Copper metabolism in retinitis pigmentosa patients

SUNKARA SANGAMESWAR RAO,¹ MADHUSUDAN SATAPATHY,²
AND ARI SITARAMAYYA¹

From the ¹Institute of Genetics, Osmania University, and ²Sarojini Devi Eye Hospital,
Hyderabad, Andhra Pradesh, India

SUMMARY Serum copper, ceruloplasmin, and urinary copper were estimated in 13 normal subjects and 24 patients with primary retinitis pigmentosa. The serum copper levels in patients appeared to be higher and ceruloplasmin levels lower than in the normal subjects. The patients seem to fall into 2 categories with regard to urinary copper. About a third of them excreted 2-4 times more copper in the urine, while in the others the excretion is comparable to normal subjects. It appears possible that there exists in India a genetic isolate of retinitis pigmentosa with altered copper metabolism. The distribution of these patients may be different between the northern and southern parts of the country.

Retinitis pigmentosa (tapetoretinal degeneration or retinal abiotrophy) is a disease whose aetiology is not yet known. Retinal degeneration occurs secondarily in such disorders like abetalipoproteinaemia, Refsum's disease, and others. Rocha and Antunes¹ suggested that primary retinitis pigmentosa may be an autoimmune disease. Gouras and Chader² proposed a possible role for retinol binding protein in retinitis pigmentosa. Gahlot *et al.*³ suggested that the primary defect in retinitis pigmentosa is in copper metabolism. They reported decreased serum ceruloplasmin and increased urinary copper excretion in every patient they examined.^{3,4} Investigators in the United States⁵ and Denmark⁶ who have conducted similar studies of copper metabolism on retinitis pigmentosa patients found no abnormalities in copper metabolism similar to those found by Gahlot *et al.* Discussing their results, Marmor *et al.*⁵ considered it possible that Gahlot *et al.* from India were studying a genetic isolate of retinitis pigmentosa patients in whom copper metabolism is altered. We considered it worth while to investigate copper metabolism in retinitis pigmentosa patients, and we report here that about a third of all patients we investigated showed abnormalities of copper metabolism of the nature described by Gahlot *et al.*

Subjects and methods

The patients in this study visited the Retinal Services Section of Sarojini Devi Eye Hospital between 19 March and 20 July 1980. To avoid bias every patient

diagnosed as suffering from primary retinitis pigmentosa was included in this study. They were all informed of the nature of the study. The normal subjects were the surgeons at Sarojini Devi Eye Hospital and the staff of the Institute of Genetics and their family members.

After the diagnosis had been made and it was established that the patients suffered from no other disease the following 3 parameters were measured on these patients: (1) serum copper was measured by the method of Giorgio *et al.*⁷; (2) ceruloplasmin concentration in serum was measured from the rate of oxidation of *p*-phenylenediamine as described by Henry *et al.*⁸; (3) copper content in 24 h urine samples was measured by the method of Giorgio *et al.*⁷ The same parameters were measured in normal subjects by the same methods.

Results

Serum copper and ceruloplasmin concentrations and urinary excretion of copper for 13 normal subjects are shown in Table 1. The values for 24 patients are shown in Table 2. (SI conversion: ceruloplasmin mg/l = mg/100 ml $\times$ 10; serum copper μ mol/l = μ g/100 ml $\times$ 0.157; urinary copper μ mol/24 h = μ g/24 h $\times$ 0.0157.)

Serum copper in normal subjects varied from 67.5 to 150.0 μ g per 100 ml. The mean value was 102.4 μ g. Values in the range of 64 to 143 μ g per 100 ml for normal subjects were reported by Marmor *et al.*⁵ and in the range of 60 to 150 μ g per 100 ml by Gahlot.⁴ The serum copper content of normal subjects in this study is comparable with values reported by these authors. In the patients the serum

Correspondence to Dr A. Sitaramayya, University of Pennsylvania School of Medicine, Department of Anaomy G3, Philadelphia, PA 19174, USA.

Table 1 Parameters of copper metabolism in control subjects

Serial number	Sex	Age (years)	Serum copper μg per 100 ml	Serum ceruloplasmin, mg per 100 ml	Urine copper in 24 hours sample, μg
1	M	25	78.7	19.80	37.7
2	M	34	105.0	21.48	25.5
3	M	32	150.0	33.50	21.7
4	M	34	120.0	28.20	43.4
5	M	35	135.0	29.76	38.0
6	M	52	90.0	69.00	40.4
7	M	40	150.0	44.28	60.2
8	F	28	75.0	31.46	26.5
9	F	42	120.0	28.02	46.2
10	M	18	75.0	21.84	29.2
11	M	60	67.5	23.52	42.7
12	F	50	78.7	26.04	23.2
13	M	30	86.2	21.72	29.7

For SI conversions see text.

