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A Screening Test for Wilson's Disease and its Application to Psychiatric Patients

DIANE WILSON COX, B.Sc., M.A., *Montreal*

Varied modes of onset make the early diagnosis of Wilson's disease difficult. A deficiency of serum ceruloplasmin, usually characteristic of the disease, was used as the basis for a screening test. Simple test materials and provision for handling about 50 plasma samples simultaneously made this test feasible for large-scale screening.

The screening test was applied to 336 persons hospitalized for psychiatric disorders, to detect patients with Wilson's disease before the classical symptoms appeared. Two patients with ceruloplasmin levels below the normal limits were detected but did not have Wilson's disease. Further application of the screening test to relatives of patients known to have Wilson's disease and to individuals with any symptoms of the disease (hepatic disease, extrapyramidal dysfunction, psychiatric disorders, behaviour problems in children) would aid in early diagnosis and more effective treatment.

La maladie de Wilson a des débuts extrêmement variables qui en rendent difficile le diagnostic précoce. L'insuffisance de céruloplasmine, généralement pathognomonique de la maladie, a constitué la base d'une épreuve de dépistage. Des accessoires simples et l'adoption de méthodes permettant de manipuler 50 spécimens de plasma simultanément ont rendu ce test de dépistage applicable sur une échelle.

On l'a appliqué à 336 sujets hospitalisés pour des troubles mentaux, pour déceler les malades souffrant de maladie de Wilson avant l'apparition des symptômes classiques. On a découvert une faible teneur en céruloplasmine, inférieure à la normale, chez deux malades qui n'avaient pas cependant la maladie de Wilson. On contribuerait à faciliter le diagnostic précoce et à améliorer le traitement en appliquant davantage le test de dépistage aux parents de malades qui ont notoirement la maladie de Wilson et aux sujets qui présentent un des symptômes de la maladie (dégénérescence hépatique, trouble fonctionnel extrapyramidal, troubles psychiatriques, comportement anormal chez l'enfant).

WILSON'S disease (hepatolenticular degeneration) is a recessively inherited disease in which copper metabolism is abnormal. The basic metabolic defect has not yet been identified. However, as a result of the defect, excess copper accumulates in certain body tissues and the toxic effect of this usually leads to neurological, hepatic and renal abnormalities, and to the appearance of Kayser-Fleischer rings.

Treatment with chelating agents has been proved effective in many cases of Wilson's disease and is most effective when instituted early in the course of the disease. However, early diagnosis is often difficult because of the great variability of symptoms with which the disease presents, e.g. those of hepatic or neurological disease or psychiatric aberrations.

The deficiency of serum ceruloplasmin, characteristic when a patient is otherwise asymptomatic, provides the basis for a simple, reproducible screening method for asymptomatic relatives of patients with Wilson's disease, especially sibs who have a one-in-four chance of developing the disease.

Application of a simple, reproducible screening test, such as the one to be presented, to patients whose illnesses have features suggestive of Wilson's disease should provide a valuable aid to early diagnosis.

From the Human Genetics Sector, Department of Genetics, McGill University, and the Department of Medical Genetics, The Montreal Children's Hospital, Montreal, Quebec.

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Reprint requests to: Diane Wilson Cox, Human Genetics Sector, Department of Genetics, McGill University, Montreal, Quebec.

The screening test developed for the present investigation is based on the detection of a serum ceruloplasmin level below the normal range. The mean ceruloplasmin level for normal adults is 30.4 mg. %, with 95% having levels between 20.7 and 40.2 mg. %.[1] About 96% of patients with Wilson's disease have levels below 20 mg. %, with the great majority less than 10 mg. %.[2] A test which detects low levels of serum ceruloplasmin is therefore suitable and desirable as a screening test for Wilson's disease.

A spot screening test, based on the oxidase activity of ceruloplasmin with paraphenylenediamine dihydrochloride (PPD) has been reported for distinguishing ceruloplasmin levels below that of a given standard.[3] In the author's experience, test strips were inconvenient to prepare and not suitable for storage, and test results were frequently difficult to interpret. The screening test described similarly depends upon oxidase activity of ceruloplasmin but overcomes previous difficulties. It is more suitable for screening large numbers of patients than a commercially available kit* because of provision for handling large numbers of samples at one time and its low cost.

For application of the screening test developed, we have selected patients hospitalized for psychiatric treatment. Psychiatric abnormalities are present in some cases of Wilson's disease,[4] although this aspect of the disease has not been emphasized. In one series of 33 cases,[5]

*Dade Reagents, Inc.

