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Alterations in calcium ATPase activity in erythrocyte membranes of non-insulin dependent diabetes mellitus patients.

Original Article

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ABSTRACT.

Introduction. The imparired Ca^{2+} metabolism in diabetes is a result of several wide spectrum of abnormalities correlation between the high levels of glucose to that of Ca^{2+} ATPase activity and erythrocyte Ca^{2+} in non-insulin depent diabetes mellitus (NIDDM) is studied in this paper.

Materials and methods. Heparinized blood samples were collected from 20 patients with NIDDM. Estimation of total Ca^{2+} was carried out with HCl/1 Lanthanum supernatents of erythrocyte suspension by atomic absorption spectrophotometry. Estimations of membrane bound Ca^{2+}ATPase activity was determined by coupled enzyme assay and of membrane glycoprotein was carried out by phenolsulphuric acid method.

Results. The levels of erythrocyte membrane Ca^{2+} ATPase was observed to be 0.532 ± 0.019 µg/mg in controls and 0.321 ± 0.041 µg/mg in NIDDM. There is a significant 0.60 fold decrease in NIDDM when compared with the controls. The levels of membrane glycoprotein was observed to be 59.86 ± 6.3 µg/mg in controls and 38.66 ± 6.9 µg/mg in NIDDM. There is a significant 0.64 fold decrease in NIDDM subjects when compared to controls. The erythrocyte membrane Ca^{2+} was observed to be 0.144 ± 0.02 µg/mg in controls and 0.067 ±0.016 µg/mg in NIDDM (0.46 folds decrease in NIDDM subjects when compared to the controls). Erythrocyte total Ca^{2+} is 0.615 ± 0.102 µg/mg in controls and 2.02 ± 0.08 µg/mg in NIDDM (3.2 folds increase in NIDDM patients when compared to controls).

Discussion. Our results sugest that the cellular Ca^{2+} overload is a major impairment in diabetes which leads to the loss of membrane integrity and the loss of membrane glycoprotein, which was observed to decrease as a result of membrane alteration, and increased osmotic fragility.

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Key words: Diabetes mellitus, ATP-ase, calcium metabolism, erytrocyte membranes.

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RESUMEN.

Alteraciones de la actividad de la ATP-asa de las membranas de los eritrocitos en pacientes con diabetes mellitus tipo II.

Introducción.- El metabolismo anómalo del Ca^{2+} en la diabetes mellitus es el resultado de un amplio espectro de anormalidades. La correlación entre los niveles altos de glucosa con la actividad de la ATP-asa del Ca^{2+} y el Ca^{2+} eritrocitario en pacientes con diabetes tipo II (DM-II) se reporta en este trabajo.

Material y Métodos.- Muestras de sangre heparinizada fueron colectadas en de 20 pacientes con DM-II. La estimación del Ca^{2+} fue realizada en lisados de eritrocitos utilizando espectofotometría de absorción atómica. La actividad de la ATPasa de Ca^{2+} fijada a la membrana por ensayo enzimático acoplado y la determinación de la glicoproteina de membrana por el método del ácido feniolsulfúrico.

Resultados.- Los niveles de la ATPasa de Ca^{2+} fijada a la membrana de los eritrocitos fueron 0.532 ± 0.019 µg/mg en el grupo control y 0.321 ± 0.041 µg/mg en DM-II. Hay un decremento significativo de 0.6 en DM-II comparado con el grupo control. Los niveles de la glicoproteina de la membrana fue de 59.86 ± 6.3 µg/mg en el grupo control y 38.66 ± 6.9 µg/mg en DM-II. Hay un decremento significativo de 0.64 en DM-II en relación al grupo control. El Ca^{2+} fijado a la membrana eritrocitaria fue de 0.144 ± 0.02 µg/mg en el grupo control y de 0.067 ± 0.016 µg/mg en DM-II. El Ca^{2+} total de los eritrocitos fue de 0.615 ± 0.102 µg/mg en el grupo control y 2.02 ± 0.08 µg/mg en DM-II (un incremento de 3.2 en DM-II con relación al grupo control).

Discusión.- Nuestros resultados sugieren que el incremento de Ca^{2+} intracelular es un defecto predominante en la DM-II que ocasiona la pérdida de la integridad de la membrana y disminución de la glucoproteina de membrana, lo que en su turno ocasiona incremento de la fragilidad osmótica. ***(Rev Biomed 2000; 11:1-5)***

Palabras clave: Diabetes mellitus, ATP-asa, metabolismo de calcio, membrana eritrocitaria.

INTRODUCTION.

Concentrations of Ca^{2+} and Ca^{2+} ATPase enzyme activity in erythrocytes is maintained by several mechanisms. It has been proposed that abnormal plasma concentrations of glucose will alter the erythrocyte membrane permeability to various cations such as Ca^{2+} ions (1). Increased erythrocyte Ca^{2+} and decreased Ca^{2+} ATPases activity represent the abnormal metabolism of Ca^{2+} in several conditions (2).

The impaired Ca^{2+} metabolism in diabetes is a result of several wide spectrum of abnormalities. However their heterogenecity may be explained by the complex interaction between the different mechanisms involved in Ca^{2+} homeostatsis (3-5).

The correlation between the high levels of glucose to that of Ca^{2+} ATPase activity and erythrocyte Ca^{2+} in NIDDM is the objective to be studied in this paper.

MATERIALS AND METHODS.

The heparinized blood samples were collected from 20 patients with NIDDM (12 males and 8 females) and processed for various parameters. The clinical diagnostic data represented in table 1 and 2 is carried out by the standard procedure according to Dacie *et al.* (6).

