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Endemic Hyperdigoxinemia Related Cardiovascular and Endocrine Mucopolysaccharidoses Syndrome

Introduction

Kerala has a high incidence of endomyocardial fibrosis (EMF), chronic calcific pancreatitis (CCP), multinodular goitre (MNG) and mucoid angiopathic lesions presenting as coronary artery disease and thrombotic strokes. EMF claims 2.5% of all cardiac patients under 40 years who attend hospitals in Kerala. Kerala has the highest incidence of tropical pancreatitis in India. From 1980-1986 it accounted for 0.2% of total admissions and 7% of all diabetic admissions to the Trivandrum Medical College Hospital. MNG accounts for nearly 1.5% of total admissions in this hospital every year. Sandhyamoni has demonstrated a high incidence of mucoid angiopathic lesions in autopsy studies of the coronary and cerebral vessels with acid mucopolysaccharides accumulating in the tunica intima and media. There is magnesium deficiency in cardiac tissues of EMF patients. Magnesium deficiency is also a risk factor in the development of diabetes mellitus. In this context it has been reported that there are elevated levels of a hypothalamic endogenous membrane sodium potassium ATPase inhibitor, digoxin in the serum of diabetic patients. Membrane sodium potassium ATPase inhibition can lead to magnesium deficiency. Hypomagnesemia has been reported to produce upregulation of GAG synthesis in experimental models. Alteration in connective tissue metabolism may underlie EMF, CCP, MNG and mucoid angiopathy which are known to coexist in the very same geographic zone. Elevated digoxin levels and hypomagnesemia are known to contribute to insulin resistance. It has been reported that there is a high incidence of insulin resistance and metabolic syndrome x in Asian populations inhabiting the very same endemic zone.

Global warming leads to dehydration and osmotic stress. Global warming also leads to increased actinidic archaeal growth. Archaea catabolizes cholesterol and synthesizes digoxin. Digoxin can inhibit sodium potassium ATPase and increase intracellular calcium load producing mitochondrial PT pore dysfunction. This leads to oxidative stress. Osmotic stress and oxidative stress can induce the enzyme aldose reductase which converts glucose to fructose. Fructose has got a low km value for ketokinase as compared to glucose. Therefore fructose gets selectively phosphorylated to fructose phosphate which can be converted to glucosamine and galactosamine. Thus aldose reductase inhibition consequent to osmotic and oxidative stress of global warming can induce glycosaminoglycan synthesis. This study was undertaken to assess the following parameters in EMF, CCP, MNG and mucoid aniopathy-serum magnesium, serum total GAG, serum levels of various GAG fractions and serum digoxin levels. A hypothesis highlighting the pivotal role of endogenous digoxin in the pathogenesis of these disorders, which could be different spectra of a common insulin resistance state is also presented. This can lead to a global warming related Lemurian cardiovascular and endocrine syndrome.

Materials and Methods

Informed consent was obtained from all the patients as well as relatives of subjects included in the study. Necessary ethical clearance was also obtained for this study from the ethical committee of the Medical College Hospital, Trivandrum. Fifteen cases of EMF, CCP, MNG and mucoid angiopathic strokes and CAD from Department of Medicine, Cardiology and Gastroenterology of Medical College Hospital, Trivandrum were chosen for the study. The patients aged 25-50 years were selected randomly over a period of 2 years as and when they were admitted to the wards. They were all freshly diagnosed cases and