Canad. Med. Ass. J.
Jan. 14, 1967, vol. 96

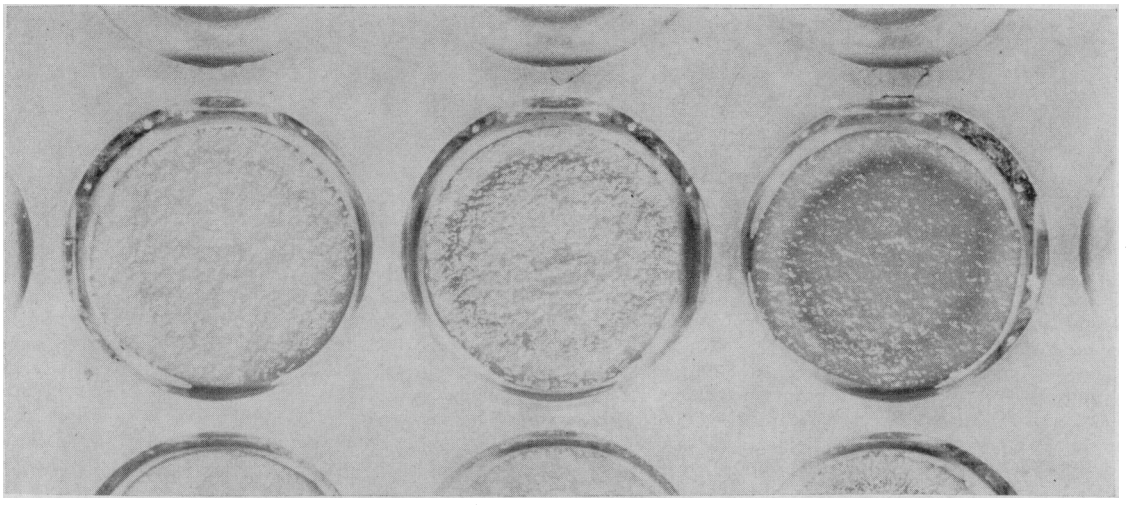

Fig. 1.—Typical appearance of test spots. Results are as follows, from left to right, with levels of ceruloplasmin as determined by measuring oxidase activity,[1] shown in parentheses: positive (11.8 mg. %); standard serum (18.8 mg. %); and negative (32.2 mg. %).

15% had psychiatric disturbances before classical symptoms made Wilson's disease a possible diagnosis and an additional 25% had emotional disturbances accompanied by typical neurological or hepatic manifestations. The possibility therefore existed that some of the hospitalized patients had unrecognized Wilson's disease.

METHODS

*Test Procedure**

A standard serum (or plasma) containing about 19 or 20 mg. % of ceruloplasmin is required. This was prepared by mixing, in suitable proportions, serum from a normal adult and serum deficient in ceruloplasmin. The latter can be obtained from the umbilical cord of a newborn[6] or from a patient with Wilson's disease. The ceruloplasmin levels of the sera used for the standard and of the final standard mixture were determined by a quantitative enzymatic method.[1] Our standard serum contained 18.8 mg. % ceruloplasmin.

A heparinized capillary tube (hematocrit tube, 1.3-1.5 mm. diameter) was filled with blood from a finger puncture. One end of the tube was plugged with plasticine and the plasma was separated by centrifugation or gravity. The cell-containing portion of the tube was broken off and discarded. Into each well of a microtitre 'U' plate (Canlab. No. 21828/4) was placed a disc of thick filter paper (1.3 cm. diameter discs cut from Whatman No. 3 MM.; or filter paper discs for antibiotic assay, Fisher No. 9-897). The microtitre plate was marked off into squares of nine wells each. The centre well of each group was used for the standard. The centre of each disc was touched with a capillary tube of standard, or plasma to be tested, allowing the absorption of about one inch of the capillary tube contents into the disc. The amount of plasma is not critical, as it determines, within limits, only the size of spot produced. On each disc were dropped two drops of a 0.4% solution of PPD (Fisher certified)* in acetate buffer (pH 5.2, 1 M), prepared immediately before testing. The plate was covered with plastic film (Saran wrap) and was placed in a shallow water bath (rectangular pyrex dish) at 35 to 40° C. for 10 minutes or until the standard discs were pale blue. The plate was then uncovered and read against a white background. A test disc as pale as or paler than the standard indicated a positive test result; that is, a low level of ceruloplasmin. A disc darker than the standard indicated a normal level of ceruloplasmin. The appearance of the discs is shown in Fig. 1.

