

## Carbonic Anhydrase I and II Levels in Erythrocytes of Chronic Renal Disease Patients<sup>\*</sup>

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### ABSTRACT

Carbonic anhydrase isozymes, carbonic anhydrase I and II, also known as carbonate dehydratase, were isolated and purified from human erythrocytes. Rabbits were then immunized and the respective types of antisera were prepared. Using the antisera obtained, the carbonic anhydrase I and II levels in erythrocytes of healthy persons and patients with chronic glomerulonephritis, primary nephrotic syndrome, lupus nephritis and those undergoing chronic hemodialysis were determined by the single radial immunodiffusion method.

Results showed that primary nephrotic syndrome, lupus nephritis and chronic hemodialysis patients had significantly higher carbonic anhydrase I levels than healthy persons, but an increase in carbonic anhydrase I level could not be demonstrated in patients with chronic glomerulonephritis.

The carbonic anhydrase II level in chronic glomerulonephritis, primary nephrotic syndrome, lupus nephritis and chronic hemodialysis patients was significantly higher than that of healthy persons.

Further, in the patients with chronic glomerulonephritis, a significant positive correlation was observed between carbonic anhydrase I and BUN, serum creatinine and total cholesterol levels, while there was a negative correlation between the PSP 15-minute value. Also a significant positive correlation was found between carbonic anhydrase II and total cholesterol levels.

It is concluded that the determination of carbonic anhydrase I and II levels in human erythrocytes are important for the follow-up and evaluation of prognosis in chronic renal disease patients.

### INTRODUCTION

Carbonic anhydrase (CA), also known as carbonate dehydratase (EC 4. 2. 1. 1.), is an enzyme which serves as a catalyzer in the  $\text{CO}_2 + \text{H}_2\text{O} \rightleftharpoons \text{H}_2\text{CO}_3$  reaction and also plays important roles in such functions as exhalation of  $\text{CO}_2$  from the lungs and maintenance of acid-base equilibrium in body fluid. It is present in erythrocytes, kidney, eyeball and gastric mucosa, and consists of 3 types, CA I, CA II and CA III, but only CA I and II have been reported to be present in human erythrocytes.

It is also known that the carbonic anhydrase

level in human erythrocytes differs in various diseases. However, there are no reports on the determination of the levels in chronic renal disease patients except for that of Mondrup et al.<sup>7)</sup> on CA I in uremic patients. Further, the acid-base equilibrium is maintained by the kidney through the action of carbonic anhydrase, and thus variation in the carbonic anhydrase level can be expected in renal disease patients.

We determined the CA I and II levels in chronic renal disease patients and compared their profiles in various renal disease states. This report was prepared on the basis of those findings.

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## MATERIALS AND METHODS

### 1. Materials

The subjects were 24 patients with chronic glomerulonephritis, 13 with primary nephrotic syndrome (8 well-controlled and 5 poorly-controlled) and 5 with lupus nephritis, 13 undergoing chronic hemodialysis and 66 healthy controls. Selected as healthy persons were those without any remarkable past medical history, subjective symptoms or abnormal physical findings, nor any abnormalities on urinalysis, stool, blood tests, chest X-ray and ECG. The breakdown by sex showed 37 males and 29 females whose ages ranged from 16 to 66. Further, the well-controlled nephrotic syndrome group consisted of those in whom proteinuria and hypoproteinemia could not currently be found, while the poorly-controlled group was composed of those in whom proteinuria persisted. Also, the primary disease in all chronic hemodialysis patients was chronic glomerulonephritis, and their residual renal function in terms of creatinine clearance was less than 5 ml/min.

### 2. Methods

#### 1) Preparation of anti-CA I and anti-CA II sera

CA I and II were isolated by affinity chromatography using sulfonamide as the ligand by the method of Osborne et al.<sup>9)</sup>, then purified by DEAE-Sephadex column and used as the antigen.

Anti-CA I and II sera were prepared by immunizing rabbits with 1 mg each of CA I and II, obtained by a modification of the method of Funakoshi et al.<sup>1)</sup>, together with Freund's complete adjuvant.

#### 2) Determination of CA I and II levels

The CA I and II concentrations in hemolysed blood were determined by a single radial immunodiffusion method in which 7 ml of 1.2% agal was mixed with 0.2 ml of anti-CA I or 0.4 ml of anti-CA II serum for the respective isozymes. A standard curve was constructed by plotting diffusion diameters against 3 different concentrations of standard solutions. The concentrations of CA I and II were read from this curve. Hemoglobin concentration was measured by the cyanmethemoglobin method, and the enzyme concentration was expressed in term of mg per gram hemoglobin

(mg/gHb).

