

The biochemical diagnosis of Wilson's disease in Iran (*)

By

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The Wilson's disease or hepatolenticular degeneration, which is thought to be a familial disease transmitted as a recessive characteristic, is resulted from deposition of the copper in tissues.

Disordeliness in the metabolism of copper is due to the deficiency of enzymatic activity of the ceruloplasmin fraction of the blood.

The disease generally appeared between the age of 10 to 20 and is associated with cirrhosis, and degeneration of the center part of lenticular brain.

In addition to the clinical features, biochemical analysis of the blood and urine, play an important part in the diagnosis of the disease.

The present communication, however, is a report on the first diagnosis of Wilson's disease in Iran based on the biochemical analysis methods.

Patients : From 29,246 to 24,448 (May 1967 to July 1969) different patients, between 6 to 28 years old, were conducted from the Pahlavy (1) and Bahramy (2) hospital to the Razi institute, with some of the following clinical symptoms:

The weakness, muscular rigidity, tremor of arms, inability of walking or eating, associated with Kayser, Fleischer ring and a cirrhosis.

The most interesting observation, were the familiarity of the mother and the father in the most of these patients.

Biochemical diagnosis of Wilson's disease: Clinical observations help generally the physician restricting to laboratories equipped by the spectrophotometric, manometric, immunochemical, electrophoretic, and chromatographic assays for the biochemical and effective diagnosis of Wilson's disease.

The three following tests were used for the blood and urine analysis.

- 1 — Determination of urinary copper.
- 2 — Determination of oxidasic activity of ceruloplasmin.
- 3 — The amino-acids chromatography of urine.

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1 — Patients M.A and S.F. from the Pediatric depart. of Pahlavy hospital, (By Prof. M. Gharib) and patient R.A. from neurology depart. of Pahlavy hospital, (By Prof. E. Tchehrazi).

2 — Patient A.S. from the Bahramy hospital, (By Dr. Mokhtar Zadeh).

Materials and methods : The following materials were analysed in this investigation:

- 1 — Blood and urine of the patients.
- 2 — Blood and urine of parents and sibs.
- 3 — Blood and urine of different subjects served as controls for biochemical analysis.

Pyrex bottles were carefully washed with bidistilled water. The blood samples withdrawn by a sterile dry syringe was transferred to a Pyrex tube, and the urine of twenty-four-hour were collected in a pyrex bottle, then samples stored in a 4° C refrigerator.

The methods of C.J. Gubler and Co-Workers (1) and G.E. Cartwright and Coll (2) were used to estimate the urinary copper, and the determination of serum ceruloplasmin oxidasic activity was performed according to the method of H.A. Ravin (3 and 4) using the P-phenylendiamin, 2 HCl. The amino-acids chromatography of urine was done by ascending, descending, and two ways technique in Shandon Universal Chromathank, using the Whatman No. 1 papers chromatography of 20x20 and 25x25 centimeter, and finally the chromatograms were developped with a ninhydrin solution (5 and 6).

1—*Determination of urinary copper* : Samples of the patients were examined, using the sodium diethyldithiocarbamate. The transmission of the solutions was read in a Coleman Junior Spectrophotometer at a wave length of 440 m μ , with the following results:

Patient M.A. = 1,61mg Cu per liter of urine.

Patient R.A. = 2,5 mg Cu per liter of urine.

Patient S.F. = 1,12mg Cu per liter of urine

Patient A.S. = 0,68mg Cu per liter of urine.

T (normal) = 0,065mg Cu per liter of urine.

2—For determination of oxidasic activity of ceruloplasmin, we have used two methods: Paper electrophoresis and spectrophotometric technique.

By the paper electrophoresis, 50 to 100 microliter of serum were fractionated with the Elphor electrophoresis apparatus, using sodium barbital/Sodium acetate buffer, and for the staining a 0,5% of P. Phenylendiamin, 2 HCl were employed. Using the electrophoresis technique for the ceruloplasmin activity, the serum of patients showed no or a trace activity in the ceruloplasmin fraction, but the normal sera has a good activity in the ceruloplasmin fraction, Fig. 1 and 2.

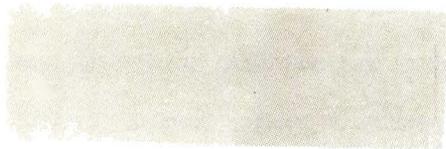


Fig. 1, Electrophoresis of a normal serum ceruloplasmin

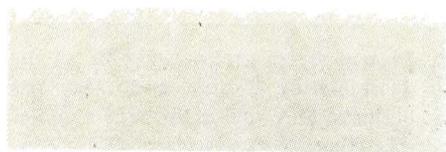


Fig. 2, Electrophoresis of a serum with Wilson's disease

For the spectrophotometric method, we have used the Coleman Junior spectrophotometer, and the following transmission results were obtained at 530 m μ .:

B = 100
 T = 12 normal serum
 Patient M.A = 99
 Patient R.A = 96
 Patient S.F = 95
 Patient A.S = 100

The results obtained by the spectrophotometric method were similar to those of electrophoresis technique.

3—*The amino-acids chromatography of Urine* An amino-acid chromatogram prepared with 5 to 10 microliter of 5 x concentrated and purified normal urine was contained from 5 to 7 pale spots related to the following amino-acids: Taurine, alanine, glycine, glutamic acid or glutamine, methionine sulfoxide, and some time cystine and histidine.

By the described above technique, we have observed from 12 to 18 differents amino-acids in the urine of the patients, with notable increase in: Serine, lysine, threonine, alanine, glycine, glutamic acid, cystine, glutamine and histidine; and less well marked were observed for aspartic acid, proline, tyrosine, valine, leucine, and iso-leucine. Fig 3 and 4.



Fig. 3, Amino-acid chromatography of normal urine.

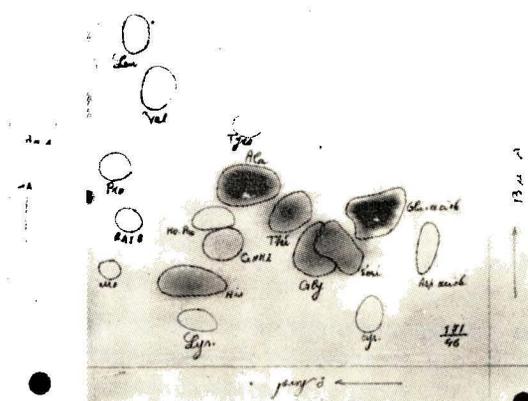


Fig. 4, Amino-acids chromatography of patient M.A with Wilson's disease.

Summary : Different cases of Wilson's disease were recognized in Iran for the first time. The diagnosis was based on different biochemical analysis of the urine and the blood of patients using spectrophotometric, electrophoretic, and chromatographic methods.

Three abnormalities, namely high excretion of copper in the urine, deficiency or absence of oxidasic activity in the ceruloplasmin fraction, and an increase level of amino-acids in the urine, were mostly manifested in all cases.

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