We have found it convenient to test 48 samples at one time. The microtitre plate contains 80 wells; however, we used six groups of nine wells each at one time. The use of one control in each group of nine compensates for differences in reaction times.

Before the screening of patients with psychiatric disorders, the screening test was evaluated on 50 sera, covering a broad range of ceruloplasmin levels, obtained from normal persons and from patients with Wilson's disease and their relatives. Two investigators examined the test discs independently without knowledge of the source of the sera, and recorded their conclusions. The amount of ceruloplasmin was

*A mimeographed description of this method was prepared for distribution on August 19, 1964.

*Recrystallization is not required when the PPD crystals are white and the solution is clear. If either is coloured, dissolve the crystals in a minimum amount of hot water, decolourize with charcoal, filter while hot, and dry the crystals obtained from the filtrate over calcium chloride.

measured by a quantitative method[1] and the results of the two tests were compared.

Patients Tested

Psychiatric patients at the Douglas Hospital (formerly Verdun Protestant Hospital) were selected for screening if they fulfilled the following criteria: (1) less than 20 years of age, or (2) between 20 and 45 years of age and patients in the hospital for less than 20 years. A total of 336 patients (208 males, 128 females) were tested. An additional four females were selected but refused the test. The patients were selected from a hospital population of about 1240 patients; wards consisting entirely of patients over 45 years of age were not included. Of the patients tested 41 (12.2%) were under 20 years of age, 118 (35.1%) were between 20 and 30 years, and 177 (52.7%) were between 31 and 45 years. The majority of patients (79.2%) had been in the hospital continuously for five years or less.

RESULTS

Evaluation of the Screening Test

The results of the evaluation are shown in Table I. Results of the screening test were classified as uncertain when the two investigators disagreed or when one or both investigators considered a result open to question. In practice, such results should be considered positive and further investigated. The screening test was found effective in detecting ceruloplasmin levels below that of the standard; in all 10 cases the pertinent sera were classified as positive in the screening test. Sera with ceruloplasmin levels up to 2 mg. % above the standard registered as false positives, as did occasionally those with levels up to 5 mg. % above the standard. The screening test therefore tended to pick up normal levels (false positives), but never missed low levels of ceruloplasmin.

Application of the Screening Test

Non-heparinized capillary tubes were used in testing the first 254 patients. This occasionally resulted in hemolyzed samples which produced a very pale area in the centre of the spot but surrounded by a dark ring. Retesting of sera from the 10 patients with such spots, using non-hemolyzed samples, indicated that all were negative. Quantitative measure of ceruloplasmin[1] in serum obtained by venipuncture confirmed the negative results. This problem was avoided for the remainder of the screening program by using heparinized capillary tubes.

TABLE I.—RESULTS OF SCREENING TEST COMPARED TO QUANTITATIVE ASSAY OF CERULOPLASMIN

Ceruloplasmin concentration (mg. %)*	*Number tested*	*Results of screening*		
		Negative	*Positive*	*Uncertain*
40.0—50.0	6	6		
30.0—39.9	19	19		
25.0—29.9	9	9		
23.0—24.9	2	1		1
21.0—22.9	2	2		
19.0—20.9	2		1	1
17.0—18.9	1		1	
15.0—16.9	3		3	
Less than 15.0	6		6	

*Measured by quantitative determination of oxidase activity.[1]

Two positive results (M.D. and Y.Y.T.) and one questionable result (R.C.) were obtained from the complete screening series. Two independent observers agreed on the interpretation of the results. The ceruloplasmin level was measured quantitatively in serum obtained by venipuncture.

R.C. was an 18-year-old Negro male. His ceruloplasmin level (20.8 mg. %) was within normal limits for his age (19.1-32.4 mg. %).[1] As he has been severely mentally retarded from infancy, his symptoms are unlikely to be caused by Wilson's disease.

The ceruloplasmin levels of M.D. (18.3 mg. %) and Y.Y.T. (16.8 mg. %) were below the limits for 95% of normal adult males (20.7-40.2 mg. %).[1] M.D. was a 33-year-old Jewish male with a diagnosis of catatonic schizophrenia. Absence of Kayser-Fleischer rings by slit-lamp examination and normal 24-hour urinary copper excretion was considered sufficient evidence to eliminate Wilson's disease, since his psychiatric disturbances had been present for at least nine years. Y.Y.T. was a 30-year-old Chinese male with schizophrenia of 12 years' duration. He had normal liver function tests, normal 24-hour urinary copper excretion and no Kayser-Fleischer rings by slit-lamp examination. Neither patient appears to have Wilson's disease.