## RESULTS

### 1. CA I level (Refer to Fig. 1)

The CA I level of the 66 healthy persons was  $10.7 \pm 1.9$  mg/gHb (mean  $\pm$  standard deviation to apply hereafter). There was no significant difference in the level according to sex, being  $10.7 \pm 1.8$  mg/gHb for males and  $10.7 \pm 1.9$  mg/gHb for females. The levels showed no significant differences by age, with those under 30 showing  $10.5 \pm 2.0$  mg/gHb (n=41), those under 50  $11.3 \pm 1.6$  mg/gHb (n=11) and those over 50  $10.7 \pm 1.7$  mg/gHb (n=14).

In chronic glomerulonephritis patients, the level was  $11.4 \pm 2.4$  mg/gHb, while it was  $12.8 \pm 3.3$  mg/gHb in well-controlled primary nephrotic syndrome patients,  $14.0 \pm 0.9$  mg/gHb in the poorly-controlled,  $15.5 \pm 3.7$  mg/gHb in lupus nephritis patients and  $15.9 \pm 2.5$  mg/gHb in those undergoing chronic hemodialysis.

That is, a significant increase as compared to healthy persons was noted in well-controlled nephrotic syndrome ( $p < 0.01$ ), poorly-controlled nephrotic syndrome ( $p < 0.001$ ), lupus nephritis ( $p < 0.001$ ), and chronic hemodialysis patients ( $p < 0.001$ ).

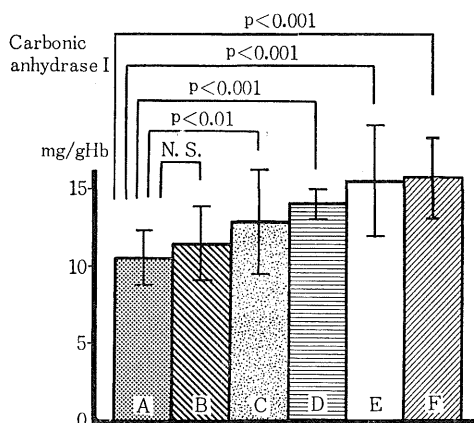


Fig. 1. Carbonic anhydrase I levels in erythrocytes of subjects with chronic renal diseases

- A : healthy
- B : chronic glomerulonephritis
- C : nephrotic syndrome (well-controlled)
- D : nephrotic syndrome (poorly-controlled)
- E : lupus nephritis
- F : chronic hemodialysis

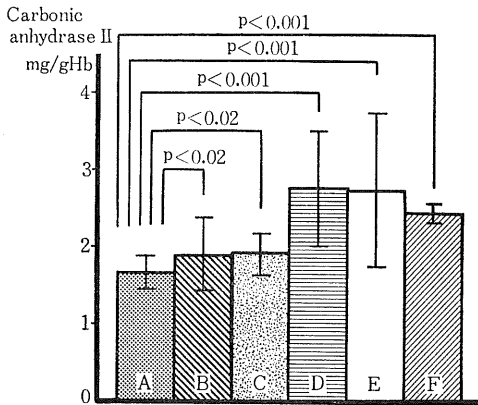
### 2. CA II level (Refer to Fig. 2)

The CA II level of healthy persons was  $1.69 \pm 0.20$  mg/gHb, and by sex was  $1.68 \pm 0.20$

