STATUS OF MICRONUTRIENTS IN PATIENTS WITH TYPE 2 DIABETES MELLITUS.

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ABSTRACT

Diabetes mellitus (DM) or simply diabetes is a group of metabolic diseases in which a person has high blood sugar. This high blood sugar produces the symptoms of frequent urination, increased thirst, increased hunger. Untreated diabetes can cause many complications. Diabetes is a metabolic disorder caused by defective insulin secretion, insulin action or both. The two major factors for the development of diabetes are pancreatic beta cell dysfunction and insulin resistance. The main types of diabetes mellitus are Type 1 DM and Type 2 DM. Type1 DM results from the body's failure to produce insulin. This form was previously referred to as "insulin-dependent diabetes mellitus" (IDDM) or "juvenile diabetes". Type 2 DM results from insulin resistance, a condition in which cells fail to use insulin properly, sometimes also with an absolute insulin deficiency. This form was previously referred to as non insulin-dependent diabetes mellitus (NIDDM) or "adult-onset diabetes". A relationship was observed between diabetes mellitus and trace elements in many research studies. In many cases, an alteration in the metabolism of these minerals was demonstrated. The objective of this work was to evaluate the status of micronutrients in patients with type 2 diabetes mellitus. T2 DM was reported associated with calcium, magnesium, zinc, and chromium. Therefore, it was decided to assess the status of Ca, Mg, Zn and Cr in the serum of patients with T2DM.

1.0 Introduction

Metals in extremely small quantities that are present in animal and plant cells and tissues are called trace metals. They are a necessary part of nutrition and physiology. Trace metals include iron, magnesium, lithium, zinc, copper, chromium, nickel, cobalt, vanadium, arsenic, molybdenum, manganese, selenium and others. Trace element deficiencies and excesses are known to affect numerous biological functions in humans, including physical growth, psychomotor development and immunity. Thus, it is very important to check trace elements

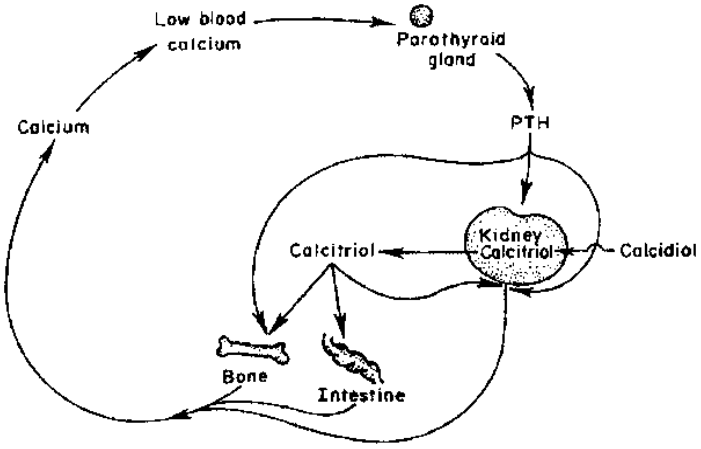
concentration regularly in the body. Trace elements though required in smaller quantities are to be taken in diet as they are required by the body for specific functions but taking them in excess causes various problems. Trace metals are known to influence hormones and enzymes at levels of secretion, activity and binding to target tissues. The role of metal in the catalytic action of the enzymes is;

- As constituents in the active site.
- As stabilizers of tertiary or quaternary structure.
- To providing assistance in forming weak bonding complex with substrates orienting the substrate for reaction.
- To stabilize charged transition states

Therefore, analysis of trace metal in biological samples can be used as diagnostic or prognostic aid in patients with different hormonal disturbance along with other biological parameters. Diabetes is a metabolic disorder caused by defective insulin secretion, insulin action or both. The two major actors for the development of diabetes are pancreatic beta cell dysfunction and insulin resistance. In type 2 diabetes mellitus, production and release of insulin is unobstructed but due to factors like ageing or obesity, the produced insulin cannot compensate for the increased demand. It can be seen that pancreatic beta cell dysfunction and insulin resistance which develop diabetes; are associated with the status of various metal ions; and all the more true with Ca, Mg, Cr and Zn. It has been suggested that a change in the composition of one ion will affect the others and the inter relationship is difficult to be predicted, though there are some known. A high intake of calcium depresses intestinal zinc absorption while an excess zinc intake depresses copper absorption. Vitamin D enhances the absorption of calcium and therefore excess intake of vitamin D would increase calcium, decreasing Mg, K, or P retention.