Estimation of total Ca^{2+} was carried out with HCl/1 Lanthanum supernatents of erythrocyte suspension by atomic absorption spectrophotometry, according to Turrini *et al.* (7). Estimation of Membrane bound Ca^{2+} ATPase activity was determined by coupled enzyme assay as described by the method of Turrini *et al.* (7). Estimation of Membrane glycoprotein was carried out by phenolsulphuric acid method (8).

RESULTS.

The NIDDM patients were diagnosed based on clinical parameters such as Hb levels, glycosylated Hb levels, Fasting and Post Prandial Blood Glucose Levels.

The clinical diagnostic data is represented in table l. The levels of Hb in controls was observed to be 13.60 ± 1.20 g/dL and in NIDDM subjects it was slightly lower. lt was 11.90 ± 2.10 g/dL. The Hb% was observed to be decreased to 0.875 folds in NIDDM when compared to controls. The glycosylated Hb is 3.45 ± 0.58 g/dL in controls and 4.24 ± 0.48 g/dL in NIDDM subjects. There is a significant 1.2 folds increase in NIDDM.

In order to check the permeability alterations of erythrocyte membrane in hyperglycemic conditions, analysis of levels of Ca^{2+} and membrane bound Ca^{2+} ATPase activities were carried out in normals and NIDDM patients.

In table 2 the levels of erythrocyte membrane Ca^{2+} ATPase was observed to be 0.532 ± 0.019 µg/mg in controls and 0.321 ± 0.041 in NIDDM. There is a significant 0.60 folds decrease in NIDDM when compared with the controls. The levels of membrane glycoprotein was observed to be 59.86 ± 6.3 µg/mg in controls and 38.66 ± 6.9 µg/mg in NIDDM subject when compared to controls.

The analysis of divalent cations is also represented in table 2 in which the erythrocyte membrane Ca^{2+} was observed to be 0.144 ± 0.02 µg/mg in controls and 0.067 ±0.016 µg/mg in NIDDM. There was a significant 0.46 folds decrease in NIDDM subjects when compared to the controls. Erythrocyte total Ca^{2+} is 0.615 ± 0.102 µg/mg in controls and 2.02 ± 0.08 µg/mg in

Table l
Levels of Haemoglobin (Hb), Glycosylated Hemoglobin, Fasting Blood Sugar (FBS), Post Prandial Blood Sugar (PLBS).

S. No.	Parameters and Units	Control (20)	NIDDM (20)
1	HB (g/dL)	13.60 ± 01.20	11.90 ± 02.10
2	Glycosylated HB (g/dL)	3.45 ± 0.58	4.24 ± 0.48
3	FBS (mg/dL)	98.0 ± 10.0	188.0 ± 10.0
4	PLBS (mg/dL)	138.0 ± 12.2	260.0 ± 10.2

Table 2
Levels of Erythrocyte membrane Ca^{2+} ATPase, Membrane Ca^{2+}, Total Ca^{2+} and Membrane Glycoprotein.

S. No.	Parameters and Units	Control (20)	NIDDM (20)
1	Erythrocyte membrane Ca^{2+} ATPase (µg/mg)	0.532 ± 0.19	0.321 ± 0.041
2	Erythrocyte membrane Ca^{2+} (µg/mg)	0.144 ± 0.02	0.067 ± 0.016
3	Erythrocyte total Ca^{2+} (µg/mg)	0.615 ± 0.10	2.020 ± 0.08
4	Erythrocyte membrane glycoprotein (µg/mg)	59.86 ± 6.3	38.66 ± 6.9

NIDDM. There is a significant 3.2 folds increase in NIDDM patients when compared to controls.

DISCUSSION.

Abnormal Ca^{2+} metabolism causes insulin resistance and impairs, insulin secretion and may be a basic common pathology of NIDDM syndrome (3,9). The NIDDM is not an immune mediated one and is determined by genetic factors. Insulin resistance plays a major role in this disorder (10).

The above studies demonstrate that when the erythrocyte is suspended in a pool of glucose, a hyperglycemic condition the concentration of total Ca^{2+} is observed to be increased and Ca^{2+}-ATPase to be decreased. The decreased activity of Ca^{2+}-ATPase would result in increased erythrocyte total Ca^{2+} levels, as a result of the permeability alterations. The decreased Ca^{2+}-ATPase also decreases the membrane bound Ca^{2+} concentrations. This also indicates that the erythrocyte permeability is altered. The increased total cytosolic Ca^{2+} may be due to the Ca^{2+} induced release of Ca^{2+} from storage sites in the membrane (11). It is already reported that this increased Ca^{2+} concentrations has an effect on osmotic fragility (1).

The cellular Ca^{2+} overload is a major impairment in diabetes which leads to the loss of membrane integrity and the loss of membrane glycoprotein which was observed to decrease as a result of membrane alteration and increased osmotic fragility. We have observed that the decreased activity of Ca^{2+}-ATPase is a result of hyperglycemia and this would result in a decreased ability of Ca^{2+}-ATPase to efflux Ca^{2+} out due to the permeability alterations. lt has been reported in rat pancreatic islet cells that the high glucose results in transient decrease in the Ca^{2+}-ATPase activity which rapidly retums to baseline (12). The function of Ca^{2+}-ATPase and Ca^{2+} may be further deteriorated in conditions of chronic hyperglycemia. This may play a significant role in the decrease responsiveness of Ca^{2+}-ATPase to glucose challenge.

The excess glucose leads to the glycosylation of several proteins such as hemoglobin and several other membrane proteins (13). Glycosylated hemoglobin may undergo auto oxidation and cause pemeability alterations which results in increased osmotic fragility (14). Therefore we could observe that glycosylation would bring about severas confirmation changes of membrane leading to altered permeability and the observed increased osmotic fragility (1).

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