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Evaluation of the alterations of Calcium and Magnesium levels in Alloxan-Induced Diabetic Rats

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Abstract

This study determined the level of magnesium and Ca^{2+} in diabetic control and Non diabetic control DM in a total of twenty (20) albino rats; diabetic (n=10) and Non-diabetic (n=10). Plasma magnesium and calcium were analyzed using indirect method of atomic absorption spectrophotphot ometer. The body weight of diabetic and non-diabetic rats of the initial weight were comparable 150.3±21.9g vs 149.1±16.9g; P>0.05; the former had significantly (P<0.05) final body weight were lower (141.4±13.7) then the initial body weight of the non-diabetic control (164.1±22.3g).The fasting plasma magnesium and calcium levels were between non diabetic and diabetic rats shows that diabetic rats had lower level of plasma calcium (37.5 ±19.3ppm), and magnesium (17.0±7.7ppm) in compassion to the non-diabetic control 49.8±38.4ppm and (18.6±9ppm) for calcium and magnesium respectively. Due to difference is the selection of subject or study design and due to prevalence of diabetes in this part of the world and not much work have been done to ascertain the relationship between trace-element (magnesium and calcium) in clinical status of patient with diabetes mellitus from this work, the pattern of change is plasma magnesium and ca' levels were similar in diabetic albino rat Clear to understand that in diabetic patients this tract element are reduced. Even though the work is consistent in some aspects with previous finding, additional and more reliable work using advance technique should be carried out with lagers number of sample in order to fully elucidate the relationship between magnesium and calcium.

Keywords: Diabetes, Magnesium, Calcium, Alloxan-Induced Diabetic Rats

Introduction

The term diabetes is assigned as two types of physiological conditions; "Diabetes insipidus" and Diabetes mellitus".

Diabetes insipidus is a term used to describe water dieresis due to deficiency of the anti diuretic hormone (ADH), while diabetes mellitus (DM) is that associated with pancreatic insulin [1].

Diabetes mellitus is not a single well defined disease entity, but is a syndrome, a cluster of symptoms, caused by any of the several endocrine gland disorders characterized by increased plasma glucose concentration, an absolute or relative insulin

deficiency and glucosuria may or may not be present.

Diabetes mellitus has been defined by the world Health organization (WHO), on the basis of Laboratory finding as a fasting venous plasma glucose concentration more than or equal to 7.0mmol/L (on more than one occasion or once in the presence of diabetes symptom) or a random venous plasma glucose concentration more than or equal to 11.1mmol/L [1].

Diabetes mellitus disorder is the seventh leading causes of death in the United States, and is responsible for 12% of all cases of end stage renal disease.

Trace element are defined as inorganic compounds that, like vitamins, are essential for health and needed only in small amounts, known as the reference nutrients intake [1].

Some trace elements act as antioxidants and prevent membrane peroxidation, others act directly on glucose metabolism. Interest in trace elements has been steadily increasing over the last twenty five (25) years trace elements are accepted as essential for optimum human health, because of their diverse metabolic characteristics and functions to serve a variety of catalytic structural and regulation functions, in which the interacts with macro molecules such as enzymes, prohormone and secretory granules biological membrance.

Magnesium is an essential mineral for the human body, principally because of its role in the regulation of cellular processes and its function as a cofactor in a wide range of metabolic reactions. Many enzymes that catalyze phoshorylation and dephosphorylation reactions, including those involved in glycolysis, are activated by the formation of $MgATP^{+2}$ complexes, which are the real substrates for these enzymes [2].

Alterations in the distribution of magnesium within the body have been associated with several disease and especially diabetes, a discover representing a global public health problem of increasingly serious concern [3]. Although some epidemiological studies have suggested that adequate magnesium intake reduces the risk of development of type 2 diabetes, there are still contradictions with respect to the role of low magnesium intake as a predictor factor for this disease [4]. The importance of magnesium for individuals with diabetes can be explained on the basis of maintenance of glucose homeostasis along with activation of the factors involved in sensitivity of tissues on insulin, the receptors of which are phosphorylated only in the presence of $MgATP^{+2}$ [5].

Some studies have shown that the magnesium intake by patients with diabetes is often below recommended levels. Additionally, there is evidence that the magnesium status of patients with diabetes tends to alter, and that low body concentrations of this mineral may influence the evolution of the disease and generate further complications [6].