Cations of Cu, Fe, Mn have unpaired electrons and hence are considered free radicals but the actual role of a metal depends not only on the chemical structure of it but also of the molecule that chelates with the metal. Zinc has been recognized as an efficient anti-oxidant. It has been suggested that Zinc metalothionaein complexes in the pancreatic islet cells provide protection against immune-mediated free radical attack. Zinc also compete with Copper and Iron for membrane binding sites and thus can reduce the potential for hydroxyl radical formation which causes the Zinc mediated inhibition of the oxidation of LDL. Antioxidant property of Zinc is also reported to protect the endothelial cells against dysfunction caused by the fatty acids. Calcium is the most abundant metal in human body which serves two major roles as structural components and regulatory agents in body fluids in the form of ions or in combination with organic and inorganic compounds. The metabolic processes like blood coagulation, muscle contractibility enzyme activation, nerve transmission, hormone function and membrane transport are all controlled by calcium. It has a vital role in the insulin storage. Crystallisation of insulin in the membrane limited granules of β cells of pancreas needs zinc and calcium. Increased risk for diabetes was most seen when patients had the highest levels of calcium.

Major calcium movements in the body



Magnesium is a cofactor to more than 300 enzymatic reactions. It critically stabilizes enzymes including many ATP generating reactions and is thus connected to glucose utilization. Hypomagnesaemia is considered as a cause or consequence of both type 1 and type 2 Diabetes. Insulin is involved in the transport of magnesium through the cellular membrane and in the intracellular supply. Different studies have indicated that diabetes is associated with increased loss of magnesium in urine when hyperglycemia is poorly controlled. A negative correlation was observed between glucose and plasma concentration of mg in a study of diurnal profile of diabetic patients and control subjects. Chromium is an essential mineral to the metabolism of lipids, proteins, carbohydrates and insulin regulation. Thyroid activity is affected by chromium status. A low thyroid activity may allow increased insulin secretion resulting in its loss. Chromium acts as a cofactor for insulin by facilitating insulin to receptor on the surface of the target cell as well as within the cell. Deficiency of Cr is associated with increases serum insulin levels, decreased no. of insulin receptors and may signs of cardiovascular disease such as elevated serum cholesterol triglycerides as well as decreased HDL. Cr deficiency in humans and other mammals is reported to show symptoms comparable to those associated with T2DM and cardiovascular disease. Cr is found effective in the treatment of various types of diabetes. Treatment of type2 diabetes with Cr led to improvement in blood glucose, insulin levels. Numerous studies have confirmed that zinc and ATP are co packaged and co-secreted with insulin. Zinc stabilizes insulin hexamers and the pancreatic storage of the hormone. It is an efficient anti-oxidant and can compete for iron and copper by site specific binding. Various mechanisms of action have been suggested to explain the improved action of insulin by Zinc, hyper zincuria which results from a glucose mediated process, interacts with impaired Zinc absorption to produce border line zinc deficiency in patients with T2DM[38]. In a prospective study using high Zinc in a large group it was found only to slightly reduce the risk of T2DM in a Zinc deficient sub group. In microalbuminuric T2DM patients Zinc is reported to lower the homocystine levels.

Atomic absorption spectroscopy (AAS) is a spectroanalytical procedure for the quantitative determination of chemical elements using the absorption of optical radiation (light) by free atoms in the gaseous state. In analytical chemistry the technique is used for determining the concentration of a particular element (the analyte) in a sample to be analyzed. AAS can be used to determine over 70 different elements in solution or directly in solid samples used in pharmacology, biophysics and toxicology research. The technique makes use of absorption spectrometry to assess the concentration of an analyte in a sample. It requires standards with known analyte content to establish the relation between the measured absorbance and the analyte concentration, the electrons of the atoms in the atomizer can be promoted to higher orbitals (excited state) for a short period of time (nanoseconds) by absorbing a defined quantity of energy (radiation of a given wavelength).This amount of energy, i.e., wavelength, is specific to a particular electron transition in a particular element. In general, each wavelength corresponds to only one element, and the width of an absorption line is only of the order of a few picometers (pm), which gives the technique its elemental selectivity. The radiation flux without a sample and with a sample in the atomizer is measured using a detector, and the ratio between the two values (the absorbance) is converted to analyte concentration or mass using the Beer-Lambert Law. In order to analyze a sample for its atomic constituents, it has to be atomized. The atomizers most commonly used nowadays are flames and electro thermal (graphite tube) atomizers. The atoms should then be irradiated by optical radiation, and the radiation source could be an element-specific line radiation source or a continuum radiation source. The radiation then passes through a monochromator in order to separate the element-specific radiation from any other radiation emitted by the radiation source, which is finally measured by a detector.