Table 2 Parameters of copper metabolism in retinitis pigmentosa patients

Serial number	Sex	Age (years)	Serum copper, μg per 100 ml	Serum ceruloplasmin mg per 100 ml	Urine copper in 24 hours sample, μg
1	F	27	198.7	20.58	56.0
2	M	15	206.2	18.12	47.5
3	M	14	99.9	24.60	31.4
4	M	32	83.3	23.52	30.0
5	F	35	92.4	33.36	105.0
6	F	34	103.1	19.50	165.9
7	F	20	125.0	18.00	149.6
8	F	18	150.0	15.54	16.6
9	F	14	172.5	19.50	168.5
10	M	28	150.0	12.12	180.5
11	F	20	187.5	15.60	146.3
12	M	20	112.5	18.18	47.9
13	M	18	112.5	24.72	25.6
14	F	19	75.0	25.74	40.6
15	M	25	93.7	12.66	42.0
16	F	12	105.0	33.00	36.9
17	M	13	90.0	30.50	34.5
18	F	16	120.0	27.80	20.0
19	M	22	112.5	20.52	20.5
20	M	17	135.0	22.50	97.5
21	M	16	105.0	30.72	30.5
22	M	38	120.0	34.98	175.5
23	M	28	75.0	26.10	27.4
24	M	15	75.0	30.96	53.2

For SI conversions see text.

copper varied from 75 to 206.2 μg per 100 ml, with a mean value of 135.7 μg . This value is considerably higher than the mean value for normal subjects and differs from that of Marmor *et al.*,⁵ who found no major difference between control subjects and patients, and also from that of Gahlot,⁴ who found that the patients showed lower serum copper content compared with normal subjects.

The serum ceruloplasmin content, measured by the diamine oxidase activity, ranged from 19.8 to 69.0 mg per 100 ml with a mean value of 30.66 mg. The ceruloplasmin concentration in the patients is lower, with a mean value of 22.81 mg per 100 ml and a range of 12.12 to 34.98 mg. The mean value for ceruloplasmin in control subjects in the study of Marmor *et al.*⁵ was 32.3 and is close to the observation in this study. However, in their study the mean value in patients was higher at 37.0 mg per 100 ml. Though the mean value for normal subjects reported by Gahlot *et al.*³ is much lower than in our observations, that the patients showed lower ceruloplasmin concentrations than normal subjects observed in this study is in agreement with the results of Gahlot *et al.*³ and Gahlot.⁴

The urinary excretion of copper in 24 hours in normal subjects ranged from 21.7 to 60.2 μg with a mean value of 35.7 μg . In the patients the range was 16.6 μg to 180.5 μg , with a mean value of 79.5, which is more than twice the average for normal subjects. The values for normal subjects in our study are in agreement with those reported by Gahlot⁴ and Marmor *et al.*⁵ However, while Marmor *et al.* found that patients excreted less than 50 μg of copper in 24 hours, as did the normal subjects, Gahlot *et al.*³ and Gahlot⁴ reported that the urinary excretion of copper in patients was elevated to about 200 μg per 24 hours. One striking difference between the present study and that of Gahlot is that, while only 8 out of 24 patients in our study showed urinary excretion of copper above 50 μg in 24 hours, the latter reported that each one of their patients showed increased copper excretion in the urine. When we isolate these 8 cases in our study (patients 5-7, 9-11, 20, and 22), their urinary copper excretion ranged from 97.5 to 180.5 μg /24 hours, with a mean value of 148.6 μg (Table 3). This value is close to the report of Gahlot. Also, when these 8 are excluded from the rest of the patients, the urinary excretion for these patients averaged 40.0 μg and is comparable with that for control subjects. The 8 patients with increased urinary copper excretion and the rest of the patients had comparable ceruloplasmin concentrations (Table 3). The serum copper was raised in the rest of the patients but not to the same level as in the 8 patients with increased urinary copper excretion (Table 3).

Table 3 Comparison of patients with raised urinary copper excretion with other patients and normal subjects

Subjects	Serum copper, μg per 100 ml	Serum ceruloplasmin, mg per 100 ml	Copper in 24 hours urine, μg
Control	102.4	30.66	35.7
Patients with elevated urinary copper excretion	135.7	21.94	148.6
Other patients	118.9	23.30	40.0

Data presented here are mean values. For SI conversions see text.

Discussion

From our observations it is evident that the serum copper levels in patients and normal subjects are not strikingly different, though the mean value for patients was higher than that for control subjects. However, the urinary excretion of copper is increased in 8 out of the 24 patients to about 4 times that of the control subjects, while in the rest of the patients the excretion is comparable to that of the control subjects. The mean value for serum ceruloplasmin levels in our control subjects agrees with the values reported,^{9,10} and individually each one of them fell in the reported range for normal subjects. The ceruloplasmin content in patients as a group is much lower than in controls, and 7 patients have ceruloplasmin content below the normal range. Three of these 7 are in the group excreting higher copper in urine.

