INTRODUCTION

Wilson's disease (WD) is a rare autosomal recessive disease that affects approximately 1 in 30,000 live births, with a predominance in males. It is characterized by a disturbance in copper metabolism that leads to the accumulation of the metal in various tissues of the body. Copper accumulation triggers a variety of clinical manifestations that most often affect neurological and liver functions, although effects have also been observed for bone, retina, kidney, and hematological tissues ***(Rosencrantz and Schilsky, 2011).***

WD is caused by mutations in the *ATP7B* gene that encodes the ATP7B protein. This protein is essential for transporting and excreting excess copper; it is expressed mainly in the liver and kidney and, at lower levels, in the brain, lungs, and placenta. ATP7B is a transmembrane protein of the trans-Golgi network that incorporates free copper into the apoceruloplasmin molecule, which in turn transports the excess metal to the excretory vesicles of the biliary ducts ***(La Fontaine and Mercer, 2010).***

Prominent clinical features of WD include hepatic and neurological/psychiatric symptoms. Hepatic symptoms range from acute and chronic hepatitis to cirrhosis and fulminant hepatic failure. Although serum levels of transaminases are high in infants with WD, hepatic disorders usually occur after 8 years of age. Neurological symptoms appear after 12 years of

age and are characterized by extrapyramidal effects, which include dysarthria, dystonia, tremor, choreoathetosis, and ataxia. The most common ocular findings is Kayser–Fleischer rings which are present in virtually all patients ***(Lorincz et al., 2010).***

Diagnosis is based on low serum copper and ceruloplasmin levels (<20 mg/dL; immunoassay), high copper concentrations in the liver (>250 μg/g dry weight), high copper excretion in the urine (>100μg/day), and by conducting a penicillamine challenge test (urinary copper excretion >1,600 or 1,057 μg/day) ***(Miyayama et al., 2011).***

In fact, serum copper levels are often high in patients with WD suffering from acute liver failure due to the release of accumulated copper in hepatocytes. Furthermore, other hepatic diseases, including autoimmune hepatitis and intrahepatic cholestasis, may affect serum copper measurements and make diagnosis difficult ***(Lin et al., 2010).***

When patients are diagnosed with WD, they should be promptly treated with chelating agents, including penicillamine and trientine, and/or zinc. Treatment should continue throughout the patient's life, with routine monitoring of serum and urine copper, blood cell counts, coagulation parameters, and testing for liver and renal function ***(Walshe, 2010).***

AIM OF THE WORK

The objective of the present study is to assess the clinical, biochemical, serological features and outcome of Wilson disease in Egyptian children and to evaluate the predictive factors for treatment response.

WILSON DISEASE

Wilson's disease (WD) is a rare autosomal recessive disease that affects approximately 1 in 30,000 live births, with a predominance in males. It is characterized by a disturbance in copper metabolism that leads to the accumulation of the metal in various tissues of the body. Copper accumulation triggers a variety of clinical manifestations that most often affect neurological and liver functions, although effects have also been observed for bone, retina, kidney, and hematological tissues ***(Manolaki et al., 2009).***

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years of age. Neurological symptoms appear after 12 years of age and are characterized by extrapyramidal effects, which include dysarthria, dystonia, tremor, choreoathetosis, and ataxia. The most common ocular findings is Kayser–Fleischer rings which are present in virtually all patients ***(Lorincz,2010).***

History:

The disease bears the name of the British physician Dr Samuel Alexander Kinnier Wilson (1878-1937), a neurologist who described the condition, including the pathological changes in the brain and liver, in 1912 ***(Rosencrantz and Schilsky, 2011).***

Cumings, 1948 made the link with copper accumulation in both the liver and the brain. Then Cumings, and the New Zealand neurologist Dr Derek Denny-Brown, working in the USA, first reported effective treatment with metal chelator British anti-Lewisite in 1951 ***(Walshe, 2009).***

Pathophysiology:

Copper is needed by the body for a number of functions, predominantly as a cofactor for a number of enzymes such as ceruloplasmin, cytochrome coxidase, superoxide dismutase ***(De Bie et al., 2007).***

