Pergamon

Clinical Hemorheology, Vol. 15, No. 3, pp. 325–333, 1995

0271-5198/95 $9.50 + .00

0271-5198(95)00018-6

# RED BLOOD CELL SODIUM CONTENT IN NID DIABETIC PATIENTS WITH HEMORHEOLOGICAL ABNORMALITIES

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(Received 26.04.1994; accepted 23.02.1995)

ABSTRACT

The aim of this study was to verify if abnormalities of sodium content can explain the decrease in the red blood cell deformability observed in diabetic patients.

Erythrocyte deformability based on filtration index, membrane chemical composition evaluated by free cholesterol, phospholipid fractions measured by thin layer chromatography, total protein, sodium, potassium and magnesium contents were investigated in 37 non insulin dependent diabetic (NIDD) patients and 26 control subjects.

The results showed that erythrocyte sodium content was higher in diabetic patients than in controls and it correlated significantly with filtration index and membrane lipid contents.

## INTRODUCTION

Evidence has accumulated over the past several years documenting the occurrence of hemorheological abnormalities in diabetes, characterized by a decrease in erythrocyte deformability ( 1, 2, 3). This impaired fundamental physical property of red blood cells (RBC) reduces the speed of microcirculation and induces a deleterious effect on tissue oxygenation. This has been attributed more particularly to modifications of the membrane lipid content (4). An increase in the sodium transport in erythrocytes of diabetic patients and a correlation between erythrocyte cations transport and the main membrane lipid fractions have been observed. Since in diabetics lipid metabolism is always disturbed and RBC rigidity increased, it appeared interesting to determine whether RBC deformability could be affected by the $Na^+$ transport abnormalities.

Sodium ( $Na^+$), Potassium ($K^+$), Magnesium ( $Mg^{++}$) erythrocyte contents, filtration index and membrane lipid composition were measured in 37 non insulin dependent diabetic (NIDD) patients and the results were compared with those in 26 controls.

Key words : erythrocytes, diabetes, deformability, sodium erythrocyte content, membrane lipids.

## MATERIAL AND METHODS

### 1. Subjects

The present study included a control group of 26 normal healthy men and women and a group of 37 NIDD patients without strict glycemia control but free of major clinical diabetic complications (lower limb arteritis, coronary heart disease, retinopathy and proteinuria). In these patients previous analysis had shown an increase in the RBC index. The subjects were not taking any drugs or hormones able to change RBC filtration index or lipid parameters.Characteristics of the diabetic patients are reported in Table I.

### 2. Red blood cell filtration measurement

Red blood cell deformability was evaluated by the method of initial filtration rate measurement, using the Hanss hemorheometer (9).
Hanss's method consists of an automatic calculation of the time taken by red blood cells to cross through a 13 mm diameter calibrated porous membrane (Nuclepores filter with a pore diameter equal to 5 μm ).

$$FI = (Ts-Tb)/ Tb \times 100/H$$

Ts : Filtration time of Hanks buffer.
Tb : Filtration time of red blood cells in Hanks solution.
H : Haematocrit

### 3. Analysis red blood cell membrane chemical composition

The erythrocyte membranes were obtained according to Dodge et al. (10). Membrane proteins were assayed according to Lowry et al. (11).
Lipids were extracted with chloroform methanol ( 2/1 v,v) The residues were washed using Folch's method. Membrane lipids were quantitatively separated by thin layer chromatography according to Pré and Garnier's method (12).

### 4. Measurement of erythrocyte cytosolic cations

Erythrocyte cytosol $Na^+$ and $K^+$ were measured by flame photometry in the total blood and plasma . $Mg^{++}$ was measured by a colorimetric method (13). Erythrocyte cation concentrations were obtained by calculation.
Nai = (Nat.2,9)-(Nap.(1-H)/H
Nai : Sodium erythrocyte content ; Nat : Total blood sodium ; Nap : plasma sodium ;
H : Heamatocrit

### 5. Measurement of biochemical plasma parameters

Plasma total cholesterol, triglycerides and glycemia were measured by an automated enzymatic method ( Börhinger Manheim , Germany).
Hemoglobin $A_{1c}$ was measured by a micro column test (Bio-Rad Diagnostic group, France).
Fructosamine was measured by a colorimetric test with nitroblue tetrazolium (Roche Diagnostic, France).

### 6. Statistical analysis

The dispersion of the data was obtained by the standard deviation. The Student's *t* -test on unpaired data was used to compare the values in the two groups. Statistical methods also included single and multiple regression analysis. The *r* correlation coefficient and the regression lines were subsequently determined.

