*ACTA MED PORT 2: 107, 1980*

# SERUM COPPER, CERULOPLASMIN AND MONOAMINE OXIDASE IN DIABETICS WITH DIFFERENT TIMES OF EVOLUTION OF THE DISEASE *

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SUMMARY

In 103 diabetics simultaneous measurements of serum copper and ceruloplasmin were made, together with the determination of glucose, cholesterol and monoamine oxidase. These diabetics were divided in two groups: 53 had a diagnosis made less than two years ago, and 50 had diabetes diagnosed for more than 10 years. A control group consisted in 50 normal individuals. Copper and ceruloplasmin of diabetic sera are more elevated than in normal individuals. This elevation is statistically significant. There is a positive correlation between copper and ceruloplasmin, both in normal individuals and in diabetics. Serum copper is more elevated in diabetics with less than two years of evolution than in diabetics with more than 10 years of evolution. The opposite happens with serum monoamine oxidase. Serum cholesterol is not significantly elevated in diabetics.

In a previous paper [1] it was demonstrated that serum monoamine oxidase (sMAO) was elevated in diabetic patients. This elevation was more marked in patients whose diabetes lasted for more than 10 years, when compared to patients with less than two years of duration of the disease. Besides, in long standing diabetes, there was a positive correlation between sMAO and glycemia.

In the present study we compare the values of serum copper (Cu) and ceruloplasmin (CPL) in both groups of diabetics. Serum cholesterol and glycemia were also determined, as accessory parameters.

## MATERIAL AND METHODS

A total of 103 patients under follow up in the Diabetes Clinic of Hospital de Santa Maria were used in this study. They were divided in two groups. Group I included 53 diabetics (24 male and 29 female) with less than two years of duration of the disease. Group II included 50 diabetics (25 male and 25 female) with more than 10 years of disease. As a control we used 50 normal individuals (22 male and 28 female) of approximately the same age range.

Patients were examined and blood was drawn by venipuncture. In each sample of blood the following determinations were done: glycemia, cholesterolemia, sMAO, Cu and CPL. The results obtained for sMAO were presented previously.[1]

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* Supported by Instituto Nacional de Investigação Científica.

Received: *28 August 1979*

Serum Cu was determined by the spectrophotometric micromethod of Stark and Dawson.[2] The results are expressed in micrograms/dl of serum.

Serum CPL activity was determined by the method of Ravin [3] using p-phenylenediamine as substract and following its oxidations in a spectrophotometer ,for 10 min. at 525 nm., and 37° The results are converted in mg CPL/dl, by comparison with pure CPL type III, obtained from Sygma Chemical Co.

Glycemia was determined by the glucose oxidase method. Total cholesterol was determined by the catalase method. Kits from Sigma and from Boehringer-Mannheim were used respectively for these two determinations.

## RESULTS

Inspection of Tables 1 and 2 brings the following conclusions:

1 — Glycemia is more elevated in diabetics than in normals, as expected. The slight difference between the two groups of diabetics is not significant.

2 — Serum cholesterol is slightly more elevated in diabetics, although this elevation has no statistical significance.

3 — sMAO shows a continuous increase in activity from normals to short term diabetics, and from these to long term diabetics. The last group shows a positive correlation with glycemia, as described previously. [1]

4 — Serum Cu is definitely elevated in both groups of diabetics, in comparison with normals. Besides, it is more elevated in short term diabetes, than in long term diabetes, in opposition to sMAO.