Despite reports describing the occurrence of hypomagnesaemia among patients with diabetes [7]. Few investigations have considered dietary intake of magnesium in the Brazilian population, and none has examined the levels of magnesium in patients with type 2 diabetes.

Due to prevalence of diabetes in this part of the world and not much work have been done to ascertain the relationship between trace elements (mg and Ca) in the diagnosis and treatment of diabetes. Hence the evaluation of the status of these elements (magnesium and calcium) in the plasma will help in recognition of complication and in management of diabetes mellitus. The Aim of this study is to evaluate the levels of magnesium and calcium in the plasma of rats with diabetes mellitus DM in an effort to clarify their role in this disease.

Material and method

Animals

Twenty Wistar albino rats comprising of 12 female and 8 males were brought from the animal house in the Department of Biochemistry of the University of Nigeria, Nsukka. They were bred for 4 weeks under well-controlled conditions at the animal house of the Department of Biochemistry Ebonyi state University unit conventional laboratory diet and tap water were provided the weight of the rats were measured and fasting blood glucose level estimated before the induction of DM.

Induction of diabetes

The rats weighing between 160-120g were randomly divided into 2 groups (i) control, which consist of 10 wistar rats (6 female and 4 males) were induced DM by intraperitoneal injection of 12omg/kg/body weight alloxan.

Diabetic control

They comprised 6 female and 4 male wistar rats. The diabetic control received equivalent volume of distilled water alone.

Sample collection

After two days, samples of blood were collected by ocular puncture with capillary tube used to puncture the eyes of the rats and samples were made to run through the capillary tube into the anticoagulant tube.

5mLs of blood were collected each from the rats into heparin anticoagulant plastic containers and fluoride Oxalate containers. The blood was centrifuged and the plasma was immediately separated from the cells.

Plasma from the fluoride Oxalate anticoagulated container were used for fasting blood glucose estimation while plasma from the heparin anticoagulated container were used for magnesium and calcium estimation.

Method of analysis

Priciple of fasting blood glucose estimation (enzymatic method)

The glucose oxidase catalyses the oxidation of glucose into hydrogen peroxide and electronic acid. In the presence of peroxidase, hydrogen peroxide is broken down into oxygen and water. The oxygen react with 4-ammo artipyrine and phenol to produce a pink coloured complex which is then read spectrophotometricaly at 515 nanometer [1].

Determination of plasma magnesium and calcium concetration

Digestion

Before the sample were be analyzed, for a trace element it is first digested so as remove all micro-element like fats and proteins which could be present in the sample.

Digestion was done to ensure that no interference was experienced since all other components of the sample is removed leaving only the trace element which is of our interest [8].

Procedure

Digestion is done in a fame cupboard using a needed and a

syringe,/mL of the sample in collected into a tube 5 m/s of 65% trioxo nitrate (V) acid and 5mLs of concentration tetraoxo sulphate (VI) acid is measured out with a measuring cylinder. Into the tube containing the sample, 5 mLs of tetroxo oxosulphate (VI) acid in added followed by adding the 5mLs of pre-heated trioxo nitrate (V) acid. The tubes are then carefully arranged into the fume cupboard and are heated until digestion in complete.

The after, the digest is then diluted with distilled water, however the trace element is then analyzed using s flame atomic absorption spectrophotometer with air acetylene gas.

Principle of atomic absortion spectrophotometer

The instrument method of analysis is based on the absorption of electromagnetic radiation in the visible and ultraviolet region of the spectrum by atoms in the vapour state (i.e it result in changes in electronic structures). When the sample in aspirates into the flame, the solvent is evaporated or burnt and the sample compound are thermally decomposed and converted into a gas of individual's atoms. They are then capable of absorbing radiation at discrete line of narrow band width. The desired element in the sample can them absorb only resonance lines, with wavelengths corresponding to transition from the minimum energy state to some high level. The narrow emission lines which are to be absorbed by the sample are generally provided by hallow cathode lamp made up of elements, such lamps emit only the spectrum of desired elements.

The difference in their determination lies in each element having its own wavelength of which it absorbs, its own lamp current, its own band pass or width and its own hallow cathode lamp. The hallow cathode lamp the element being determined has first miscalled into the instrument, the wavelength, lamp current slit width or band pass, burner height were all adjusted.

The spectrophotometer was then switched on and left about 15 minutes to warm up. The gases, oxidants and the fuel were then opened. The standard solutions of the elements under analysis were then aspirated starting with minimum to maximum concentration. The absorbance and concentration were record for each element.