TABLE I
Mean and standard deviation of Plasma Biochemical Parameters in non insulin dependent diabetic Patients

| | AGE | F.GLY | PP.GLY | FRUCT | $HbA_{1c}$ | TCL | TRG |
|---|---|---|---|---|---|---|---|
| PLASMA BIOCHEM. PARAM. | 54,0 +/- 6,7 | 12,6 +/- 3,2 | 17,78 +/- 4,07 | 382,9 +/- 73,3 | 8,83 +/- 1,82 | 5,69 +/- 1,16 | 2,79 +/- 2,48 |
| NORMAL VALUES | | <6 mmol/l | <9 mmol/l | <285 µmol/l | <6% | <6,4 mmol/l | <2 mmol/l |

PLASMA BIOCHEM.PARAM. : Plasma Biochemical Parameters ; F.GLY : Fasting Glycemia ; PP.GLY : Post Prandial Glycemia ; FRUCT : Fructosamine ; $HbA_{1c}$ : Glycosylated Hemoglobin ; TCL : Total Cholesterol ; TRG : Triglyceride.

TABLE II
Mean and Standard Deviation of RBC Cations in 26 Controls and 37 non insulin dependent diabetic patients (NIDD's)

| | CONTROLS ( n=26) | NIDD's (n= 37) |
|---|---|---|
| Nai mEq/l | 17,9 +/- 10,1 | 28,8 +/- 9,6 |
| Ki mEq/l | 109,3 +/- 9,6 | 112,1 +/- 10,3 |
| Mgi mmol/l | 2,7 +/- 0,9 | 2,6 +/- 0,6 |

Nai : Erythrocyte sodium content ; Ki : Erythrocyte potassium content ;
Mgi : Erythrocyte magnesium content

TABLE III
Mean and Standard Deviation Values of RBC Index Filtration and Principal Membrane Lipid Fractions obtained in 26 Normal Subject and 37 non insulin dependent diabetic patients (NIDD's)

| | CONTROLS (n=26) | NIDD'S (n=37) |
|---|---|---|
| FILTRATION INDEX | 10,42 +/- 2,20 | 11,5 +/- 1,8 |
| FREE CHOLESTEROL µmol/mg proteins | 0,66 +/- 0,13 | 0,53 +/- 0,06 |
| P. CHOLINE µmol/mg proteins | 0,27 +/- 0,07 | 0,30 +/- 0,04 |
| SPHINGOMYELINE µmol/mg proteins | 0,14 +/- 0,04 | 0,20 +/- 0,03 |
| P. ETHANOLAMINE µmol/mg proteins | 0,25 +/- 0,05 | 0,23 +/- 0,03 |

P.CHOLINE : Phosphatidylcholine ; P.ETHANOLAMINE : Phosphatidylethanolamine

## RESULTS

In the diabetic patients RBC sodium levels were higher (+61%) than those in the controls ($t = 3.56$ ; $p<0,001$). Eighteen NIDD patients had a RBC sodium level greater than the mean + 1 SD value in the controls. $Mg^{++}$ and $K^{+}$ were similar in diabetic patients and controls (Table II).

The RBC filtration index in the diabetic group (11.5 +/- 1.8) was significantly higher (+10.5%) than in the control group (10.42 +/- 2.20) ($t = 3.49$ ; $p<0,01$).

Lipid composition of the RBC membrane was different in diabetic patients and controls. Free cholesterol content was 20% lower ($t = 3.49$ ; $p<0.01$) and sphingomyeline content was 43% higher ($t = 5.33$ ; $p<0.01$), resulting in a 46% decrease in the RBC cholesterol / phospholipid ratio (table III).

Plasma- erythrocyte Correlations:

Statistical analysis according to the unpaired Student's *t* test for data analysis and using linear regression showed a significant correlation between plasma $Mg^{++}$ values and $Mg^{++}$ RBC content but these values were not different in diabetic patients and controls.

In diabetic patients $Na^{+}$ RBC content correlated significantly with fasting glycemia ($r = 0.53$ ; $p<0.05$) and the filtration index correlated significantly with post prandial glycemia ($r = 0.49$ ; $p=0.014$).

Correlations between RBC $Na^{+}$ content and the filtration index:

A significant positive correlation appeared between RBC $Na^{+}$ content and the filtration index in diabetic patients ($r = 0.40$ ; $p=0.015$) (fig 1a).

