BIBILE MEMORIAL ORATION

IMPACT OF MINERAL HOMEOSTASISON THE PATHOGENESIS OF SOME COMMON DISEASES IN SRI LANKA

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Abstract

Minerals play an important role in the homeostasis of human body functions. Increased levels of such minerals may lead to their precipitation or accumulation at different body sites leading to diseases. In this oration, effects of Manganese on sulfation of neurotransmitters, work on gall stones and renal stones in Sri Lankan population is discussed.

Oration

Professor Senaka Bibile was born in 1920 and educated at Trinity College, Kandy. He obtained his medical degree from University of Colombo with first class honours. He obtained distinctions and gold medals for Medicine and Surgery. He completed his Ph.D. from the University of Edinburgh. He was the first Dean Faculty of Medicine at University of Peradeniya for three years in from 1967.

Homeostasis is the secret of a healthy life. Minerals play an important role in metabolism and metabolic disorders. Metals are found in the earth's crust and the amount vary according to the geographical location. Heavy metals have a specific density of more than 5 g/cm^3 and adversely affects many living systems[1]. Minerals play a key role in our body as regulators for metabolic functions, acid base balance, maintaining normal cellular functions, as structural units of bones and teeth and as some cofactors in the enzymatic reactions. Kidney and liver functions play a key role in their metabolism and excretion respectively. In this oration, some important aspects related to minerals and electrolytes, as discovered and experimented by our work, from 1996 would be discussed.

Effect of Manganese on sulfation of neurotransmitters

Manganese promote the production of Dopamine Sulfate and decrease of Dopamine in Sprague-Dawley Rats

Manganese is an important metal in living organisms[2] and an important component of

a number of enzymes and proteins such as photosynthesis system II and superoxide dismutase[3]. But at high concentrations it can be neurotoxic[4]. Occupational exposure to manganese dust in the miners has resulted in neurological symptoms mimicking Parkinsonism[5]. Manganese effects the enzymatic conversion dopamine to norepinephrine by influencing the enzyme responsible for this conversion[6]. Majority of dopamine in human plasma circulates as sulfate-conjugated form. Following manganese exposure, dopamine/Dopa/ tyrosine-sulfating sulfotransferase may be activated. Dopamine, L-Dopa, and L-p-tyrosine may be sulfated and excreted, lowering the levels eventually giving neurological disorders. Therefore, the manganese-poisoned individuals may show symptoms as of Parkinson's disease[5], and relieving of

serum samples by HPLC is shown in Table 1. In manganese treated rats, serum level of dopamine sulfate has been elevated than in untreated control rats or rats treated with sodium sulfate. There was a decrease in the serum level of un sulfated dopamine in manganese-treated rats and L-Dopa sulfate and L- p-tyrosine sulfate were also increased. The higher combined amounts of unsulfated and sulfated dopamine may be due to the stimulatory effects of manganese on tyrosine hydroxylase [9,10].

Finding manganese in internal organs of the exposed animals is an important issue. As shown in Table 2, brain appeared to be the organ with the highest degree of manganese accumulation in manganese exposed rats. The reason for higher degree of manganese accumulation in the brains of treated rats may be due to abnormal

Table 1: Unsulfated and sulfated dopamine, L- Dopa, and L-p-tyrosine in the serum of different experimental groups of rats

Substrate	Group 1 (Control)	Group 2 (20 mg NaCl)	Group 3 (20 mg Mn)	Group 4 40 mg Mn
Dopamine	0.041 ± 0.018	0.022 ± 0.010	0.014 ± 0.004	0.008 ± 0.005
Dopamine Sulfate	0.014 ± 0.009	0.016 ± 0.010	0.0179 ± 0.048	0.158 ± 0.062
L- Dopa	0.191 ± 0.058	0.227 ± 0.101	0.239 ± 0.033	0.316 ± 0.152
L- Dopa Sulphate	0.013 ± 0.006	0.20 ± 0.003	0.117 ± 0.009	0.082 ± 0.034
L- p-Tyrosine	47.99 ± 7.63	42.67 ± 13.55	61.35 ± 14.91	48.89 ± 19.56
L-p-Tyrosine Suphate	0.142 ± 0.040	0.135 ± 0.023	0.173 ± 0.015	0.248 ± 0.097

Data shown represent mean SD derived from five experiments.

some of these symptoms has been observed with L-Dopa[7]. To test the validity of this hypothesis, an animal study was carried out to investigate the effects of manganese on excretion of the sulfated form of m-tyrosine[8]. Further, patterns of deposition of Manganese in major organs was determined.