Data analysis

All data was analysis using statistical package for social sciences (SPSS version 10), Data were analyzed for mean and standard deviation.

Results

Table 1: Comparison of body weights between non-diabetic and diabetic rats

Body weight (g)	Non-diabetics (n=10)	Diabetics (n=10)	p-values
Initial	150.3 ± 21.9	149.1 ±16.9	0.892
Final	164.1 ± 22.3	141.4 ± 13.7	0.013*
Change	+13.8 (9.2%)	-7.7(5.2)	

*p < 0.05 is considered significant

Table 1 shows the comparison of body weights of diabetic and non-diabetic rats. Even though the initial body weights of the diabetic and the non-diabetic rats were comparable (150.3 ± 21.9g vs. 149.1 ± 16.9 g, p > 0.05) the former had significantly (p < 0.05) lower final body weight (141.4 ± 13.7) than the latter (164.1 ± 22.3g).

Table 2: Comparison of fasting plasma glucose between non-diabetic and diabetic rats

Fasting plasma glucose (mmol/L)	Non-diabetics (n=10)	Diabetics (n=10)	p-values
Initial	5.6 ± 0.9	5.5 ± 0.4	0.782
Final	5.7 ± 0.6	15.7 ± 0.8	0.001*
Change (%)	+0.1 (1.8)	+10.2 (185.5)	

*p < 0.05 is considered significant

From Table 2, although there was no significant difference (p > 0.05) in the initial body weight of the diabetic (5.5 ± 0.4mmol/L) and non- diabetic (5.6 ± 0.9 mmol/L) rats, the former had significantly (p < 0.05) higher final fasting plasma glucose in comparison to the latter (15.7 ± 0.8 mmol/L vs. 5.7 ± 0.6 mmol/L)

Table 3: Comparison of plasma calcium and magnesium between non-diabetic and diabetic rats

Parameters	Non-diabetics	Diabetics	p-values
Calcium (ppm)	49.8 ± 38.4	37.5 ±19.3	0.378
Magnesium (ppm)	18.6 ± 9.6	17.0 ± 7.7	0.686

Comparison of plasma calcium and magnesium between non-diabetic and diabetic rats (Table 3) shows that diabetic rats had lower plasma calcium (37.5 ± 19.3ppm) and magnesium (17.0 ± 7.7ppm) in comparison to the non-diabetic rats (49.8 ± 38.4ppm and 18.6 ± 9.6ppm for calcium and magnesium respectively).

Discussion

Trace element are uniquely inorganic compounds that are essential for health, maintained of life and growth development. Inadequate supply of such nutrients produces a functional impairment and exception of any one of the trace element can result to imbalance of the nutrient which may cause alterations of the macromolecules and can result in disease condition. The clinical significance and evaluation of trace elements such as magnesium and calcium as regards to diabetes mellitus remains a controversial and conflicting if trace element have any beneficial effort in maintaining and play a good role in diabetes mellitus.

In experimental DM has been related alteration in the tissue retribution of magnesium spontaneous diabetes rats showed increased urinary excretion of magnesium correspondent to 1.5 times compaired with controls and reduction in the lungs, kidneys, testicles and adiposities in the early stages of the diabetes [8]. Hypo magnesium in diabetic is usually observed in patients with deficient metabolic control, or associated to the Diabetes mellitus, chronic complications, according to chemical and epidemiological studies [9]. Despite of the deduction that the hypomagnesaemia is caused by the diabetes and not the opposite, the magnesium deficiency were also demonstrated in the onset of this disease. This was predominantly on prospective study The mg deficit interferes on enzymatic reactions that use or produced Adenosine triphosphate (ATP), in consequence modifies the enzymatic cascade that involved the same way as metabolism triggering DM, in the way as observed by Delva *et al* [10].

Experimental researches have shown that patients with diabetic retinopathy present low concentration of plasma mg disposing to a higher risk of advanced retinopathy. And

cardiovascular, hypertension and which magnesium at as a beneficial antioxidant since it is among the essential trace element.

Conclusion

Diabetes mellitus in diabetic albino DM showed lower level of magnesium and calcium as compared to the non-diabetic healthy control. In the light of the importance of magnesium and calcium in health and disease, the America diabetic Association has recommended the replacement of mg and Ca^{2+} based on the reduction of serum concentration of this element and is additions to food diversification supplemental magnesium and ca^{2+} may be considered as part of diabetic management.

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