The set of proteins regulating copper distribution within the cells are thought to be the same in all tissues (Figure 1). The mammalian Cu-transporting P-type ATPases ATP7A and

ATP7B are two key proteins that regulate the Cu status of the body. They transport Cu across cellular membranes for biosynthetic and protective functions, and to prevent a toxic build-up of Cu inside cells ***(La Fontaine and Mercer, 2010).***

ATP7A and ATP7B expression patterns are somewhat complementary. ATP7A is expressed in the majority of tissues except for the liver. ATP7B expression is more restricted, with highest expression in the liver ***(Miyayama et al., 2011).***

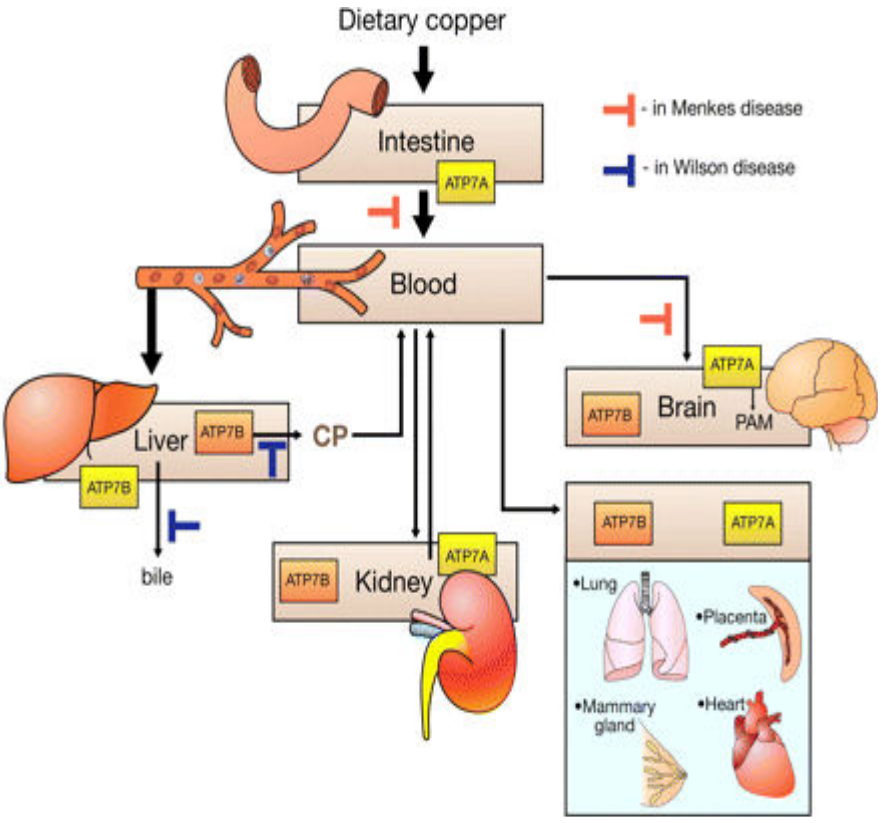

Figure (1): Copper distribution in the body. Disruptions to the pathways are marked for Wilson disease and Menkes disease. CP: ceruloplasmin, PAM: Quoted from ***(Lutsenko et al., 2007)***.

Copper enters the body through the digestive tract via a transporter protein; copper membrane transporter 1 (CMT1 or CTR1) to inside the enterocytes. Once bound to it, copper is delivered to its target proteins where some is bound to metallothionein and part is carried by antioxidant protein 1 (ATOX1) which is a copper protein required for the excretion pathway and required for subsequent copper transfer to ATP7A ***(Lonnerdal, 2011)***.

Copper is exported from the enterocytes into the blood by ATP7A. ATP7A has six copper binding domains at its N-terminus that bind copper prior to its transfer across the membrane for trafficking to the basal membrane ***(La Fontaine and Mercer, 2010)***.