Correlations between RBC $Na^{+}$ content and RBC membrane major lipid fractions:

In diabetic patients RBC $Na^{+}$ content values correlated with:

- potassium erythrocyte ($r = 0.52$ ; $p=0.001$)
- membrane phosphatidyl choline values ( $r = -0.45$ ; $p=0.008$) (fig 1b)
- membrane proteins content ($r = 0.39$ ; $p=0.021$)

The sphingomyeline values discriminated patients from controls but no correlations existed with the filtration index or $Na^{+}$ content of RBC.

RBC $Mg^{++}$ content and $K^{+}$ content did not correlate with the filtration index or membrane major lipid fractions.

## DISCUSSION

These results show evidence of a substantial difference in RBC sodium content, RBC filtration index and membrane lipid composition between the NIDD patients and the controls.

Erythrocyte sodium content values in controls are in agreement using those previously reported with similar technology by flame spectrophotometry (14).

In controls there was no relationship between filtration index and RBC $Na^{+}$ content or major membrane lipid fractions. These results agree with those found by P. Lijnen et al., who reported significant correlations of the different Na + transport mechanisms but not for internal cations content (8).

In contrast, the results obtained in the diabetic patients (shown in Table III and fig.1a), demonstrate that the alteration of the red blood cell membrane dynamic properties, measured by the filtration index, correlated with RBC $Na^{+}$ content. Since RBC $Na^{+}$ content was markedly higher, this disorder might be involved in the decrease in RBC deformability.

FIG: 1a

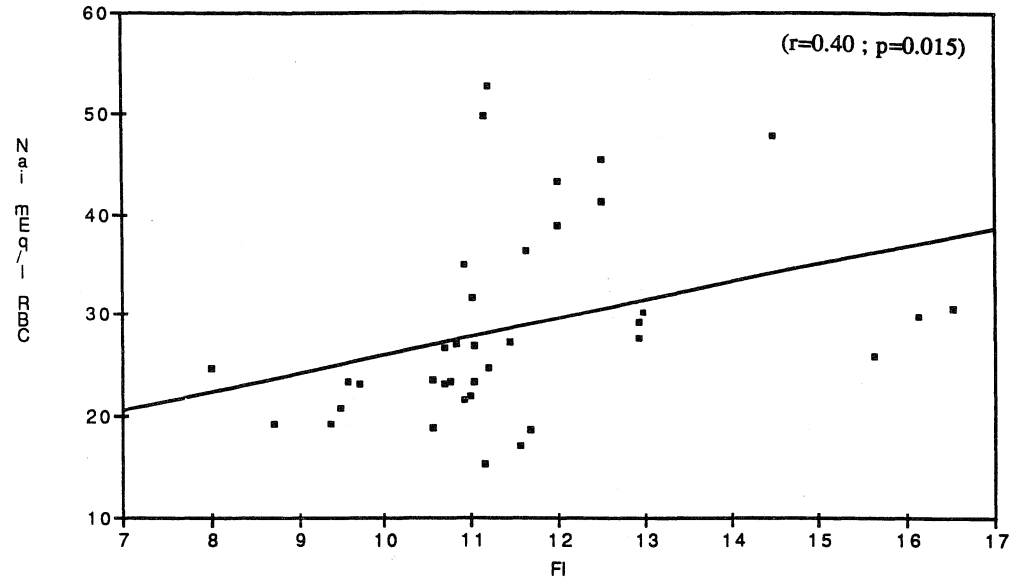
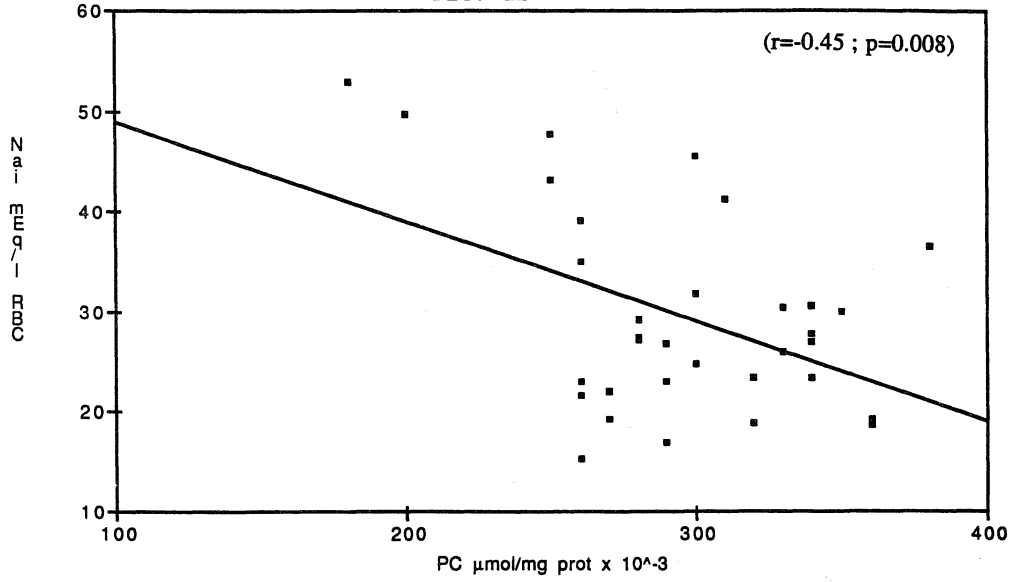