Table 1

*Mean ($\overline{X}$) and Standard deviation (s) for normals, diabetics < 2 years, diabetics > 10 years*

| Parameters | | Normals | Diabetics <2 years | Diabetics > 10 years |
|---|---|---|---|---|
| Number cases | | 28 F/22 M | 29 F/24 M | 25 F/25 M |
| Glycemia (mg/dl) | $\overline{X}$ | 73.14 | 194.387 | 188.362 |
| | s | 13.542 | 102.435 | 81.696 |
| Cholesterol (mg/dl) | $\overline{X}$ | 171.569 | 180.579 | 182.813 |
| | s | 39.846 | 42.429 | 42.669 |
| MAO (u. McEwen) | $\overline{X}$ | 23.804 | 43.298 | 52.590 |
| | s | 9.445 | 17.088 | 27.568 |
| $Cu^{++}$ (µg/dl) | $\overline{X}$ | 134.406 | 180.603 | 157.590 |
| | s | 37.296 | 52.530 | 46.367 |
| CPL (mg/dl) | $\overline{X}$ | 16.693 | 24.968 | 20.778 |
| | s | 4.788 | 7.998 | 6.214 |

F — females; M — males

Table 2

*Comparison between the results obtained in normal individuals, diabetics < 2 years, diabetics > 10 years by the Students test*

| Parameters | Normals/diab <2y | | Normals diab > 10y | | Diab <2y/diab> 10y | |
|---|---|---|---|---|---|---|
| | t | p | t | p | t | p |
| MAO | −7.106 | <0.0005 | −6.978 | <0.0005 | −2.064 | <0.025 |
| $Cu^{++}$ | −5.084 | <0.0005 | −2.615 | <0.01 | 2.198 | <0.025 |
| CPL | −6.126 | <0.0005 | −3.517 | <0.0005 | 2.857 | <0.005 |
| Glycemia | −8.299 | <0.0005 | −9.180 | <0.0005 | 0.327 | n. s. |
| Cholesterol | −1.097 | n. s. | −1.337 | n. s. | −0.240 | n. s. |

5 — CPL follows the same pattern as Cu. Besides, looking at Table 3, it is noticed that there is always a positive statistically, significant correlation, betwenn both of them.

Table 3

*Correlation $Cu^{++}$/CPL*

| | r | p |
|---|---|---|
| Normals | 0.351 | <0.02 |
| Diabetics < 2y | 0.637 | <0.01 |
| Diabetics > 10y | 0.472 | <0.01 |

In Tables 4 and 5 the results for serum copper are divided by sexes.

Table 4

*Comparison of the results obtained for serum copper in normals, short term diabetics and long term diabetics, when divided by sexes*

| Sex | Classification | N | $\bar{X}$ | S |
|---|---|---|---|---|
| Males | Normal | 22 | 120.495 | 38.389 |
| | Diabetes < 2y | 24 | 171.592 | 51.222 |
| | Diabetes> 10y | 25 | 159.572 | 35.725 |
| Females | Normal | 28 | 145.579 | 34.484 |
| | Diabetes < 2y | 29 | 188.062 | 53.313 |
| | Diabetes> 10y | 25 | 156.009 | 54.339 |

N=number of cases; $\bar{X}$=mean; s=standard deviation

Table 5

*Results of the Students test for the above values*

| Groups compared | t | p |
|---|---|---|
| Normal M/normal F | −2.370 | <0.025 |
| Diab M <2y/diab F <2y | −1.139 | n. s. |
| Diab M > 10y/diab F > 10y | 0.240 | n. s. |
| Normal M/diab M < 2y | −3.600 | <0.0005 |
| Normal M/diab M >10y | −3.237 | <0.005 |
| Diab M <2y/diab M> 10y | 0.851 | n. s. |
| Normal F/diab F <2y | −3.558 | <0.0005 |
| Normal F/diab F > 10y | −0.827 | n. s. |
| Diab F <2y/diab F> 10y | 2.109 | <0.025 |

We notice a statistically significant difference in serum copper in normal individuals, according to sex, but not when corresponding groups of diabetics are compared.

In the group of male individuls there is a highly significant difference between normals and either group of diabetics, but the comparison between short term and long term diabetics has no significance.

In the group of females, also the difference between normals and short term diabetics is highly significant, although not between normal and long term diabetics. But the two groups of diabetics show a moderate degree of significance in their difference.