**Table 1.** Chronic Glomerulonephritis Patients by Age, Sex, Laboratory Findings and Medication

| No. | Case | Age | Sex | Carbonic anhydrase I mg/gHb | Carbonic anhydrase II mg/gHb | Degree of proteinuria | Hemoglobin g/dl | BUN mg/dl | Creatinine mg/dl | Total cholesterol mg/dl | PSP (15) % | Creatinine clearance ml/min | Medication    |
|-----|------|-----|-----|-----------------------------|------------------------------|-----------------------|-----------------|-----------|------------------|-------------------------|------------|-----------------------------|---------------|
| 1   | Y.N. | 36  | F   | 10.44                       | 2.30                         | (-)                   | 13.4            | 13.0      | 1.0              | 224                     | 51.0       | 68.0                        | (-)           |
| 2   | H.T. | 20  | F   | 9.36                        | 1.96                         | (+)                   | 12.5            | 18.0      | 1.1              | 245                     | 40.0       | 75.2                        | (-)           |
| 3   | Y.H. | 19  | M   | 7.56                        | 1.90                         | (+)                   | 16.6            | 18.0      | 1.2              | 192                     | 40.5       | 109.0                       | Dipyridamole  |
| 4   | K.Y. | 24  | F   | 9.49                        | 1.47                         | (-)                   | 13.4            | 13.0      | 1.2              | 190                     | 38.4       | 100.0                       | (-)           |
| 5   | K.S. | 31  | M   | 12.56                       | 1.50                         | (±)                   | 14.6            | 20.0      | 1.2              | 202                     | 35.0       | 151.7                       | Dipyridamole  |
| 6   | Y.H. | 46  | F   | 9.76                        | 1.46                         | (±)                   | 12.1            | 26.0      | 1.7              | 178                     | 30.0       | 87.0                        | Dipyridamole  |
| 7   | E.N. | 49  | F   | 8.84                        | 1.83                         | (++)                  | 13.8            | 18.0      | 1.0              | 146                     | 40.0       | 123.0                       | Dipyridamole  |
| 8   | K.N. | 24  | F   | 8.60                        | 1.90                         | (+++)                 | 13.5            | 10.0      | 1.3              | 202                     | 35.5       | 60.7                        | Dipyridamole  |
| 9   | K.I. | 35  | M   | 10.06                       | 1.64                         | (+++)                 | 16.7            | 14.0      | 1.7              | 213                     | 40.0       | 109.0                       | Dipyridamole  |
| 10  | H.U. | 49  | M   | 12.92                       | 2.32                         | (++++)                | 12.6            | 39.0      | 3.0              | 267                     | 5.0        | 15.0                        | α-methyl dopa |
| 11  | T.I. | 45  | M   | 15.36                       | 1.74                         | (+++)                 | 12.9            | 47.0      | 5.2              | 245                     | 5.0        | 13.0                        | α-methyl dopa |
| 12  | K.Y. | 63  | F   | 11.68                       | 1.89                         | (+)                   | 14.3            | 14.0      | 1.3              | 229                     | 29.0       | 41.9                        | α-methyl dopa |
| 13  | Y.F. | 46  | M   | 10.24                       | 2.39                         | (-)                   | 14.8            | 16.0      | 1.8              | 182                     | 25.0       | 54.4                        | (-)           |
| 14  | T.K. | 56  | F   | 8.52                        | 1.97                         | (-)                   | 13.2            | 15.0      | 1.0              | 230                     | 30.0       | 92.0                        | α-methyl dopa |
| 15  | H.N. | 38  | M   | 15.12                       | 3.65                         | (+++)                 | 12.2            | 54.0      | 4.8              | 218                     | 5.0        | 14.2                        | α-methyl dopa |
| 16  | C.M. | 45  | F   | 13.80                       | 2.03                         | (±)                   | 11.5            | 14.0      | 1.4              | 221                     | 20.0       | 51.7                        | Dipyridamole  |
| 17  | H.S. | 37  | M   | 13.24                       | 1.20                         | (+++)                 | 10.8            | 40.0      | 3.9              | 200                     | 15.0       | 52.0                        | Dipyridamole  |
| 18  | I.O. | 66  | M   | 11.72                       | 1.49                         | (++)                  | 14.8            | 30.0      | 2.0              | 205                     | 25.0       | 55.2                        | Dipyridamole  |
| 19  | S.N. | 35  | M   | 16.04                       | 2.32                         | (+)                   | 15.9            | 21.0      | 1.3              | 223                     | 40.0       | 104.0                       | Dipyridamole  |
| 20  | T.H. | 28  | M   | 15.32                       | 1.35                         | (++)                  | 12.4            | 63.0      | 4.7              | 218                     | 7.0        | 25.4                        | α-methyl dopa |
| 21  | T.T. | 40  | M   | 9.80                        | 1.55                         | (++)                  | 15.0            | 29.0      | 2.9              | 192                     | 5.0        | 43.9                        | Dipyridamole  |
| 22  | T.S. | 37  | M   | 11.25                       | 1.64                         | (+)                   | 15.1            | 11.0      | 1.1              | 189                     | 33.0       | 152.0                       | Dipyridamole  |
| 23  | M.Y. | 20  | F   | 10.31                       | 1.80                         | (-)                   | 12.8            | 12.0      | 1.4              | 126                     | 25.0       | 111.0                       | (-)           |
| 24  | S.S. | 32  | M   | 11.68                       | 1.82                         | (±)                   | 12.2            | 26.0      | 2.8              | 144                     | 30.0       | 46.1                        | Dipyridamole  |

Carbonic Anhydrase Levels



**Fig. 2.** Carbonic anhydrase II levels in erythrocytes of subjects with chronic renal diseases

A : healthy

B : chronic glomerulonephritis

C : nephrotic syndrome (well-controlled)

D : nephrotic syndrome (poorly-controlled)

E : lupus nephritis

F : chronic hemodialysis

mg/gHb for males and  $1.70 \pm 0.22$  mg/gHb for females, indicating there was no significant difference between the sexes.