The analysis of un-sulfated and sulfated dopamine, L-Dopa, and L-p-tyrosine in

dopamine metabolism and, whether it is due to the involvement of manganese dopamine-sulfating sulfotransferase enzyme(s) in neurons of the brain.

It can be concluded that a dramatic increase in serum levels of dopamine sulfate, dopa, and -tyrosine; and the concomitant decrease in dopamine is seen in those exposed to manganese. This study shows the occupational risk of the

Table 2: Manganese accumulation in different organs in different experimental groups of rats

Organ	Group 1 (Control)	Group 2 (20 mg NaCl)	Group 3 (20 mg Mn)	Group 4 40 mg Mn
Liver	6.20 ± 0.01	5.83 ± 0.18	10.45 ± 0.24	13.11 ± 0.01
Brain	1.06 ± 0.01	2.52 ± 0.63	3.04 ± 0.91	5.58 ± 0.017
Heart	1.89 ± 0.63	1.61 ± 0.18	2.79 ± 1.36	6.46 ± 3.31
Kidney	1.59 ± 0.01	2.09 ± 0.79	3.35 ± 0.57	7.65 ± 0.31

Data shown represent mean derived from five experiments.

people who are being exposed to manganese long term.

Stereoselective and manganese-dependent sulfation and urinary excretion of D-form and L-form meta-tyrosine O-sulfate by Sprague–Dawley rats

In this study, three groups of six male rats were treated with different concentration of $MnCl_2$ (3 mM, 10 mM and 30 mM) with 0.2 mM DL-m-tyrosine per day for 7 days. Urine was analyzed for the sulfated DL-m-tyrosine (DL-m-TyrS) by HPLC. Fig 1. Urinary DL-m-TyrS, was present in the urine of the $MnCl_2$-treated rats but not control rats, The D-form and L-form m-TyrS present in the urine sample of $MnCl_2$-treated rats were detected by Chiral HPLC. These results give evidence for the occurrence of stereoselective and manganese - dependent sulfation of DL – m-tyrosine. D tyrosine cannot be used for protein synthesis, the sulfation maybe used for increasing the water solubility and excretion.

Calcium Phosphorous and other metals as constituents in Gall stones

Chemical characterization of gallstones to explore the pathogenesis of gallstone disease in Sri Lanka

Gallstones (GS) are formed in the gallbladder (GB) and bile duct, from bile constituents[11]. Cholesterol and calcium

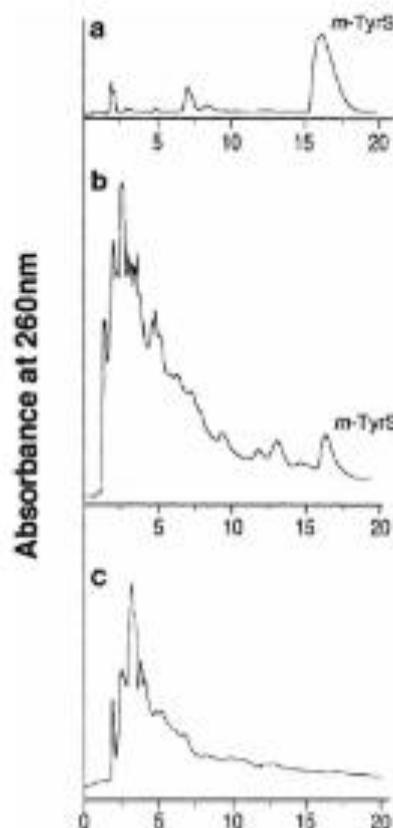bilirubinate are the main chemical compounds that precipitation in bile

Fig 1
Ion-pair HPLC (a)syntheticdl-m-TyrS and (b) urine.

induced by multiple aetiological factors. Therefore, it is likely that chemical composition of GS would indicate the process of development of these GS[12,13].

Heavy metals are also detected in varying concentrations in different types of GS [14] and some trace minerals are suspected to be involved in the development of pigment GS as well.