Ceruloplasmin is involved in various functions including: oxidation of organic amines, iron oxidation and the regulation of cellular iron levels, free radical scavenging and other antioxidant processes ***(Healy and Tipton, 2011).***

Both functions of ATP7B are impaired in Wilson disease. Copper accumulates in the liver tissue; ceruloplasmin is still secreted, but in a form that lacks copper (apoceruloplasmin) and rapidly degraded in the bloodstream ***(De Bie et al., 2007).***

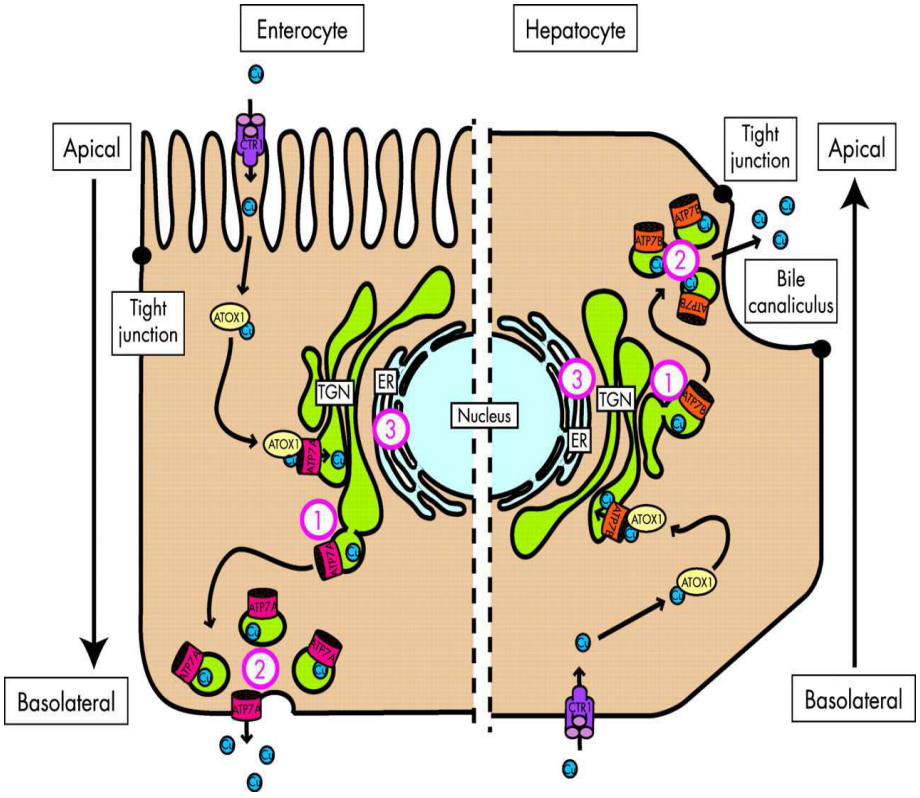

Figure (2): Copper metabolism. Schematic presentation of copper-induced relocalisation of ATP7A and ATP7B. Left side shows an enterocyte, and right side represents and hepatocyte. In both cells, copper enters through the copper transporter 1 (CTR1), and is then distributed via the ATOX1 to ATP7A or ATP7B residing in the TGN (Trans Golgi Network). After a rise in copper concentrations, ATP7A and ATP7B relocalise from the TGN to the cell periphery and in the case of ATP7A also the plasma membrane, to facilitate excretion of copper. The main difference in these two copper transport pathways lies in the direction. In the enterocyte, ATP7A facilitates excretion of copper into the bloodstream at the basolateral side, whereas in the hepatocyte copper is excreted at the apical side into the bile. The numbers indicate localisation defects of ATP7A and ATP7B due to MD-causing (Menkes Disease) and WD-causing mutations, respectively: (1) lack of copper responsiveness, resulting in constitutive localisation at the TGN, (2) constitutive localisation at the cell periphery, and (3) mislocalisation at the ER (Endoplasmic Reticulum) ***(Qouted from De Bie et al., 2007).***

When the amount of copper in the liver overwhelms the proteins that normally bind it, it causes oxidative damage; this damage eventually leads to chronic active hepatitis, fibrosis and cirrhosis. This free copper precipitates throughout the body but particularly in the kidneys, eyes and brain. In the brain, most copper is deposited in the basal ganglia. Damage to these areas produces the neuropsychiatric symptoms seen in WD ***(De Bie et al., 2007)***.