fasting blood was removed from them before any treatment was started as was from the equal number of age and sex matched healthy controls. The healthy normal controls were selected randomly from the general population of the Trivandrum district. Autopsy samples of heart tissue from EMF patients were supplied by the Sree Chitra Tirunal Institute for Medical Sciences and Technology. Autopsy samples of thyroid tissue from MNG cases were obtained from the Department of Pathology, Medical College Hospital, Trivandrum and autopsy samples of pancreatic tissue from chronic calcific pancreatitis patients were supplied by the Diagnostic and Research Centre, Trivandrum. Carotid arteries and aortic specimens with histopathologically demonstrated mucoid angiopathy were obtained from the Department of Forensic Pathology, Medical College, Alleppey. For comparison cardiac, thyroid, pancreas, aortic and carotid tissue from accident subjects (25-50 yrs) were used and these were also obtained from the Department of Forensic Pathology, Medical College, Alleppey. Concentration of total GAG and various GAG fractions were determined by methods described earlier. The tissue GAG was extracted by Folch's procedure. The dry defatted tissue was digested with papain and GAG was estimated as described before. Total cholesterol, HDL cholesterol and triglycerides were estimated in the serum by enzymatic methods. The kits were supplied by Sigma Chemicals, USA. Serum magnesium was determined by atomic absorption spectrophotometry. RBC membrane for estimation of sodium potassium ATPase was prepared according to the procedure of Blostein. For estimation of sodium potassium ATPase activity of the erythrocyte membrane, the procedure described by Wallach and Kamat was used. Digoxin in the serum was determined by the procedure by Wallah and Kamat was used. Digoxin in the serum was determined by the procedure described by Wallah and Kamat was used. Digoxin in the serum was determined by the procedure described by HPLC. Statistical analysis was carried out by ANOVA.

Results

1. Lipid profile of patients with EMF, CCP, MNG and mucoid angiopathy showed normal serum LDL cholesterol, low serum HDL cholesterol, elevated serum triglycerides and normal total serum cholesterol

2. Serum magnesium was decreased in patients with EMF, CCP, MNG and mucoid angiopathy

3. Serum total GAG was elevated in patients with EMF, CCP, MNG and mucoid angiopathy

4. The individual serum GAG fractions showed the following patterns in mucoid angiopathy - ChS forming 76.19% followed by DS forming 10% of GAG. The other fractions - HA, HS and H are present only in small amounts

5. Tissue total GAG was elevated in patients with EMF, CCP, MNG and mucoid angiopathy

6. RBC membrane sodium potassium ATPase activity was reduced in patients with EMF, CCP, MNG and mucoid angiopathy.

7. Serum digoxin level was elevated in patients with EMF, CCP, MNG and mucoid angiopathy.

Discussion

Endogenous digoxin like activity (EDLA) has been reported in several pathological conditions by reaction with digoxin antibodies. We have recently shown that the EDLA is in the fact due to the steroidal glycoside, digoxin itself.

Endogenous digoxin which is reported to be synthesized by the hypothalamus and other tissues, is a potent inhibitor of membrane sodium potassium ATPase. It is also reported that several plant sources contain digoxin like steroidal glycosides and the vegetarian diet consumed by populations in this geographic zone may be a rich source for digoxin. The increase in digoxin levels in the serum of these patients may therefore be due to increased endogenous synthesis and/or that derived from dietary sources. The decrease in membrane sodium potassium ATPase activity in the patients of CCP, EMF, MNG and mucoid angiopathy may be due to this increase in digoxin levels. The inhibition of membrane sodium potassium ATPase by digoxin is known to cuae an increase in intracellular calcium resulting from increased sodium calcium exchange, increased entry of calcium via the voltage gated calcium channel and increased release of calcium from intracellular endoplasmic reticulum calcium stores. This increase in intracellular calcium by displacing magnesium from its biding sites, causes a decrease in the functional availability of magnesium. The decrease in the availability of magnesium can cause decreased mitochondrial ATP formation which along with low magnesium can cause further inhibition of membrane sodium potassium ATPase, since the ATP-magnesium complex is the actual substrate for this reaction. Cytosolic free calcium is normally buffered by two mechanisms, ATP dependent calcium extrusion from the cell and ATP dependent sequestration of calcium within the endoplasmic reticulum. The magnesium related mitochondrial dysfunction results in defective calcium extrusion from the cell. There is thus a progressive inhibition of membrane sodium potassium ATPase activity first triggered by digoxin. Low intracellular magnesium and high intracellular calcium consequent to membrane sodium potassium ATPase inhibition appear to be crucial to the pathophysiology of these disorders. Membrane sodium potassium ATPase inhibition could in part be contributed to by the low level of background irradiation from the mineral