DISCUSSION

The screening test described can conveniently be carried out on a large scale. The test is reliable for detecting ceruloplasmin levels equal to or below a standard (standard serum: 18.8 mg. %); there were no false negative results. False positives are likely to occur when the ceruloplasmin level is up to 2 mg. % above the standard; however, less than 2% of the normal population have levels in this range.[1] We encountered one such false positive in screening 336 patients. False positives may sometimes be encountered up to 5 mg. % above the standard.

Canad. Med. Ass. J.
Jan. 14, 1967, vol. 96

but we did not encounter any in applying the test.

While a low level of ceruloplasmin usually is found in patients with Wilson's disease, some proved cases have normal levels.[7] In one series of 111 patients,[2] 96.4% of the patients had ceruloplasmin levels below 20 mg. %. The screening test presented would therefore detect most individuals with Wilson's disease.

Two individuals with positive test results, indicating below-normal levels of ceruloplasmin, were detected among the 336 patients tested. When positive results occur, further diagnostic tests must be carried out to determine whether the patient has Wilson's disease. Further study of our two patients with positive tests failed to show evidence of Wilson's disease. The frequency of patients with ceruloplasmin levels below 19 mg. % is similar to that expected in a normal population. The low ceruloplasmin levels of the two patients may be familial, as in two families reported;[1] however, their relatives were not available for study. These patients may be heterozygous carriers of the gene for Wilson's disease, although this is improbable because of the low gene frequency (perhaps one in 1000[8]).

The mental patients tested were on drug therapy in which the most commonly used drugs were trihexyphenidyl hydrochloride (Artane), tropine benzohydryl ether methanesulfonate (Cogentin), procyclidine hydrochloride (Kemadrin), trifluoperazine dihydrochloride (Stelazine) and chlorpromazine (Largactil). Chlorpromazine has been reported to cause increases up to 50% in the serum copper level of some individuals on the drug for several weeks.[9] Estrogen therapy, which is particularly effective in increasing serum copper and ceruloplasmin, caused a rise to normal ceruloplasmin levels in two of 11 patients with Wilson's disease and a further rise in one patient who already had a normal level.[10] Copper levels were more frequently increased than were ceruloplasmin levels after estrogen administration. If any of the drugs used produced a doubling of the ceruloplasmin level, which seems unlikely, the majority of patients with Wilson's disease could still be detected by the screening test, since about 81% of such patients have ceruloplasmin levels below 10 mg. %.[4]

Wilson's disease apparently does not occur sufficiently often in those forms likely to be confused with psychiatric disorders to be detected in a sample of the size tested in our series. Screening of larger numbers of mental patients may uncover cases of the disease.

The test reported is particularly useful for quick and easy screening of large numbers of persons. The test is also suitable for rapid screening of a few samples; the standard serum can be frozen and stored in small aliquots which can be used and refrozen repeatedly unless turbidity develops. The test is suitable for use in hospital laboratories where a minimum amount of equipment is desired for a preliminary screening. The more extensive testing, which must be carried out on individuals having a positive result, could then be referred to centres equipped for such investigation.

The capillary tubes of blood, if well sealed, can be sent conveniently by mail. We found that two days in transit apparently does not affect the ceruloplasmin level or the test results.

This screening test for Wilson's disease could be easily applied to suspects: individuals with liver disease, extrapyramidal dysfunction or psychiatric disorders, children with behaviour problems, and relatives of patients with Wilson's disease. The application of a screening test for Wilson's disease to all normal children has been suggested.[3] Such extensive screening may be unwarranted because of the low incidence of the disease (perhaps one in a million[8]), but more systematic screening than is presently carried out, of all the individuals listed above, would be advantageous.

SUMMARY

A simple screening test for Wilson's disease has been described. Large numbers of sera can be screened conveniently to detect those with a ceruloplasmin concentration below the normal range.

The screening test was applied to 336 hospitalized psychiatric patients. Two patients with levels below the limits of normal were detected, but further tests eliminated a diagnosis of Wilson's disease.

Application of this screening method is urged for individuals with liver disease, extrapyramidal dysfunction, or psychiatric disorders; children with behaviour problems; and relatives of patients with Wilson's disease.

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