iron deficiency treatment.

Zinc induces intestinal production of metallothioneins that blocks copper absorption. The treatment with Zinc sulfate is effective in case of 24 hour urine copper excretions < 75mcg (1.2mcmol.), and the concentration of copper non-bounded with ceruloplasmin diminishes. It is used in daily doses of 150mg, divided into two or three intakes (50mg.) before or after nutrition. Side effects include exacerbation of Chronical gastritis, suppression of the chemotaxis of the WBC, increased serum concentration of amylase and lipase. Treatment with Zinc sulfate does not worsen the neurological symptomatic and does not harm the renal function. Together with Trientine, Zinc sulfate is appropriate for treatment of patient with decompensated liver disease. In the new guidelines zinc treatment has replaced D-penicillamine as first-line therapy for Wilson disease. That is because, if not started

correctly, D-penicillamine treatment with initial high doses can lead to releasing excessive quantity of free copper in the blood and in that way to inducing oxidative stress in the body and iatrogenic deteriorations. The new paradigm in the treatment of Wilson disease is that it is necessary to reduce the free form of copper in the blood, and not to de-copper the excessive copper in the liver.

Tetrathiomolybdate is a chelator, used for initial treatment of patients with leading neurological symptomatic. During this treatment, the concentration of copper non-bounded with ceruloplasmin diminishes in the first 8 weeks, without worsening the neurological signs.

As patients with Wilson disease have liver disease as a result of copper toxicity, producing free oxygen radicals, using antioxidants is expedient. It can be applied vit. E-300-600mg. daily.

5.4. Treatment of patients in special groups of patients

Asymptomatic. As asymptomatic brothers and sisters develop the disease, in 25% they could be treated with Zinc sulfate 150mg. daily or D-Penicillamine 750-1000mg. daily.

Pregnant. The dosage of D-Penicillamine and Trientine must be reduced with 25% to 50% in the third trimester of the pregnancy as in that way the side effects and the toxicity over the embryo diminishes and the operative wound recovers better and quicker. Caesarean section is recommended to those patients. Zinc sulfate does not have toxic effects and its daily dose may not be reduced.

Patients with fulminant form of Wilson disease. Liver transplantation is recommended to those patients. Before this plasmapheresis is applied. It reduces the intravascular hemolysis, improves the renal failure, and diminishes the serum copper concentration.

Monitoring of the patients. At the beginning of the therapy with chelators, full blood picture needs to be taken**,** proteinuria. Clinical examination, blood picture, biochemical tests, coagulation, copper concentration in the serum, ceruloplasmin, proteinuria (quantity protein) need to be done each 3 months. Copper urine excretion must be done once a year during treatment with chelators and Zinc sulfate. Treatment is effective, if copper urine excretion is 3-8mcmol in 24 hour urine when taking chelators or 1.2mcmol in 24 hour urine when treated with Zinc.

When change of therapy is necessary, the decision must be taken from experts.

6. CONCLUSION

Prognosis depends on many factors - age of diagnosing, complications - neurological, hepatic, psychiatric, renal failure, toxicity of drugs used and so on. Around 10% of the children died 4 years after starting therapy because of late diagnosing [7]. In these cases, the first method of choice is liver transplantation. The liver cirrhosis and its complications worsen the prognosis. That is why the early diagnose is decisive for the prognosis and many patients can live an almost normal life.

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