The chemical composition of GSs vary from population to population[15]. There are multiple aetiological factors in the pathogenesis of GS. Fourier Transform Infrared Spectroscopy (FTIR) is widely used in the analysis of chemical composition of GSs[16,17]. X—ray Powder Diffraction (XRD)[18] and colorimetric assays[19] are also available. Microstructure of the GS is identified by Scanning Electron Microscopy[20] and, it is the best method for describing the microstructure of GSs. Elemental composition of these GSs is analyzed by using Atomic Absorption Spectrophotometry and Particle Induced X-Ray Emission [21]. Previous studies have shown that mixed cholesterol and black pigment stones were the predominant types in South Asian region[22].

Our work described the chemical composition of GSs in patients presenting to Teaching Hospital, Peradeniya and was carried out at Teaching Hospital, Peradeniya, Sri Lanka from May 2011 to December 2012. We recorded the appearance of the GSs and FTIR, XRD and AAS analysis was used to determine the composition of GSs.

We included 102 patients in our study and 80% of the patients had GSs in GB and 20% had GSs both in GB and bile ducts. Cross sectional appearance of the GS are shown in Figure 2. GS which had crescentic layers of dark (black or brown) and light (pale white) colour material from center to periphery (Figure 2B) were named as mixed cholesterol GS (n = 38, 37%)[23]. All remaining GSs (n = 54, 54%) which had homogenously distributed black material (Figure 2C) were categorized as pigment GSs[23].

The chemical compositions of different types GS are shown by FTIR (Table 3) and the SEM images and calcium distribution of three different types of gallstones are shown in Figure 3. The composition obtained by Energy Dispersive X-ray Spectroscopy (EDS) revealed that, majority of GSs (68%) were composed of multiple chemical compounds, while only 32% of GSs were composed only of a single compound. Calcium bilirubinate, calcium carbonate and calcium phosphate were the common calcium salts in the GS samples. Core of mixed cholesterol GSs had calcium salts namely, bilirubinate, carbonate and phosphate.
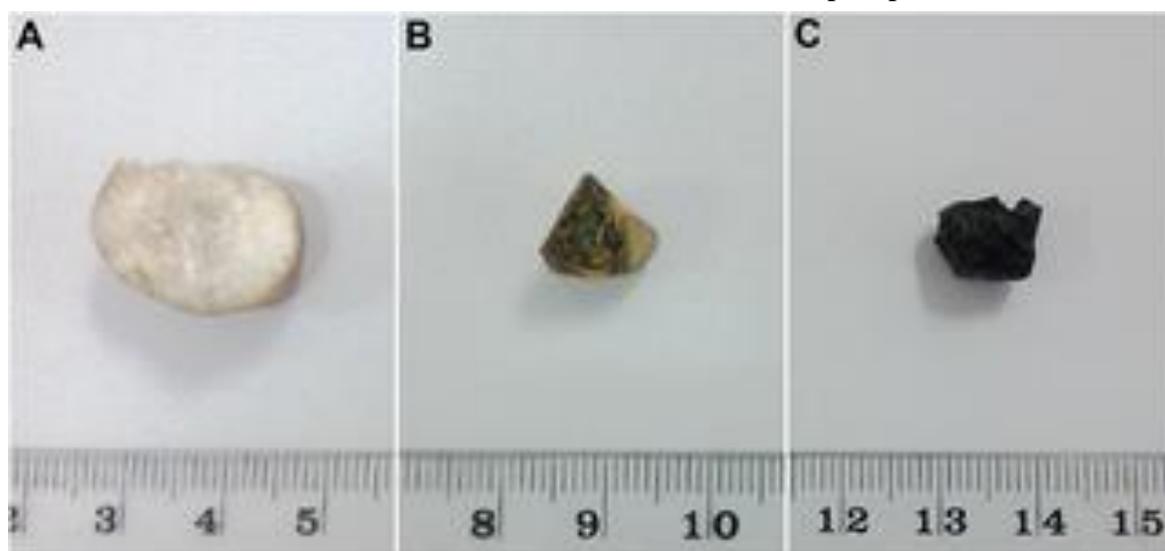

Figure 2: Cross sectional appearance of each type of gallstone.

A—Pure cholesterol B—Mixed. C—Pigment stone

Table3: Chemical composition of gallstones by FTIR.