## DISCUSSION

Ceruloplasmin has been for long time a protein of dubious function. A role as ferroxidase was attributed to it [4] among others, such as the transport of copper [5] and the neutralization of superoxide ions.[6] Besides, CPL is an acute phase reactant.[7]

The importance of serum copper variations is still unclearly defined. An ambiguous role in inflammatory diseases has been appointed to this metal, varying, according to Whitehouse,[8] form inert to pharmacologically active, or toxic.[8] It has been described as elevated in rheumatoid arthritis, together with CPL, with a possible protective antioxidant activity[9] although, in counterpart, animals deficient in copper are more resistant to the inducement of adjuvant arthritis.[10] Copper is involved in several metabolic pathways, related to inflammation, such as prostaglandin synthesis and biosynthesis of collagen and elastin.[11]

Copper complexes exert an efficient action in the scavenging of noxious oxygen radicals.[12] Serum copper is also elevated in smokers.[13] Finally, there is a sex difference, females having higher values than males.[14]

In what concerns diabetics, two contradictory results are described. Mateo [15] described a statistically significant elevation of serum copper, CPL and zinc, in a diabetic

group, whereas Pidduck [16] showed no significant difference between serum copper of normals and diabetics.

As copper seems to have an important role in the mechanism of glucose uptake, by oxidating thiols into disulfides [17-19] a new study, with a larger number of patients was justified.

The selection of diabetics with less than two years of evolution and with more than ten years was intented to try to obtain a sharp demarcation in the values of the two groups that could allow a statistically significant difference instead of a continuous variation, that could be expected, if patients with the intermediate duration were included.

The demonstration of a different behavior of serum copper and CPL in diabetics, according to the duration of disease and the inverse relation with sMAO, seems well established in our study, at least when larger groups, including both sexes, are analyzed. It seems that serum copper and CPL are elevated to a higher degree in diabetics of short duration and tend to become less elevated in long standing diabetes, in opposition to sMAO. Some results without significance found in Table 5, are probably due to a small number of samples, when the patients are divided by sexes.

These facts led us to hypothesize that, possibly, there are two different phases in the evolution of diabetes, the first characterized by elevated serum copper may be prodominantly destructive, possibly inflammatory. In the second phase, the destructive process would became less intense, serum copper and CPL would tend to decrease, and, at the same time, sMAO tends to become elevated, and possibly influence the increased synthesis of collagen, underlying the atherosclerotic process of evolution of long standing diabetes.

A differentiation between insulin dependent diabetes (IDD) and NIDD and/or well and badly regulated diabetes in the study of serum Cu and CPL is the obvious future step of this investigation.

Anyhow, the results referred in Table 1, namely a statistically non significant difference between the glycemias in both groups, and the lower standard deviation for the group of longer duration, allow us to speculate that the differences eventually encountered shall not affect the results already verified

## RESUMO

Em 103 diabéticos foram feitos doseamentos simultâneos de cobre e ceruloplasmina do soro, bem como de glucose, colesterol e monoaminoxidase. Estes diabéticos estavam divididos em 2 grupos: 53 tinham o diagnóstico feito há menos de 2 anos e 50 tinham a diabetes diagnosticada há mais de 10 anos. Utilizou-se um grupo controlo de 50 indivíduos normais.

Quer o cobre quer a ceruloplasmina do soro dos diabéticos apresentam valores mais elevados que nos indivíduos normais. Esta elevação é estatisticamente significativa. Há uma correlação positiva entre o cobre e a ceruloplasmina, tanto em indivíduos normais como em diabéticos.

A cuprémia está mais elevada em diabéticos com menos de dois anos de evolução da doença do que em diabéticos com mais de 10 anos de evolução. O contrário se passa com a monoaminoxidase.

O colesterol não está elevado no soro de diabéticos duma forma estatisticamente significativa.

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