Further, a significant difference by age could not be demonstrated, those under 30 showing levels of  $1.67 \pm 0.20$  mg/gHb ( $n=41$ ), those under 50  $1.80 \pm 0.28$  mg/gHb ( $n=11$ ) and those over 50  $1.67 \pm 0.14$  mg/gHb ( $n=14$ ).

The CA II level was  $1.88 \pm 0.48$  mg/gHb in chronic glomerulonephritis,  $1.90 \pm 0.27$  mg/gHb in well-controlled primary nephrotic syndrome,  $2.75 \pm 0.75$  mg/gHb in the poorly-controlled,  $2.73 \pm 1.00$  mg/gHb in lupus nephritis and  $2.45 \pm 0.29$  mg/gHb in chronic hemodialysis patients.

Thus, a significant increase in CA II level as compared to healthy persons was noted in chronic glomerulonephritis ( $p<0.02$ ), well-controlled nephrotic syndrome ( $p<0.02$ ), poorly-controlled nephrotic syndrome ( $p<0.001$ ), lupus nephritis ( $p<0.001$ ) and chronic hemodialysis patients ( $p<0.001$ ).

### 3. CA I and CA II levels in chronic glomerulonephritis patients and laboratory test results

The CA I level in chronic glomerulonephritis patients failed to show a significant increase when compared to the healthy persons, but as a significant difference was noted between chronic hemodialysis patients, a comparative

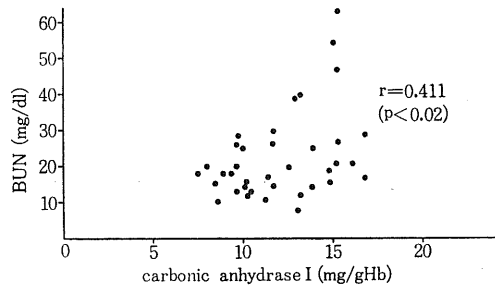
review based on CA I and II levels and laboratory test results, primarily those involved in renal function, was carried out.

Table 1 shows the CA I and II levels of each patient, as well as the degree of proteinuria, hemoglobin concentration, BUN, serum creatinine, total cholesterol, PSP 15-minute value, creatinine clearance value and the medication administered.

#### 1) Relationship between BUN

A significantly positive correlation ( $p<0.02$ ) was observed between BUN and CA I levels with CA I increasing together with BUN (Refer to Fig. 3).

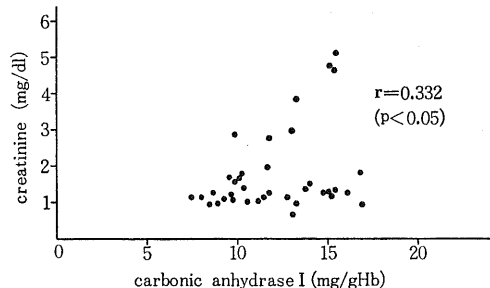
However, such a significant relationship did not exist between CA II.



**Fig. 3.** Correlation between carbonic anhydrase I and BUN in patients with chronic glomerulonephritis

#### 2) Relationship between serum creatinine

As in the case of BUN, a significantly positive relationship ( $p<0.05$ ) was observed between serum creatinine and CA I levels (Refer to Fig. 4), but such was not present between CA II.



**Fig. 4.** Correlation between carbonic anhydrase I and creatinine in patients with chronic glomerulonephritis

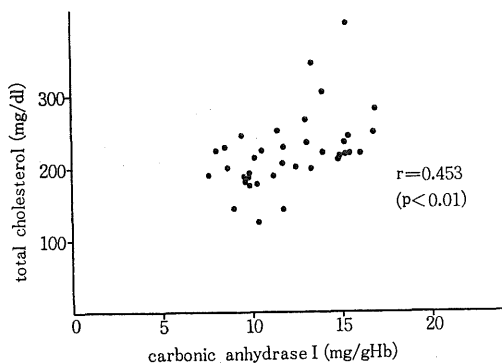
#### 3) Relationship between hemoglobin concentration

A significant relationship between hemo-

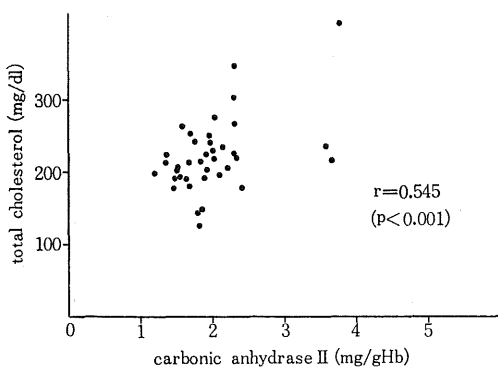
globin concentration and CA I and II levels could not be demonstrated.

