

## Original Article

# Serum Ceruloplasmin in Wilson's Disease in Indian Children- what should be the cut off?

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### ABSTRACT

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**Background:** To determine the cut-off level of serum ceruloplasmin when screening for Wilson's Disease (WD) in Indian Children.

**Methods:** This retrospective study was conducted in 40 children from 2012-2015. All children suspected of WD who were screened with ceruloplasmin were included in the study. All patients with acute liver failure (ALF) were excluded from the study. As per EASL guidelines, patients were divided into 2 groups: those having WD and those who were non-WD. Sensitivity and specificity of ceruloplasmin to diagnose and rule out WD at various cut-off levels were analysed.

**Results:** Eighteen (45%) children had WD. Low ceruloplasmin was present in all patients (100%) with WD whereas it was low in 10(45%) in patients without WD ( $p=0.001$ ). The mean ceruloplasmin level in patients with WD was  $6.5 \pm 4.8$  mg/dl and in those without WD was  $21 \pm 5.5$  mg/dl ( $p=0.0001$ ). Ceruloplasmin cut off levels of 20mg/dl had a high sensitivity (100%) with low specificity (54.6%) with high negative predictive value (NPV:100%) and low positive predictive value (PPV :64.3%). When cut off was taken as <10 mg/dl then sensitivity was 72.2% and specificity was 95.5% with PPV of 92.9% and NPV of 80.8%. However, with a cut-off of <5mg/dl, the sensitivity was 55.6% and specificity was 100% with PPV of 100% and NPV of 73.3%.

**Conclusion:** Serum ceruloplasmin of >20 mg/dl rules out WD. However, low ceruloplasmin may also be seen in 45% of patients without WD. Ceruloplasmin level of <10mg/dl is predictive of WD in 93% of patients and <5mg/dl is diagnostic of WD in 100% of patients.

**KEYWORDS:** Ceruloplasmin; Wilson's disease; children.

## Introduction

Wilson's disease is an autosomal recessive disorder of copper metabolism which leads to increase in tissue copper and can cause abnormalities in several systems, but the most common clinical manifestations are signs of hepatic and neurological dysfunction. The prevalence of Wilson's disease is 1 in 30000.<sup>1</sup> Untreated Wilson's disease is universally fatal, with most patients dying from liver disease. Timely treatment with chelation becomes essential for survival. Thus proper identification of Wilson's disease is essential. Diagnosis of Wilson's disease is challenging. More than 500 distinct mutations have been described in the ATP7B gene (mutations in this gene causes Wilson's disease); from which 380 have a confirmed role in the pathogenesis of the disease.<sup>2</sup> Thus gene analysis is usually not feasible to make a diagnosis of Wilson's disease. Usually a combination of various laboratory parameters is necessary to firmly establish the diagnosis. As per European Association for Study of Liver Diseases (EASL) guidelines, Wilson's disease can be diagnosed normally on the basis of combination of low ceruloplasmin ( $<0.1\text{g/L}$ ) and presence of Kayser – Fleischer ring (K-F ring).<sup>3</sup> K-F ring is present in 95% of patients with neurologic symptoms and somewhat over half of those without neurologic symptoms.<sup>4,5</sup> They are not entirely specific for Wilson's disease, since they may be found in patients with chronic cholestatic diseases including children with neonatal cholestasis.<sup>6</sup> Also serum ceruloplasmin can be decreased in severely malnourished subjects and in heterozygous carriers of the Wilson's disease gene. Very low levels were found in a patient with autoimmune hepatitis, which increases following steroid treatment.<sup>6</sup> Thus, not a single test per se is specific and, thus, a range of tests has to be applied to make a diagnosis of Wilson's disease. A prospective study on serum ceruloplasmin, as a screening test for Wilson's disease in patients referred with liver disease, showed that subnormal ceruloplasmin had a positive predictive value of only 6%. In children with Wilson's disease, 15-36% had ceruloplasmin in the normal range.<sup>7</sup> In one series, 12 out of 55 Wilson's disease patients had normal ceruloplasmin and no Kayser–Fleischer rings.<sup>4,8</sup> We undertook this study

to determine the cut-off range of serum ceruloplasmin for screening pediatric patients for Wilson's disease.