sands in Kerala coast which produces membrane disruption. Earlier it was postulated that the cerium from the mineral sands is ingested and is exchanged for magnesium leading to magnesium depletion had postulated a protein deficiency model for this group of disorders. But there is no nutritional data to support any but marginal protein deficiency in the Kerala population. It is interesting to note that intracellular magnesium deficiency can produce ribosomal disruption and inhibition of protein synthesis. Therefore protein deficiency could be a functional correlate of magnesium depletion. The population of Kerala consumes a high carbohydrate and high fibre diet. The total dietary fibre intake is around 20 g per day. Fibre has been reported to bind to magnesium producing magnesium depletion via the stools. Cyanide from dietary sources has also been implicated in the etiology of this group of disorders. Cyanide can produce inhibition of cytochrome oxidase and produce a mitochondrial dysfunction leading to ATP depletion and membrane sodium potassium ATPase inhibition. Serum magnesium was assessed in all these disorders and was found to be reduced.

Increased calcium within the cell can open the mitochondrial PT pore, disrupt the inner membrane hydrogen gradient and uncouple oxidative phosphorylation. This results in volume dysregulation of the mitochondrial matrix, hyperosmolality of the matrix, outer membrane rupture and release of cyto C and AIF (apoptosis inducing factor) into the cytosol. This activates procaspase 9 to caspase 9 which produces apoptosis and replacement fibrosis. The intracellular magnesium depletion related inhibition of glycolysis, citric acid cycle and oxidative posphorylation can channel more glucose-6 phosphate for the synthesis of GAG precursors. Magnesium deficiency in fact has been shown by us to result in glycosaminoglycan accumulation in tissues. Glysosaminoglycan accumulation has been described in these disorders, which can be explained as being due to

magnesium deficiency. Increase in beta cell calcium can contribute to increased insulin release from beta cells and hyperinsulinemia. Hypomagnesemia has been reported to markedly increase glucose stimulated insulin secretion by the perfused pancreas. Magnesium deficiency can also lead to insulin resistance. Magnesium translocation appears to be an early event in insulin action. Decrease in intracellular magnesium can block the phosphorylation reactions involved in protein tyrosine kinase receptor activity leading to insulin resistance. Elevated fibre content of the diet can also mimic the action of insulin leading to hyperinsulinism. On the other hand magnesium deficiency produced by dietary fibre can lead to insulin resistance. Insulin administration has been reported to lead to an upregulation of GAG synthesis. The lipid profile of the patient with EMF, MNG, CCP and mucoid angiopathy is similar to that obtained in the insulin resistance state. It is tempting to speculate that all these diverse group of disorders are due to insulin resistance.

Increase in intracellular calcium can activate the G-protein coupled signal transduction system of the contra-insulin hormones - glucagon, adrenaline, noradrenaline and the growth hormone via the growth hormone releasing factor. This results in increased production of glucose. Decrease in intracellular magnesium can lead to inhibition of glycolysis causing defective glucose utilization and hyperglycemia. Increase in intracellular calcium can open up the mitochondrial PT pore, disrupt the hydrogen gradient across the inner membrane and block mitochondrial oxidative phosphorylation. Intracellular magnesium deficiency can also lead to a ATP synthase defect. All this leads to defective glucose utilization and hyperglycemia. As already described above increased intracellular calcium and reduced intracellular magnesium can lead to hyperinsulinism and insulin resistance described in CCP. Again the increased intracellular calcium can activate the T cells via the calcium dependent

calcineurin pathway producing an increase in TNF alpha secretion. This results in immune activation and the inflammatory changes noticed in the pancreas and other tissues especially peripheral nerve in CCP. Increase in TNF alpha secretion. This results in immune activation and the inflammatory changes noticed in the pancreas and other tissues especially peripheral nerve in CCP. Increase in TNF alpha can also contribute to insulin resistance. Decrease in intracellular magnesium can result in a glycosylation defect since magnesium is a cofactor for formation of dolichol-1 phosphate required for N-glycosylation and nucleoside diphosphate sugars required for O-glycosylation. The defective glycoproteins may be responsible for micro and macroangiopathy of diabetes mellitus in CCP. Defectively processed N-glycosylated glycoproteins can also lead on to a defective insulin sensing mechanism of the beta cells. Intracellular magnesium deficiency can lead to upregulation of GAG synthesis and their accumulation in the pancreatic tissues. MPS accumulation in the pancreas has been described in CCP.