Type of GS	Chemical Composition	Frequency n%
Pure Cholesterol	Cholesterol	10 (09)
Mixed Cholesterol	Cholesterol, Calcium bilirubinate, Calcium carbonate, Calcium phosphate	38 (37)
	Calcium bilirubinate, carbonate, phosphate	23 (23)
Pigment	Calcium bilirubinate, palmitate	06(06)

GS disease in Kandy district, Sri Lanka is common among middle aged females. Presence of different calcium salts (calcium bilirubinate, carbonate and phosphate) was a common feature.

Heavy metal Pb^{2+} was found in all the tested GS samples while Cd^{2+} was detected only in 70% pigment and 21% cholesterol GS samples.

Possible prediction of type of GSs with known risk factors

Different types of cholesterol GSs, mixed cholesterol and black pigment GSs are identified as the major type of GS in South Asia[22]. Therefore, the identification of aetiopathogenesis of GSs of both mixed cholesterol and black pigment GS is important in implementing preventive measures for GS among South Asians. Mixed cholesterol and black pigment stones have the same chemical constituents namely cholesterol, calcium bilirubinate, phosphate and carbonate[23]. Cholesterol is the main constituent in mixed cholesterol GS while calcium bilirubinate is the main constituent in black pigment GS. Apart from that gall bladder hypomotility[24], and effects of trace elements[25] and other factors such as intestinal hypermobility and phospholipid concentrations[26] are under investigation as the causes for pathogenesis of black pigment GS, as most of the incidence of this type of stone cannot be explained by the classic causes.

In our studies[23] we found that mixed cholesterol GSs were commoner among females and that Moors with a BMI over 25 kg/m^2 also had mixed cholesterol stones. We also found that black pigment GSs were commoner among patients with type II diabetes. Therefore, we concluded that models could be made with further studies, to identify risk groups for different types of GS diseases. These could be used in developing preventive strategies.

Impact of minerals on the pathogenesis of urinary stones

Anatomical location of human renal stones, mechanism of nucleation and growth and composition in Sri Lankan population

Urolithiasis is defined as any calculi originating within urinary system from kidneys to urethra. Calculi at initial stages are usually asymptomatic and symptoms arise depending on the size, anatomical location and chemical composition[27]. Classification of renal stones are mainly based on their anatomical location and chemical composition. Although rare, classifications based on morphological appearance of renal stones may give more information on the history[28]. Risk factors

for renal stone formation can be classified as dietary, metabolic, environmental and urinary factors[29]. Many attempts have been made to elucidate the process of stone formation in humans[30]. Binding of calcium to urinary proteins such as Tamm Horsfall glycoprotein may provide a nucleus for the formation of urinary stones and may
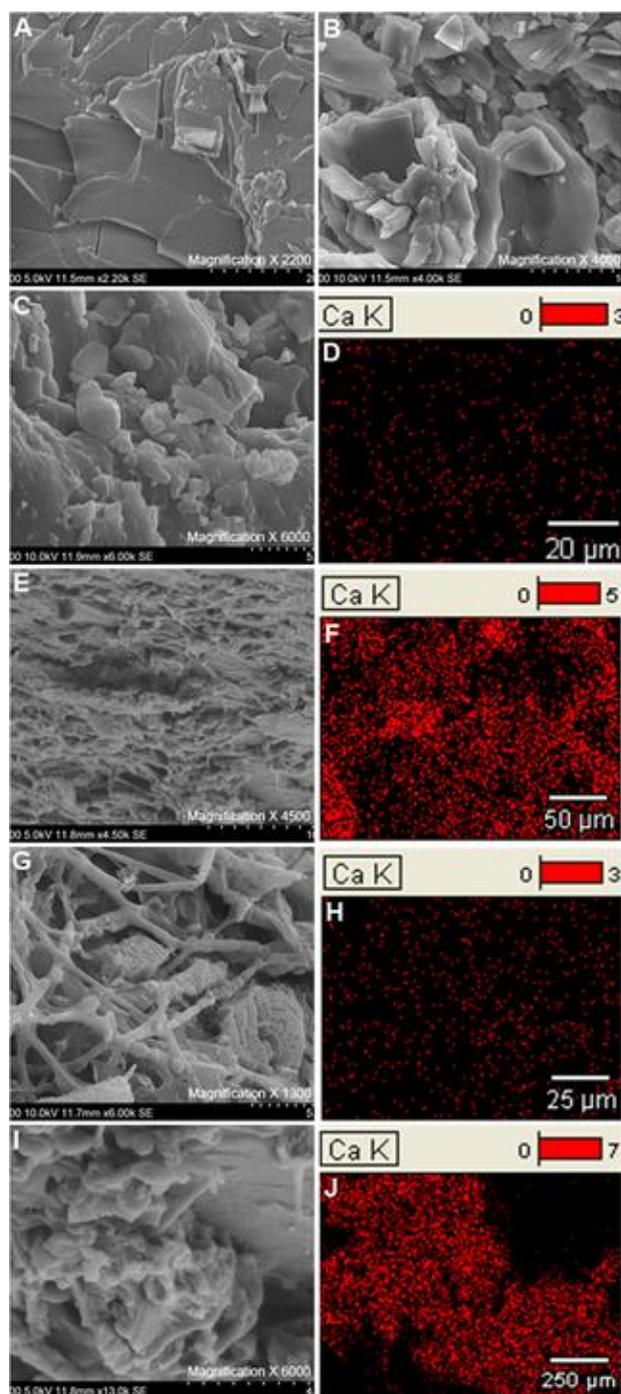
Fig 3. Microstructure and calcium distribution of different types of gallstones.