## Materials and Methods

This is the retrospective study conducted at the outpatient Paediatric Liver Clinic in a tertiary referral children's hospital in Mumbai from January 2012 to December 2015. All patients screened for Wilson's disease by serum ceruloplasmin were included in the study. Serum ceruloplasmin was done by nephelometry and levels  $<20\text{mg/dl}$  were considered to be low. The various tests used to make a diagnosis of Wilson's disease were hepatic copper content, 24 hours urine copper excretion and presence of K-F ring in addition to clinical manifestations such as neuropsychiatric manifestations and presence of coombs negative haemolytic anaemia. A symptomatic siblings of patients with Wilson's disease were screened with serum ceruloplasmin, 24 hours urine copper and K-F ring. Only those siblings with low serum ceruloplasmin underwent a hepatic copper estimation. A diagnostic score based on all available tests was proposed by the Working Party at the 8th International Meeting on Wilson's disease, Leipzig 2001 was used to make a diagnosis of Wilson's disease.<sup>9</sup> A score 4 or more established a diagnosis of Wilson's disease whereas a score 2 or less ruled out Wilson's disease. As per Leipzig 2001 meeting, with a score of 3, diagnosis possible and more tests are needed.<sup>9</sup> Patients who were hospitalised with acute liver cell failure were excluded from the study. All patients underwent liver function tests and Doppler of the portal system. Siblings who were not detected to have Wilson's disease did not undergo estimation of liver function tests or ultrasound examination. Various parameters such as serum ceruloplasmin levels, 24 hours urine copper content, and SGOT:SGPT ratio were analysed in Wilson's and non-Wilson's group.

Statistical analysis was done by Medcal online calculator using chi-square and Fischer exact test for proportions and anova-1 and t test for variables. P value  $<0.05$  was considered as significant. Sensitivity and specificity of serum ceruloplasmin at various levels to diagnose Wilson's disease were analysed.

## Results

Out of 40 patients included in the study, 18 (45%) had Wilson's disease and the remaining were ruled out to be Wilson's disease. Total siblings who were screened for Wilson's disease were 10 (25%) and 2 (20%) were detected to have Wilson's disease. Low ceruloplasmin was present in all patients (100%) with Wilson's disease whereas it was low in 10 (45%) in patients without Wilson's disease ( $p=0.001$ ). Twelve (66.7%) in the Wilson's group had presence of K-F ring and none in the non-Wilson's group had presence of K-F ring. ( $p=0.0005$ ). Neurological manifestations were present in 6 patients with Wilson's disease. Liver biopsy was done in total 9 patients of which 4 had Wilson's disease and 2 (22.2%) had hepatic copper content more than 100 mcg/g. The various parameters associated with Wilson's disease and those who did not have Wilson's disease are included in **Table 1**. Serum ceruloplasmin ranges in Wilson's and non-Wilson's group are depicted in **Table 2**. Sensitivity of serum ceruloplasmin when using cut-off of 20 mg/dl and more to detect Wilson's disease was 100% with a specificity of 54.6% and positive predictive value of 64.3% and negative predictive value of 100%. Sensitivity of serum ceruloplasmin to detect Wilson's disease when a cut off of <15 mg/dl is used was found to be 94.4% and specificity was 90.9% with positive predictive value

of 89.5% and negative predictive value of 95.2%. When cut off serum ceruloplasmin was taken as <10 mg/dl then sensitivity was 72.2% and specificity was 95.5% with positive predictive value of 92.9% and negative predictive value of 80.8%. However, with a cut-off of <5mg/dl, the sensitivity was 55.6% and specificity was 100% with positive predictive value of 100% and negative predictive value of 73.3%.