4) Relationship between total cholesterol (Refer to Fig. 5, 6.)

A significantly positive correlation was observed between both CA I and II (CA I:  $p < 0.01$ , CA II:  $p < 0.001$ ).

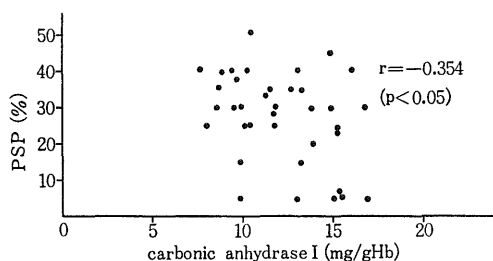


**Fig. 5.** Correlation between carbonic anhydrase I and total cholesterol in patients with chronic glomerulonephritis



**Fig. 6.** Correlation between carbonic anhydrase II and total cholesterol in patients with chronic glomerulonephritis

5) Relationship between PSP (15-minute) value



**Fig. 7.** Correlation between carbonic anhydrase I and PSP in patients with chronic glomerulonephritis

A significantly negative correlation ( $p < 0.05$ ) was noted between PSP and CA I (Refer to Fig. 7), but no such significant correlation was found between CA II.

6) Relationship between creatinine clearance value

A significant correlation could not be observed between creatinine clearance value and CA I and II levels.

## DISCUSSION

The carbonic anhydrase level in human erythrocytes is known to be increased in hypothyroidism<sup>2,6,10,12</sup>, chronic obstructive pulmonary disease<sup>2,3</sup> and anemia<sup>5,8</sup>, and decreased in hyperthyroidism<sup>2,4,6,10,12</sup> and polycythemia<sup>2</sup>. However, reports on this enzyme level in renal disease patients are limited to that on the increase in CA I in uremic patients by Mondrup et al,<sup>7,8</sup> and no other reports on the levels of CA I and II in any other types of renal conditions have been found. Therefore, we carried out a study to determine the CA I and II levels in erythrocytes of patients with kidney ailments such as chronic glomerulonephritis, primary nephrotic syndrome and lupus nephritis, and those undergoing hemodialysis.

Results showed that well- and poorly-controlled primary nephrotic syndrome, lupus nephritis, and chronic hemodialysis patients had significantly higher CA I levels than the controls. Significant increases in CA II level was noted in chronic glomerulonephritis, well- and poorly-controlled primary nephrotic syndrome, lupus nephritis and chronic hemodialysis patients. On the other hand, the CA I level was not significantly increased over the controls in patients with chronic glomerulonephritis, but there was a significant by positive correlation between CA I level and BUN, serum creatinine and total cholesterol levels respectively, while a significantly negative correlation was observed between the PSP 15-minute value. A significantly positive correlation was noted between the CA II and total cholesterol levels. In view of these findings, we conclude that the determination of erythrocyte CA I and II levels may be important in the follow-up and evaluation of prognosis in chronic renal disease patients.

The mechanism responsible for increase in CA I and II levels in chronic hemodialysis patients most probably is a compensatory re-

sponse to the disordered acid-base equilibrium in the renal tubules.

Further, in such cases, as there is severe anemia which causes a decrease in CO<sub>2</sub> transport from the tissue to the lungs, the increase in these levels may also be considered a compensatory mechanism.

Mondrup et al.<sup>8)</sup> also reported that the CA I level, in particular, is increased in uremic patients who had compensatory acidosis and anemia.

Although patients with poorly-controlled primary nephrotic syndrome and lupus nephritis showed significant increases in CA I and II levels as in chronic hemodialysis patients, severe disorders of acid-base equilibrium and anemia were not necessarily observed. Thus, the mechanism responsible for the increase is not clear, but the possible effects of corticosteroids and immunosuppressive agents used in the treatment of both entities must also be considered.

Further, as pointed out by Wählstrand et al.<sup>11)</sup>, purifying the carbonic anhydrase present in various parts of the renal tissue and determining the respective enzyme levels may serve as a clue in elucidating the cause for increase and/or decrease in the level in chronic renal disease patients.

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