Wilson disease causes hemolysis, but various lines of evidence suggest that high levels of free (non-ceruloplasmin bound) copper have a direct effect on either oxidation of hemoglobin, inhibition of energy-supplying enzymes in the red blood cell, or direct damage to the cell membrane ***(La Fontaine and Mercer, 2010)***.

Copper Toxicity, Copper is a metal that is able to release or accept an electron easily, in the intestinal lumen and blood it is thought to be in its oxidized form. However, within the cell it is in the reduced form. Copper is considered a potential potent cytotoxin when allowed to accumulate in excess inside the cell ***(Knopfel and Solioz, 2012)***.

Genetics of Wilson Disease:

The gene causing WD is ATP7B, as identified in 1993 by two independent groups ***(Bull et al, 1993; and Tanzi et al., 1993)***.

Wilson disease is the most common of a group of hereditary diseases that cause copper overload in the liver. All can cause cirrhosis at a young age. The other members of the group are Indian childhood cirrhosis (ICC), endemic Tyrolean infantile cirrhosis and idiopathic copper toxicosis. These are not related to ATP7B mutations, but ICC has been linked to mutations in the KRT8 (Keratin8) and the KRT18 (Keratin18) gene located on chromosome 12q13 ***(Panichareon et al., 2011).***

Molecular diagnosis is feasible and important for reliable diagnosis. In some cases, mutation detection is essential for confirmation of diagnosis, so documentation of mutations must be as complete as possible. Differentiation of disease from non disease variants is crucial, and documentation of mutation analysis in patients is important. More than 400 mutations have been reported in patients with WD from many different populations ***(Kenney and Cox, 2009).***

ATP7B is an important transporter of copper out of the cell. When mutations occur in the ATP7B gene, copper accumulates particularly in the liver, kidney, brain, and cornea. Gene discovery and subsequent mutation analysis demonstrates that WD is a diagnostic challenge, because of its great phenotypic variability ***(Panichareon et al., 2011).***

Because of the large size of the gene and the potential for a mutation anywhere along its entire length, identification of mutations is very challenging. Identification of the prevalent mutations in a given population is therefore desirable in order to provide direct mutation-based molecular diagnosis for a larger segment of the affected population.

The Wilson disease gene ATP7B has been mapped to chromosome 13 (13q14.3) and is expressed primarily in the liver, kidney, and placenta. The gene codes for a (cation transport enzyme) ATPase that transports copper into bile and incorporates it into ceruloplasmin ***(Ala et al., 2007).***

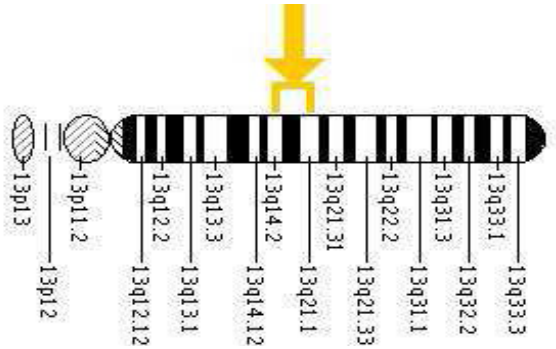

Figure (3): Illustrates the location of the ATP7B gene on Chromosome 13.**Quoted from Genetics Home Reference, 2012.**

The condition is inherited in an autosomal recessive pattern, which means both copies of the gene have mutations. In order to inherit it, both of the parents of an individual must carry an affected gene. Most patients have family history of the condition ***(Panichareon et al., 2011).***

Mutational analyses of WD patients and their relatives have identified more than 518 distinctive variants as reported in the WD mutation database**.** Most of these variants are probable disease-causing variants (379 out of 518 variants) ***(Kenney and Cox, 2009)*****.**

Diagnosis of Wilson Disease:

There is no totally reliable test for WD, but levels of ceruloplasmin and copper in the blood, as well of the amount of copper excreted in urine during a 24 hour period, are together used to form an impression of the amount of copper in the body. The gold standard or most ideal test, however, is a liver biopsy ***(Ala et al., 2007)*****.**