The intracellular deficiency of magnesium, which is required for protein transcription and translation, results in a nuclear and ribosomal dysfunction. The thyroid hormone receptor belongs to the steroid receptor family and has a DNA binding site. The intracellular magnesium deficiency results in a thyroid hormone receptor defect. This results in defective feed back inhibition to the hypothalamus and pituitary and increased TRH and TSH secretion. There is also increased activity of TSH and TRH owing to increased intracellular calcium and increased G protein coupled signal transduction of these hormones. The multinodular goiter arises owing to TSH dependent thyroid proliferation. The inhibition of sodium potassium ATPase produces inhibition of the sodium potassium ATPase coupled neutral amino acid tyrosine transport into the thyroid follicular cell. The iodide transport into the thyroid follicular cell also

depends on ATP. The intracellular magnesium depletion produces defective ATP synthesis and a mitochondrial dysfunction in the thyroid follicular cell. This results in defective iodide transport into the cell. Defective iodide and tyrosine transport into the cell can produce defects in thyroid hormone synthesis and the resulting hypothyroidism can lead on to increased TSH levels. The depleted intracellular magnesium can stimulate mucopolysaccharide biosynthesis and fibrosis of the thyroid gland. MPS accumulation has been demonstrated in the thyroid tissue in MNG.

CCP and MNG are precancerous states. CCP has been found to lead to carcinoma of the pancreas and multinodular goitre is a predisposing factor for thyroid malignancies. Membrane sodium potassium ATPase inhibition can lead to oncogenesis. Intracellular calcium activates phospholipase C beta which results in increased production of diacylglycerol (DAG) with resultant activation of protein kinase C. The protein kinase C (PKC) activates the MAP kinase cascade resulting in cellular proliferation. The decreased intracellular magnesium can produce dysfunction of GTPase activity of the alpha-subunit of G protein. This results in RAS oncogene activation, as more of the RAS is bound to GTP rather than GDP. Phosphorylation mechanisms are required for the activation of the tumour suppressor gene P_{53}. The activation of P_{53} is impaired owing to intracellular magnesium deficiency producing a phosphorylation defect.

Membrane sodium potassium ATPase inhibition can produce a decrease in the intramyocardial cell magnesium and an increase in the intramyocardial cell calcium. The increase in intramyocardial cell calcium can open the mitochondrial PT pore, destroy the hydrogen gradient across the inner membrane and uncouple oxidative phosphorylation. The decrease in intramyocardial cell magnesium can inhibit ATP synthase and produce a

defective mitochondrial oxidative phosphorylation. This results in a myocardial mitochondrial dysfunction and increased generation of free radicals due to incomplete reduction of molecular oxygen. Free radicals can produce damage to myocardium. The opening of the mitochondrial PT pore can produce osmotic dysregulation and hyperosmolality of the mitochondria, rupture the outer mitochondrial membrane and release AIF (apoptosis inducing factor) and cyto C in to the cytosol. This activates procaspase 9 to caspase 9 producing myocardial apoptosis and replacement fibrosis. The decrease in intracellular magnesium can result in increased channelling of glucose 6-phosphate to the synthesis of GAG precursors and increased glycosaminoglycan biosynthesis. Decrease in intracellular magnesium can produce changes in collagen and elastin biosynthesis and result in replacement fibrosis. MPS accumulation has been demonstrated in EMF tissues by previous studies. The increase in intracellular calcium can produce T cell activation via the calcium dependent calcineurin pathway releasing TNF alpha, from the activated T cell. Elevated levels of TNF alpha have been related to myocardial dysfunction. Impairment of calcium homeostasis, decrease in magnesium ATPase activity and troponin I phosphorylation, cytokine expression especially TNF alpha and aberrant induction of apoptosis have been described in cardiomyopathies. The basic defect in cardiomyopathy is thus interstitial/endocardial fibrosis and MPS/elastin deposition which can occur in magnesium deficiency. Increase in intracellular calcium can activate the G protein coupled signal transduction system of the platelet activating factor and thrombin. Magnesium deficiency can produce increased platelet aggregation and release reaction and thrombosis resulting in the formation of a LV or RV thrombus which can get fibrosed leading on to endocardial fibrosis.