The reports of Gahlot *et al.*³ and Gahlot⁴ that each one of the retinitis pigmentosa patients they examined showed increased excretion of copper in urine and showed decreased ceruloplasmin in serum and the report of Marmor *et al.*⁵ and Ehlers and Bulow⁶ that their patients showed no abnormal values of copper metabolism distinguishable from the normal subjects show the extent of controversy on this subject. However, the suggestion of Marmor *et al.*⁵ that there could be a genetic isolate of retinitis pigmentosa patients in India who either have a deranged copper metabolism or show such derangement under the influence of some external factors deserves serious consideration. The present observations reveal that 8 out of 24 patients showed signs of altered copper metabolism. It is hard to explain the difference between our results and those of Gahlot *et al.*³ Of the 24 cases in our study 5 were Muslims, 17 were Hindus, and 2 were Christians. Of the 8 cases with increased urinary copper excretion 2 were Muslims and 6 were Hindus. From these observations it is clear that retinitis pigmentosa with altered copper metabolism is not confined to any particular religious group (though we did have

only two Christian patients). Regionally, 23 of our 24 patients were from the southern state of Andhra Pradesh (AP) and 1 (patient 5) was from Uttar Pradesh (UP) in North India. Seven of the patients from AP and the patient from UP showed elevated copper excretion in urine. It is interesting that our only patient from the northern part of India had an increased excretion of urinary copper, and it is noteworthy that only about a third of our patients showed altered copper metabolism in contrast to every patient in the study of Gahlot from northern India.⁴ If we consider that there is a genetic isolate of retinitis pigmentosa patients in India, this group constituted only a third of the population in the southern part of this country. More studies from the north as well as south will be useful in determining this distribution.

The dietary copper is absorbed from the intestine and transported to the liver mainly bound to albumin. There copper is incorporated into ceruloplasmin and released into the blood stream. The ceruloplasmin-bound copper represents about 90% of the total serum copper. Urinary excretion of copper is minimal, while the major mode of excretion is through faeces. However, in Wilson's disease, where copper accumulates in tissues, the ceruloplasmin content of serum drops to below 10 mg per 100 ml in about 80% of the patients, and the urinary copper excretion is often as high as 1000 μg or more per day.¹¹ Wilson's disease is treated with decoppering agents like D-penicillamine. Gahlot⁴ reported treatment of retinitis pigmentosa patients with a decoppering agent and low copper diet and suggested that both acuity and fields of vision improved after a few months of treatment. We feel that the decrease in ceruloplasmin concentration and the rise in urinary copper in the 8 patients we isolated are not comparable in severity to those of Wilson's disease to warrant treatment with decoppering agents. We are advising low copper diet therapy to these 8 patients to see if that alone brings about the desired improvements.

The authors thank the staff at the Institute of Genetics and Sarojini Devi Eye Hospital for their excellent co-operation extended during this study. The Department of Science and Technology, Government of India, provided generous funds to the Institute of Genetics. We thank Drs P. Venkateswar Rao, O. S. Reddi, C. S. R. Sharma, and P. Siva Reddy for discussions.

References

- 1 Rocha H, Antunes L. Primary retinitis pigmentosa—autoimmunity? *Metabol Ophthalmol* 1977; 1: 153–6.
- 2 Gouras P, Chadar G. Retinitis pigmentosa and retinol binding protein. *Invest Ophthalmol Visual Sci* 1974; 13: 239–42.
- 3 Gahlot DK, Khosla PK, Makashir PD, Vasuki K,

- Basu N. Copper metabolism in retinitis pigmentosa. *Br J Ophthalmol* 1976; **60**: 770-4.
- 4 Gahlot DK. Retinitis pigmentosa—a state of copper toxicity? *Indian J Ophthalmol* 1979; **27**: 170-3.
- 5 Marmor MF, Nelson JW, Levin AS. Copper metabolism in American retinitis pigmentosa patients. *Br J Ophthalmol* 1978; **62**: 168-71.
- 6 Ehlers N, Bulow N. Clinical copper metabolism parameters in patients with retinitis pigmentosa and tapeto-retinal degenerations. *Br J Ophthalmol* 1977; **61**: 595-6.
- 7 Giorgio AJ, Cartwright GE, Wintrobe MM. Determination of urinary copper by means of direct extraction with zinc dibenzylthiocarbamate. *Am J Clin Pathol* 1964; **41**: 22-6.
- 8 Henry RJ, Chiamori N, Jacobs SL, Segalove M. Determination of ceruloplasmin oxidase in serum. *Proc Soc Exp Biol Med* 1960; **104**: 620-4.
- 9 Lewis KO. The nature and excretion of biliary copper in man. *Clin Sci* 1973; **45**: 133.
- 10 Gollan JL. Reply to Lewis, 1973. *Clin Sci* 1973; **45**: 133-4.
- 11 Sass-Kortsak A, Bearn AG. Hereditary disorders of copper metabolism. In: Stanbury JB, Wyngaarden JB, Fredrickson DS, eds. *The Metabolic Basis of Inherited Disease*. New York: McGraw-Hill, 1978: 1098-126.