## Discussion

The most common presentations are with liver disease or neuropsychiatric disturbances. A symptomatic patients are most often detected by family screening. Ceruloplasmin is a copper-carrying protein that is bound to 90% of the circulating copper in normal individuals. The normal concentration of ceruloplasmin is 200-400 mg/L, and a serum ceruloplasmin level below 200 mg/L (20 mg/dL) is suggestive of Wilson disease.<sup>10,11</sup> Ceruloplasmin concentrations under 200 mg/L can be found in 1% of controls, in 10% of heterozygous Wilson disease carriers and in patients with copper deficiency, Menkes disease, hereditary hypoceruloplasminemia, malabsorption, Nephrotic syndrome and chronic liver failure. What's more, normal ceruloplasmin concentrations are recorded in about 20% of Wilson disease patients.<sup>6</sup> Levels of serum ceruloplasmin are physiologically very low in

**Table 1: Various clinical and laboratory parameters in Wilson's and Non-Wilson's group**

Parameters	Wilson's Disease (n =18)	Not Wilson's Disease (n =22)	P value
Age (years)	8.2 ± 3.2	8.8 ± 4.4	0.6
Sex	Female: 6 (33.3%) Male: 12 (66.7%)	Female: 11 (50 %) Male: 11(50%)	0.4
Serum ceruloplasmin (mg/dl)	6.5 ± 4.8	21 ± 5.5	0.0001
24 hours urine copper (mg/dl)	268 ± 227.7	57.3 ± 68.2	0.0003
Bilirubin (mg/dl)	3.6 ± 4.5	3.2 ± 4.2	0.7
SGOT	115.6 ± 78.6	146.1 ± 141.4	0.4
SGPT	70.9 ± 56.1	123.5 ± 141.4	0.1
Alkaline phosphatase	251.3 ± 233.8	309.6 ± 248	0.5
Albumin	3.1 ± 0.8	3.6 ± 0.8	0.09
SGOT : SGPT	1.9 ± 1.2	1.8 ± 1.6	0.9
Jaundice	9 (50%)	4 (18.2%)	0.07
Portal hypertension	8 (44.4%)	1 (4.5%)	0.008

**Table 2: Range of serum ceruloplasmin in both groups**

Serum ceruloplasmin (mg/dl)	Wilson's disease (n=18)	Not Wilson's Disease (n =22)
Age (years)	8.2 ± 3.2	8.8 ± 4.4
> 20	0 (0%)	12 (54.5%)
15- 19.9	1 (5.5%)	8 (36.4%)
14.9-10	4 (22.2%)	1 (4.5%)
<9.9 – 5	3 (16.7.%)	1 (4.5%)
< 5	10 (55.6%)	0 (0%)

early infancy to the age of 6 months, peak at higher than adult levels in early childhood (at approximately 300-500 mg/L), and then settle to the adult range.<sup>12</sup> An extremely low serum ceruloplasmin level (<50 mg/L or <5 mg/dL) should be taken as strong evidence for the diagnosis of Wilson's disease. Modestly subnormal levels suggest further evaluation is necessary.<sup>12</sup> Similarly in our study a serum ceruloplasmin of <5mg/dl was diagnostic of Wilson's disease in 100% of patients. However, low serum ceruloplasmin in the range of 15-20 mg/dl was also seen in 36% of patients without Wilson's disease. Thus with a positive predictive value of 93%, a serum ceruloplasmin of <10 mg/dl would require extensive work up for Wilson's disease as per our study. Normal serum ceruloplasmin was found in patients with acute liver failure (ALF) in other studies.<sup>13</sup> However in our study, no patient with Wilson's disease had a normal serum ceruloplasmin probably as we had excluded the ones with ALF. In Korea, a study was done on values of serum ceruloplasmin in 2,834 children who had hepatitis of which 181 students were diagnosed as Wilson's disease. They found sensitivity of 93.4% and specificity of 84.2% when ceruloplasmin level of <20 mg/dL was taken as cut-off. In a study by Jung et al, ceruloplasmin level of ≤16.6 mg/dL showed sensitivity of 91.2%, a specificity of 94.9% for diagnosis of Wilson's disease.<sup>14</sup> This was not seen in our study and in fact our sensitivity and specificity improved only when cut-off of <10 was taken. This may be due to ethnic differences as our population were Indian children. This is a retrospective analysis and is a small study group, thus more extensive studies in Indian population would be required to determine the cut-off of serum ceruloplasmin

to make a diagnosis of Wilson's disease. Also decreasing the cut-off would lead to lesser sensitivity.

## Conclusion

Ceruloplasmin level of <5mg/dl is diagnostic of WD. However decreasing the cut-off for screening patients for Wilson's disease may improve the specificity of the test but make it less sensitive. Thus, a serum ceruloplasmin level of half the lower normal range would be more predictive of Wilson's disease.

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