(r=0.40 ; p=0.015)
Nai meq/l RBC
60
50
40
30
20
10
7
8
9
10
11
12
13
14
15
16
17
FI

FIG: 1b


(r=-0.45 ; p=0.008)
Nai meq/l RBC
60
50
40
30
20
10
100
200
300
400
PC µmol/mg prot x 10^-3

Moreover, abnormalities of RBC $Na^+$ metabolism were often observed, particularly in the $Li^+/Na^+$ counter transport, in diabetic patients or in patients with essential hypertension (15). These abnormalities have also been reported in diabetic patients for other blood cells such as platelets (16), or white cells (17) and even in skin fibroblasts culture (18).
These results are of great interest when compared with those obtained in dogs turned diabetic with alloxane. In these animals the authors found a 77% increase in the $Na^+$ content of the left ventricle cardiomyocyte (19). Now, anatomic abnormalities like an increase in the left ventricle mass have been described in normotensive diabetic patients (20,21,22)
These observations, together with the results of this study suggest there may be widespread general disturbance of sodium metabolism in diabetic patients.
The mechanism of this disorder remains unknown and needs further investigation. It may result from an intrinsic cellular abnormality or from biochemical abnormalities of carbohydrate and lipid metabolism induced by diabetes itself.
In vitro the incubation of RBC of normal subjects with high glucose or insulin concentrations was accompanied by a 25% increase in $Na^+$ RBC sodium content (23). The results of the present study in diabetic patients also showed a relationship between fasting glycemia and RBC $Na^+$ content. These results are a variance with the hypothesis of an intrinsic cellular abnormality. It is noteworthy that abnormalities in the sodium transport mechanism seem to be associated with insulin resistance (24).
Recently, it has been shown that the decrease in RBC deformability in diabetic patients was not a gradual phenomenon but occurred very early, just after the RBC entered the bloodstream or the bone marrow (25). Since the mature RBC are unable of synthesis, lipid abnormalities observed in the RBC of diabetic patients must be related to plasma lipoprotein exchanges. The increase in RBC $Na^+$ content could be induced by primary membrane lipid alterations and both could be involved in the increase in the RBC filtration index.
The decrease in RBC free cholesterol membrane content and the increase in sphingomyeline content differentiate the RBC of diabetic patients from controls. The decrease in membrane free cholesterol is a finding consistent with previous studies, even if the phospholipid changes were not consistent (1). The data concerning the alterations in the chemical composition of the diabetic RBC membrane seem to be conflicting, but lipid composition is rarely unchanged in diabetic patients (26). Since these alterations seem to improve quickly by short-term strict control of diabetes (4) it is important that, in future clinical investigations, results should be expressed according to glycemia and insulinemia levels.
In the controls there was no relationship between filtration index and RBC $Na^+$ content or major membrane lipid fractions. In contrast, in the diabetic patients, statistically significant correlations can be shown between these parameters. These findings suggest that the presence of lipidic abnormalities in diabetic patients predisposes them to changes in cellular sodium metabolism.

## CONCLUSION

This study has shown evidence of several changes in the lipid composition and $Na^+$ content of red blood cells in diabetic patients. These abnormalities are probably involved in the decrease in erythrocyte deformability. As sodium transport disturbances in several different tissues of diabetic patients are often found, associated with anatomical and functional cardiac abnormalities, it would be very interesting to determine whether measurements of sodium content and the RBC filtration index can identify diabetic patients who will develop to cardiac abnormalities.

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