More recently, molecular diagnostic studies have made it feasible either to define patterns of polymorphisms of DNA surrounding ATP7B which are useful for identification of first-degree relatives of newly diagnosed patients or to examine directly for disease-specific ATP7B mutations on both alleles of chromosome 13 *(**Roberts and Schilsky, 2008)*****.**

A scoring system (Leipzig score) has been devised to aid diagnosis ***(Ferenci et al., 2003)***. A molecular genetic strategy using haplotype analysis or direct mutation analysis may be effective in identifying affected siblings of probands *(**Roberts and Schilsky, 2008)*****.**

Table (1): Clinical manifestations of wilson's disease ***(Pfeiffer, 2007)***

Hepatic • Persistently elevated serum aminotransferases • Chronic hepatitis • Cirrhosis (decompensated or compensated) • Fulminant hepatic failure (+/- haemolytic anaemia)
Neurological • Tremor • Choreiform movements • Parkinsonism or akinetic rigid syndrome—ie, partial parkinsonism • Gait disturbances • Dysarthria • Pseudobulbar palsy • Rigid dystonia • Seizures • Migraine headaches • Insomnia
Ophthalmic • K-F rings • Sunflower cataracts
Psychiatric • Depression • Neuroses • Personality changes - Psychosis
Rare renal abnormalities: aminoaciduria and nephrolithiasis

A- Clinical picture:

- ***Age:***

Even when presymptomatic siblings are excluded, the age at which WD may present or be diagnosed is both younger and older than generally appreciated, though the majority present between ages 5 and 35. WD is increasingly diagnosed in children younger than 5 years old ***(Caprai et al., 2006)***, and in a 3-year-old children with cirrhosis ***(Wilson et al., 2000)***.

Although the upper age limit for consideration of WD is generally stated as 40 years, when other concurrent neurologic or psychiatric symptoms and histologic or biochemical findings suggest this disorder, further evaluation should be carried out in older individuals ***(Ala et al., 2007).***

- ***Liver disease:***

Hepatic dysfunction is the most common initial manifestation in childhood, with patients in this category presenting at an average age of 10- 13 years, a decade or more sooner than those presenting with neurologic symptoms. The type of the liver disease can be highly variable, ranging from asymptomatic patients with only biochemical abnormalities to acute liver failure ***(Rosencrantz and Schilsky, 2011).***

An acute presentation with rapid deterioration may also occur in patients who were previously treated but stopped their medications. Suspicion for acute WD should be particularly high in patients with deep jaundice, low haemoglobin, only mildly increased transaminases, and low alkaline phosphatase and others may present with features indistinguishable from autoimmune hepatitis ***(Eisenbach et al., 2009)***.

Patients may present with isolated splenomegaly due to clinically inapparent cirrhosis with portal hypertension. Some patients have transient episodes of jaundice due to hemolysis. Low-grade hemolysis may be associated with WD when liver disease is not clinically evident ***(Walshe, 1989).***

Hepatocellular carcinoma is a rare consequence of WD. About 5% of all patients are diagnosed only when they develop fulminant acute liver failure, often in the context of a Coombs-negative hemolytic anemia. The deranged protein metabolism leads to the accumulation of ammonia in the bloodstream, which irritate the brain and the patient develops hepatic encephalopathy that manifested by confusion, coma, seizures and finally life-threatening swelling of the brain ***(Ala et al., 2007).***

Wilson disease accounts for 6–12% of all patients with acute liver failure who are referred for emergency transplantation ***(Eisenbach et al., 2009)***.

- ***Neuropsychiatric symptoms:***

About half the patients with Wilson disease have neurological or psychiatric problems. Most patients initially have mild cognitive deteriorations, as well as changes in behavior. Specific neurological symptoms then follow, often in the form of Parkinsonism with or without atypical hand tremor, ataxia or dystonia. Seizures and migraine appear to be more common in WD.

Psychiatric problems due to Wilson disease may include behavioral changes, depression, anxiety and psychosis ***(Ala et al., 2007).***