Macular degeneration associated with age and drusen, an important cause of visual loss, is associated clinically with alterations in the retinal pigmented epithelium. Because the pigmented epithelium is a copper-rich tissue with antioxidant properties, the copper economy in patients and controls were studied by measuring ceruloplasmin. Ceruloplasmin, a multifunctional, copper-binding α-globulin, was significantly elevated in non-related patients as compared with controls (691 ± 153 mg/L vs 312 ± 64; \( P < .001 \)), both by the p-phenylenediamine oxidation technique and radial immunodiffusion assay. When 53 members of a large family were divided clinically into persons with and without macular degeneration, the ceruloplasmin concentrations were not significantly different from each other, but were elevated as compared with non-related controls (\( P < .001 \)). These differences were not due to an intragroup age mismatch. A group of patients with retinitis pigmentosa had normal serum ceruloplasmin concentrations.

This study suggests a relationship between serum ceruloplasmin, trace metals, and the tissue alterations associated with macular degeneration that deserves further investigation. Invest Ophthalmol Vis Sci 27:1675-1680, 1986

Macular degeneration associated with age and drusen is a leading cause of visual loss among aged individuals in the United States and western Europe. The etiology of macular degeneration is unknown, although phototoxicity and oxidative free radicals may be of importance. Based on clinical observations, the retinal pigmented epithelium (RPE) is significantly involved in the disease process. The RPE has a variety of specialized functions, including antioxidant activity, and many of its enzymes are copper-dependent. In addition, the RPE has one of the highest copper concentrations in the body. Thus, a better knowledge of factors relating to copper balance could be important to furthering our understanding of macular degeneration.

Ceruloplasmin is a multifunctional, copper-binding α-globulin that appears to be involved in the regulation of copper utilization by enzymatically mediating the transfer of copper to copper-containing enzymes in various parts of the body. Ceruloplasmin also has antioxidant capabilities, due to the discharge of electrons, especially during the course of its catalytic activity in converting Fe(II) to Fe(III). These electrons can be used to reduce molecular oxygen directly to H\(_2\)O. Blood concentrations of ceruloplasmin can be influenced by nutritional and pathological conditions, resulting either in hypoceruloplasminemia (e.g., malnutrition, Wilson's disease) or hyperceruloplasminemia (e.g., leukemia, rheumatoid arthritis).

The relationship between blood and RPE concentrations of copper and between antioxidant activity in the blood and similar activity in the RPE is not clear. Consequently, alterations in circulating ceruloplasmin could influence both the transport of copper and the functioning of the RPE. For this reason, we compared the serum ceruloplasmin concentrations of normal individuals and those with macular degeneration in a population from northern Utah, as well as those of a group of patients with retinitis pigmentosa and other ocular conditions. Our results show that individuals with macular degeneration have elevated ceruloplasmin.
Materials and Methods

Study subjects were enrolled after obtaining informed consent using documents and procedures that had been approved by the intramural review board. Participants with macular degeneration and their unaffected controls were born, reared, and dwell in the northern Utah area. All subjects were Caucasian. Fifty-three of our subjects were blood relatives distributed among three generations of one family, called here Family A. Detailed genealogic histories were well known for all participants. Thus, we know that our study group does not represent a sample of an inbred population. This study was not population-based; therefore, we do not know what proportion of the area's total macular degeneration population our sample represents. Retinitis pigmentosa (RP) subjects were from various regions of the continental U.S. After informed consent was obtained, a detailed history of ocular and general medical status, medications, family lineage, and diseases were elicited by a combination of interview and questionnaire techniques. All participants received thorough ocular examinations. Corrected Snellen acuity at 20 feet, and near acuity with Jaeger type, were determined. Anterior-segment examination, Schiotz intraocular-pressure determinations, and dilated direct and indirect funduscopy were also performed. Fundus appearance was recorded via a portable (Kowa Optimed, Torrance, CA) or stationary (Carl Zeiss Co., Thornwood, NY) fundus camera on Kodacolor (Eastman Kodak, Rochester, NY) ASA 64 or 25 film, respectively. Subjects with findings requiring immediate attention were so informed, and referred to their personal ophthalmologists.

Affected Groups

The diagnosis of age-related maculopathy was made if drusen and pigmentary changes were present and the best corrected visual acuity was 20/25 or worse with no other apparent explanation. The presence of drusen and pigmentary changes greater than those in a standard photograph (Fig. 1) was confirmed by an independent experienced reader who reviewed the subjects' photographs in a masked fashion.

Eye diseases other than macular degeneration included macular holes, macular pucker, retinal detachment, progressive myopia, and RP. RP was diagnosed on the basis of a history of night blindness, findings of typical pigmentary changes, including intraretinal pigment migration, depigmentation of the RPE with macular sparing, arteriolar narrowing, vitreous abnormalities, preservation of relatively good central acuity until late in the course of the disease, and the presence of peripheral visual field defects, as assessed by Goldmann perimetry. Both simplex (no affected siblings) and multiplex (one or more affected siblings) cases were included.

Unaffected Control Group

These subjects had no macular degeneration and were not blood relatives of affected subjects. This group included some spouses of affected subjects.