Membrane sodium potassium ATPase inhibition induced hypomagnesemia related increased glycosaminoglycan synthesis can contribute to mucoid angiopathy. Increased intracellular calcium within the endothelial cell leads to fragmentation of the elastic membrane and calcification. Increased calcium within the arterial wall alters elastin synthesis, turnover and composition. Increase in arterial wall cellular calcium can open the mitochondrial PT pore, produce osmotic dysregulation and hyperosmolality of the mitochondrial matrix, rupture the outer membrane and release cyto C and AIF to the cytosol producing vascular wall cellular apoptosis. In mucoid angiopathy there is a uniform hose pipe like narrowing of large, medium sized and small sized arteries with deposition of acidic mucopolysaccharides in the tunica intima and media. Increase in intracellular calcium can activate the G-protein coupled thrombin receptor and platelet activating factor producing the thrombosis observed in mucoid angiopathy. Decreased intracellular magnesium can lead to increased thrombin and ADP/collagen induced platelet aggregation. Membrane sodium potassium ATPase inhibition related increased smooth muscle calcium and decreased magnesium can contribute to the vasospasm and ischaemia observed in mucoid angiopathic, stroke and CAD. Decreased intracellular magnesium can produce dysfunction of lipoprotein lipase producing defective catabolism of triglycerides rich lipoproteins and hypertriglyceridemia. In hypomagnesemia, lecithin cholesterol acyl transferase (LCAT) is defective and there is reduced formation of cholesterol esters in HDL. This results in reduced HDL cholesterol levels described in mucoid angiopathy. The increase in intracellular calcium can activate phospholipase A_2 producing increased amounts of arachidonic acid for thromboxane A_2 synthesis via the cyclooxygenase pathway. This can lead on to increased platelet aggregation and thrombosis in mucoid angiopathy.

MNG, CCP, EMF and mucoid angiopathy belong to the same geoendemic zone. The study shows that they share the same etiological factors of causation.

1. Low membrane sodium potassium ATPase and magnesium depletion consequent to increased digoxin from exogenous or endogenous sources

2. Hypomagnesemia related elevated GAG synthesis

3. Digoxin induced hypomagnesemia related insulin resistance state/hyperinsulinism producing elevated GAG synthesis.

Therefore these disorders could be termed as the endemic hyperdigoxinemia related cardiovascular and endocrine mucopolysaccharidoses syndrome. Global warming leads to dehydration and osmotic stress. Global warming also leads to increased actinidic archaeal growth. Archaea catabolizes cholesterol and synthesizes digoxin. Digoxin can inhibit sodium potassium ATPase and increase intracellular calcium load producing mitochondrial PT pore dysfunction. This leads to oxidative stress. Osmotic stress and oxidative stress can induce the enzyme aldose reductase which converts glucose to fructose. Fructose has got a low km value for ketokinase as compared to glucose. Therefore fructose gets selectively phosphorylated to fructose phosphate which can be converted to glucosamine and galactosamine. Thus aldose reductase inhibition consequent to osmotic and oxidative stress of global warming can induce glycosaminoglycan synthesis. The fructose phosphorylation depletes the cell of ATP. This results in inhibition of glucose phosphorylation, glycolysis and mitochondrial oxidative phosphorylation. The depletion of cellular ATP results in oxidative stress. Oxidative stress can open the mitochondrial PT pore, release cytochrome c and activate the caspase cascade of cell death. Oxidative stress generated by cellular depletion of ATP consequent to fructose phosphorylation and digoxin induced mitochondrial dysfunction can produce

further aldose reductase induction. This results in more conversion of glucose to fructose. The fructose can produce mitochondrial dysfunction on its own. The fructose can also fructosylate proteins making them antigenic and the oxidative stress induced by fructose phosphorylation and depletion of cellular ATP can activate NFKB producing immune activation. The fructose phosphate can get converted to glucosamine and galactosamine increasing GAG synthesis. This results in increase in heparan sulphate which can combine with proteins producing amyloidosis. Thus the cell death, glycosaminoglycan synthesis, amyloidogenesis, immune activation and autoimmunity produced by fructosemia or fructositis can contribute to mucoid angiopathy, endomyocardial fibrosis, chronic calcific pancreatitis and multinodular goitre. This can be called a global warming related mucopolysaccharidotic syndrome.

References

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