1.1 Materials and Method

Atomic Absorption Spectrophotometry (AAS) and Inductively Coupled Plasma Emission Spectroscopy (ICPES), Inductively Coupled Plasma Mass Spectroscopy (ICPMS), and Neutron Activation Analysis are the important techniques used for the determination of trace elements in biological specimens. AAS which involves the aspiration of the diluted specimen in to the flame is very effective for the determination of metals. Graphite furnace method using as little as 10µL samples are used for the determination of metals which are below 50 ng/g. In the cases of manganese, chromium, vanadium etc, background corrections are necessary for which matrix modifiers are often added to the serum or plasma samples [45].

- Chemicals

Nitric acid (70%) and hydrochloric acids were of analytical grade. Triton X -100 polyoxyethylene sorbitanmonooleate) was from Fisher Scientific. Lanthanum Chloride (sigma Aldrich) was used for preparing the serum samples for the analysis of calcium and magnesium. Standard certified solutions in nitric acid containing 1000mg/L were purchased (Perkin Elmer) and appropriately diluted with Millipore water to give 0.5,1.0,1.5 and 2ppm solutions of zinc ; 50,100,200 ppm solutions of calcium and 10, 15,20,30 ppm solutions of magnesium. Working standards of zinc was prepared with 5% (v/v) Glycerol and that of chromium was prepared using a 0.2% solution of Nitric acid containing 0.2% Triton X-100.

- Collection of serum samples for analysis

5 ml of serum samples irrespective of the gender was collected from the outpatients of the Mahatma Gandhi Medical College and Research institute during July-August 2013. 250µL of serum was extracted by 250µL methanol isopropanol (90:10V/V) solution. The amount of vitamin D was estimated at the Biochemistry laboratory of the college

- Calibration of the instrument

Inorder to analyse the concentration of Zinc, Calcium, Magnesium and Chromium in serum sample it should be noted that the instrument is calibrated using the standard solutions of Zn, Ca, Mg and Cr.

- Preparation of standard solutions

For the preparation of standard Mg solution, first we have to prepare 10ppm Mg solution by pipetting out 1ml AAS standard Mg solution and then making up to 100ml using Lacl3. The 10 ppm solution is diluted to 0.5, 1, 1.5, 2, 2.5, 3 ppm solution. Then the concentration of Mg in these solutions are analysed by AAS. For the preparation of standard Ca solution, first we have to prepare 1ppm Ca solution by pipetting out 1µl Ca solution from AAS Ca solution into a 100 ml standard flask followed by adding 20ml , 1ppm Mg solution and then making up to 100ml using Lacl3 . Similarly prepare 2, 3, 4 ppm solutions for the determination of Ca concentrations. [Preparation of 0.1% Lacl3: Dissolve 1g Lacl3 using Millipore water in a 1000ml standard flask].For the preparation of standard Cr solution, prepare 3,5,10ppb Cr solution from 1ppb Cr solution by making up to 100ml using 0.2% HNO3 in 0.2% Triton-X solution in a standard flask .Then the concentration of Cr in these solutions are analysed by AAS. For the preparation of standard Zn solution, 0.4 ml, 1000ppm Zn solution is pipette out using an ependorff and made up to 100ml using 0.2% HNO3 in 0.2% Triton-X solution. Then the concentration of Zn in these solutions are analysed by AAS.[Preparation of 0.2% HNO3 in 0.2% Triton-X solution: Take 2ml Triton-X and 2ml fuming Nitric acid in 1L standard flask and made up to the mark using Millipore water.]