Blood relatives of affected subjects formed the “unaffected relatives” control group. Macular findings in this group were less than the standard photograph. The remainder of the examination, with the exception of lens changes in some subjects, was normal.
samples with a visible precipitate were not used, since after an additional 30 min incubation at 37°. The ab-
solution. Reaction mixtures were terminated with azide
terminated by the addition of 50 μl 1.5 M Na azide
sorbed the reaction mixtures and corresponding
blanks was determined immediately at a wavelength
of 530 nm in a Beckman (Beckman Instruments, Inc.,
Irvine, CA) DU-8 recording spectrophotometer using a
cuvette of 1 cm optical-path length. Serum cerulo-
plasmin concentration was determined by comparison
of the blank-corrected absorbance values to the stan-
dard curve, and the data expressed as mg/l.
Ceruloplasmin concentrations also were determined
for all control subjects (normals, other macular and
retinal, and RP), and some of the affected subjects by a
commercial radial immunodiffusion technique (Bio-
science Laboratories, Columbia, MD). This assay does
differentiate between the holo- and apo-cerulo-
plasmin, whereas the oxidase assay detects only holo-
ceruloplasmin.
For comparison, determinations were made of the
centration of transferrin, a plasma protein capable
of binding iron, copper, and zinc. These determinations
were made by a commercial laboratory (Bioscience
Laboratories) using a single radial immunodiffusion
technique with a 200–400 mg/dl reference range.
Liver-function studies were also performed on aliquot
portions of the sera used for ceruloplasmin de-
terminations. Both alanine and aspartate aminotrans-erase (SGPT and SGOT) activities were determined
by a commercial laboratory (Bioscience) using a stan-
dard spectrophotometric technique to measure the
conversion of NADH to NAD at 320 nm. Three per-
sions with values above 35 IU (at 37°) out of a total of
149 were not included in this study, because elevated
levels of these enzymes may indicate conditions asso-
ciated with altered levels of ceruloplasmin.

Results

Clinical Observations

Macular changes in affected individuals ranged from
sparse drusen and pigmented changes to old disciform
scars. We encountered drusen that varied both in size
and apparent consistency from soft to crystalline. Of
the legally blind eyes with macular degeneration, 71%
(26/36) had exudative lesions, and 29% had areolar atrophy.

The non-Family A affected group was generally older than the non-Family A unaffected group. Of the former group, 65% were age 60 yr or older, while only 33% of the latter were that old (Table 1). Affected and unaffected members of Family A had similar age distributions (Table 1).

**Associated Diseases**

None of the individuals in the study had conditions known to be associated with reduced ceruloplasmin (e.g., malnutrition, Wilson's disease). Small, similar numbers in affected and unaffected groups had conditions that might be associated with ceruloplasmin elevation (see Discussion), such as osteoarthritis (12 of 68 affected; 8 of 39 unaffected) or history of thyroid disease (5 of 68 affected; 2 of 39 unaffected).

**Table 2.** Comparison of mean serum transferrin concentrations

<table>
<thead>
<tr>
<th>Group</th>
<th>Number of Persons</th>
<th>Mean ± S.D.</th>
<th>Range</th>
<th>P-Value of Difference With Controls</th>
</tr>
</thead>
<tbody>
<tr>
<td>Unaffected, non-Family A</td>
<td>24</td>
<td>276 ± 30</td>
<td>180-344</td>
<td>—</td>
</tr>
<tr>
<td>All affected</td>
<td>68</td>
<td>278 ± 60</td>
<td>151-368</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>Affected</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Family A only</td>
<td>35</td>
<td>270 ± 44</td>
<td>180-358</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>Unaffected Family A only</td>
<td>18</td>
<td>296 ± 30</td>
<td>180-356</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>Other eye diseases</td>
<td>10</td>
<td>278 ± 65</td>
<td>176-351</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>RP</td>
<td>16</td>
<td>288 ± 30</td>
<td>230-388</td>
<td>&gt;0.05</td>
</tr>
</tbody>
</table>

**Ceruloplasmin and Transferrin**

A wide range of serum ceruloplasmin concentrations was found for both macular degeneration and control groups when all study participants were analyzed together (Fig. 2). In general, ceruloplasmin concentrations were higher for macular degeneration (μ = 690 mg/l; σ = 154 mg/l) than for the unaffected controls (μ = 628 mg/l; σ = 103 mg/l). This difference is marginally significant (t = 1.99; df = 96; P < 0.05). Since ceruloplasmin levels do not correlate with age for the macular degeneration group (r = 0.19), the control group (r = 0.0002), or for the two groups combined (r = 0.21), we conclude that the observed difference in ceruloplasmin concentrations between groups was not due to the age mismatch.

An interesting observation relating to the unaffected group was the apparent bimodal distribution of ceruloplasmin levels. It is possible that there could be a genetic component to this variation, since approximately one-half of the unaffected group (n = 18) and one-half of the macular degeneration group (n = 34) were blood relatives (Family A). When data for Family A members and non-Family A members were analyzed separately, several points were apparent (Fig. 2). First, the bimodal distribution seen when all unaffected controls were analyzed is now segregated into two groups, with Family A unaffected members having high ceruloplasmin and non-Family A unaffected controls having ceruloplasmin within normal limits. Second, the differences in ceruloplasmin levels between non-Family A macular degeneration and control groups is quite striking (641 ± 153 mg/l for macular degeneration vs 312 ± 64 mg/l for control) (Fig. 1, Table 1). Third, ceruloplasmin levels in both macular degeneration groups were high, and did not split into two groups, as did unaffected individuals. Fourth, there is
no apparent difference in ceruloplasmin levels between the Family A macular degeneration group (641 ± 143 mg/l) and the Family A unaffected group (655 ± 87 mg/l) (Table 2), of which approximately 35% had drusen, but not to the degree that would allow for classification as macular degeneration (Fig. 2, Table 1). However, it should be noted that, in comparing the two groups of Family A subjects to the two groups of non-Family A subjects, we find, from an analysis of variance, that the concentration differences are significant (F[3, 94] = 4.2, P < 0.01).

Although some individual variability with time was seen, persons with an elevated ceruloplasmin concentration initially, or a normal concentration initially, tended to maintain that level (Fig. 3). Elevated ceruloplasmin in macular degeneration may be specific for this condition, since patients with a variety of other ocular conditions, including RP, had normal ceruloplasmin levels (Fig. 2, Table 1). All controls (including RP subjects) and about 10% of the macular degeneration subjects also had serum ceruloplasmin concentration determined by quantitative radial immunodiffusion assay. With rare exception, the immune-determined values were within 10% of the quantities based on oxidase activity (not shown).

To identify the source of significance, multiple t-tests were performed. Since six comparisons were made, the t values must occur at P < .01 to be regarded as significant. Using this criterion, both Family A and non-Family A—solid circles; (2) unaffected, non-Family A—open circles; (3) Family A, macular degeneration—solid triangles; and (4) Family A, unaffected—open triangles.

Ceruloplasmin concentrations were variable (coefficient of variation ranges from 13–22%) within each of our groups. The degree of variability is consistent with other reported series.9,10,15,21 However, the actual concentrations in most macular degeneration subjects in this study were higher than reported values for normal. Preliminary results on macular degeneration patients from the mid-Atlantic region have also shown significantly elevated ceruloplasmin in comparison with controls living in the same region (data not shown). The absence in both studies of elevated transferrin, another liver-synthesized transport protein, supports the notion that ceruloplasmin changes may be suggestive of a specific relationship to macular degeneration, while not necessarily being diagnostic.

In contrast to the data obtained from unrelated normal and affected individuals, the results of the family study showed that the unaffected, as well as the affected, members of the large family had elevated ceruloplasmin levels. The group elevation was significant when compared to that of unaffected, unrelated controls from the same area, as well as to published “normal” values (female 170–360 mg/l; male 160–320 mg/l).9,10,15,21 This is, to our knowledge, the first report of a familial macular degeneration-related occurrence of elevated ceruloplasmin. The reason for the apparent discrepancy between the two groups of data is not clear. It is possible that there is one form of macular degeneration that is a genetic predisposition related to altered ceruloplasmin, and that those unaffected individuals with ele-
vated ceruloplasmin levels are responding to a chronic stimulus which will ultimately manifest itself as clinically recognizable, age-related maculopathy. This seems possible, because at least some of these individuals (35% of unaffected Family A members) showed subthreshold evidence of macular change. Alternatively, this finding could be a covariant with no causal relationship. Following these unaffected family members as they age will be extremely important in determining if they were predisposed to this condition at a younger age.

Normally, there is a correlation between serum copper and ceruloplasmin levels. For example, in individuals with rheumatoid arthritis and in women taking estrogen, there is a co-elevation of both. However, preliminary data indicating normal copper concentrations were not elevated, even in the presence of elevated ceruloplasmin. Copper-free ceruloplasmin is not known to have oxidase activity. However, since we measured the amount of ceruloplasmin based on this activity, these patients presumably have increased serum antioxidant capacity. The relative amounts of ceruloplasmin in affected and unaffected individuals are confirmed by the immunological assay, which is not dependent on either copper content or oxidase activity. Therefore, the elevated concentrations of ceruloplasmin are real, and do not merely reflect anomalously increased oxidase-specific activity. However, preliminary data indicating normal serum copper concentrations in affected individuals raise some interesting questions regarding the molecular heterogeneity of ceruloplasmin and the binding capacities for copper and other cations in our subjects. Investigations to study some of these possibilities are currently underway.

Key words: macular degeneration, drusen, serum ceruloplasmin, serum copper, retinal pigment epithelium, blood antioxidant activity

Acknowledgments

We gratefully acknowledge the invaluable assistance of Mrs. Florence Butler in subject recruitment and scheduling, of Marge Hoffman, RN, in conducting field clinics, of Dr. John W. Carlisle for making facilities available for field clinics, of Dr. Legrand Shupe for continued support and assistance, of Elsa Pina in performing some ceruloplasmin determinations, and of Dr. Robert Masoff in discussing the manuscript.

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