- Serum sample preparations

Calcium and Magnesium were estimated using the flame ionization technique where, 500µL serum was diluted to 5ml (10 times dilution) using 1% Lanthanum Chloride, vortexed for 30sc. and aspirated in to the flame. For the estimation of zinc 50µL of the serum sample was diluted to 5000µL with 0.2% Triton X-100 in 0.2% Nitric acid (70%) vortexed well for 30sc, 500µL transferred in to a vial.0.1% Magnesium nitrate was used as the matrix modifier. Average of two values was taken. For chromium estimation by the Graphite Furnace Technique, 200µL of the serum samples were mixed with 200µL of 0.2% Triton X-100 in 0.2% Nitric acid (70%) in a 1.5 ml microvial. The contents microvial was then vortex mixed for 30sc. and transferred in to pre washed poly propylene tubes (1.5ml).The instrument is first calibrated using different known concentrations of the ions. Ca, Mg, Zn are present in the range of ppm (parts per million) eg: Zn: 1ppm, whereas Cr is in the range of ppb (parts per billion) eg: Cr: 1-2 ppb

1.2 Results and Discussion

The aim of the project was to study the status of calcium, magnesium, zinc and chromium of T2DM patients of the age-group from the South Indian population and to check whether any significant correlation exist among the parameters. The data obtained from the analysis of serum sample is given in table below;

Sl.No.	Calcium (ppm) 90-110	Magnesium (ppm) 17-28	Zinc(ppm)0.7-1.	Chromium(ppb) (0.12- 2.1 ppb) also (3.2-7.7ppb)
1	54.5	12.9	1.5	1.204
2	119	15	1.59	1.488
3	97.5	16.4	1.43	0.622
4	101.9	10.7	1.68	1.48
5	113.8	11.8	1.35	0.638
6	121.5	12.1	1.39	1.02
7	87.1	15	1.37	0.46
8	113.5	12.5	1.59	2.86
9	76	10.5	1.43	1.94
10	109.1	10.9	1.11	2.86
11	62.1	15.2	1.79	2.47
12	111.6	11.6	1.43	1.78
13	113	12.5	1.64	1.9
14	28.7	11.3	1.37	1.83
15	54.4	13.3	1.68	2.16
16	92.5	11.1	1.63	2.95
17	43.1	11.6	1.62	2.52
18	58.9	10.2	1.58	2.14
19	23.8	10.8	1.17	2.98

20	116.8	15.7	1.57	0.2
21	106.8	13.2	1.43	1.92
22	109.6	10.2	1.71	1.94
23	72.9	16.4	1.78	0.76
24	44.5	10	0.94	0.45
25	67.6	12.1	1.05	0.81
26	90.8	13	1.1	0.8
27	110.7	10.3	1.49	1.5
28	111.9	12.1	1.08	1.27
29	112.9	11.5	1.41	1.23
30	48.8	12.5	1.14	0.64
31	70.7	14.5	1.85	0.39
32	108	13.3	1.7	1.36
33	103.6	11.9	1.67	1.63
34	96.3	11.6	1.55	0.9
35	122.4	10.5	1.13	1.68
36	43.1	11.6	1.62	2.53
37	123	13.4	1.61	0.83
38	117.5	11.6	1.29	2.16
39	80.2	12.5	1.38	2.04
40	93.1	16.4	1.61	1.71
41	114.5	11.1	1.68	1.82
42	101.9	10.6	1.31	1.49
43	93.8	13.2	1.44	2.34
44	110.7	13.1	1.2	1.55

45	107.6	12.2	1.52	1.41
46	94.8	13.8	1.63	0.44
47	70.8	9.4	1.36	1.36
48	77.9	10	1.1	1.46
49	70.8	13.9	1.6	2.58
50	99.9	11	1.6	0.27
51	106.2	13.5	1.63	1.58
52	104.1	11.1	0.74	0.57
53	45.1	12.7	1.07	1.11
54	112.2	7.9	1.58	0.98
55	82.9	10.8	0.8	1.68
56	117.3	9.6	1.2	1.35
57	101.7	10.2	1.69	1.05
58	117	12.4	1.63	0.66
59	76.4	17.6	1.5	1.25
60	113.6	13.1	1.64	1.6
61	120	12	1.47	1.8
62	74.6	9.3	1.7	2.54
63	111.7	8.2	1.55	1.92
64	101.8	11.2	1.7	3
65	105	12.6	1.41	1.87
66	94.5	14	1.99	0.85
67	91.3	12.9	1.5	1.9
68	72.3	14	1.34	0.93
69	24.3	10.5	1.33	1.06

70	47.5	12.6	1.408	0.75
71	44.9	10.7	1.46	1.44
72	79.3	17.8	1.6	1.5
73	75.5	17.3	1.8	0.54

The results of the study show that the concentrations of Ca, Zn, and chromium are within the normal ranges present in human serum, whereas Mg concentration was found lowered. The reported ranges of the parameters in healthy individuals and the observed ranges are given in table below;

Parameter	Reported Range in healthy subjects	Observed Range
Calcium	90-110 mg/L (ppm)	88.97397ppm
Magnesium	17-28 mg/L(ppm)	12.32877ppm
Zinc	0.7-1.4 mg/L(ppm)	1.460795ppm
Chromium	0.12-2.1µg/L(ppb)	1.489068ppb

1.3 CONCLUSION

Normal range of values:
Calcium : 90-110 ppm (mg/l)
Magnesium : 17-28ppm
Zinc : 0.7-1.4ppm
Chromium : 0.12-2.1ppb

It is concluded that

- The level of Zinc is within the normal ranges present in human serum.
- The status of chromium in patients with type 2 Diabetes mellitus neither increases nor decreases from the normal level.
- In patients with type 2 Diabetes mellitus, the level of calcium is found to be in the normal level.
- In the case of magnesium, the level is found to be decreased

REFERENCES

1. Hussain F, Trace element status in type 2 diabetes, Bangladesh journal of medical Science, Volume-8, June 2009, No. 3.
2. Kazi T G, Afridi H I, Kazi N, Jamali M K, Arain M B, Jalbani N, et al: Copper, chromium, manganese, iron, nickel, and zinc levels in biological samples of diabetes mellitus patients, Biol Trace Elem Res, 2008, 122:1-18.
3. Wijesekara N, Chimienti F, Wheeler M B, Zinc, a regulator of islet function and glucose homeostasis. Diabetes Obes Metab. 11(Suppl 4), 2009, 202-214.
4. Carafoli E, Santella L, Brace D & Brisi M. Generation, control, and processing of cellular of calcium signals. Crit. Rev.Biochem.Mol.Biol. Volume-36, 2001, 107-260.
5. Larsen C S, Knudsen T E,John H E, The role of calcium in stimulation of activated T lymphocytes with interleukin 2, Scandinavian Journal of Immunology, volume 24(6), 1987, 689-97.
6. Gorlach A, Klappa P, Kietzmann T. The endoplasmic Reticulam: Folding, Calcium Haemostasis, Signaling, and Redox control, Antioxidants and Redox Signaling, Vol. 8, September 2006, No 9-10: 1391-1418.
7. Miller III A, Bronner F, Calcium uptake in isolated brush-border vesicles from rat small intestine, Biochem J.1981,196:391-401.
8. Barger-Lux M J, Heaney R P, Lanspa S J et al. Clin Endocrinol Metab, 1995, 80:406-11.
9. Mc Alindon T, La Valley M et-al. Effect of vitamin D supplementation on progression of Knee pain and cartilage Volume loss in Patients with symptomatic Osteoarthritis. A randomnized controlled trial. JAMMA, Vol 309, January 9, 2013, No.2.
10. Tang B M, Eslick G D, Nowson C Lancet vol- 370, 2007, 657-66.
11. Song Y, Wang L, Pittas A G, Del Gobbo L C, Zhang C, Manson J E, Hu FB. Blood 25 –hydroxy vitamin D levels and incident type 2diabetes: a meta-analysis of prospective studies, Diabetes Care. , vol- 36(5), 2013 May , 1422-8.
12. Lorenzo C et al, Cardiovascular Type 2 Diabetes. European Association for the Study of Diabetes, 2013 September, 697:23-27
13. Misra S, Padmanaban P, Deepti G N, Sarkar G, Sumati S, Toora B D, Serum Magnesium and Dyslipidemia in Type -2 Diabetes Mellitus. Biomedical Research, volume- 23(2), 2012, 295-300.
14. Fox C, Ramsoomair D Carter C. Magnesium. its proven and potential clinical significance. South Med , volume- 94(12), 2001, 1195-201.
15. Toello L, Hypomagnesemia and Diabetes Mellitus. Arch Intern Med. Volume-156, 1996, 1143-8

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~ Ardra C Mathew