A, B—Pure cholesterol stone C—Mixed cholesterol stone. D—Calcium distribution of the mixed cholesterol GS. E- Pigment stone, F—Calcium distribution of the pigment GS G—Pigment stone H—Calcium distribution of the pigment stone I—Pigment stone J— Calcium distribution of the pigment stone

precipitation is known as heterogeneous nucleation[32].

A study based on artificial urine has revealed that the possibility for the formation of calcium oxalate is higher than that of calcium phosphate. Further, calcium oxalate is the commonest and an important constituent for renal calculi[33]. About 85% of the stones consists of calcium oxalate associated with minor calcium phosphate although phosphate salts are a commonly found chemical constituent in urine. It indicates that precipitation of calcium oxalate is much favorable despite their lower concentrations[28]. So far, many studies have been carried out on morphological differentiation of renal stones based on chemical composition. However morphological differentiation with respect to the anatomical location, nucleation, lamination and crystal arrangements has not yet clearly been established. Despite extensive studies have been performed in relation to occurrence of renal stone in many parts of the world in the recent past, only few are available from Sri Lanka[34]. These studies described the variation of chemical composition of renal stones with respect to the geographical location of the country and the incidence rate of stag horn calculi.

There is no reported literature regarding the correlation of anatomical location and the morphological appearance even in other parts of the world. However, it can be assumed that there should be a strong relationship between the morphological features of stones (both external and internal) and anatomical locations due to different environments of these locations. Therefore, correlation of such data is useful to interpret the origin and growth of stones. Thus we characterized the kidney stones with respect to the anatomical locations and chemical composition. Further we aimed to assess the nucleation

and the rate of growth of kidney stones at different anatomical locations.

Sample collection was carried during March 2013 to December 2013 from patients who underwent open surgeries for renal stone disease, irrespective of their age, sex and they were sealed until the analyses were performed. Seventy six (76) samples were collected[35]. Analyses included basic petrographic studies, including visual estimation of grain size, shape, appearance of surface, availability of crystals on the surface. The outer surface of stones was observed using optical stereoscopic microscope. Then the stones were cut by Fred's saw perpendicularly to the surface and the nucleus and the periphery zones of the stones were selected for most of sections.

Microscopic studies were carried out to assess the size of crystals and to interpret the abundances and nature of amorphous materials, internal textural characteristics and the porosity of stones nucleation, crystallinity and laminations. Nucleus of renal stone mainly consisted of brown - black substances with irregular shapes. The brown, black substances were mainly free of crystals. So they were not sensitive to the depolarized light. Substances which contained high amount of organic matter have this feature. It indicated that nucleus of renal stone mainly consisted of organic matter rather than inorganic crystals. Ureteric stones frequently interact with ureteric wall which causes the haematuria. Therefore, the darker colour of them may be due to accumulation of iron derived from haemoglobin

Colour of a stone is mainly determined by the chemical composition of urine. However, we identified that their anatomical location also plays the major role on the variation of the colour. Amorphous areas which is supposed to be rich in organic matter are normally high in staghorn calculi. The lowest amounts of

organic matter were noted from bladder stones and their cavity content was remarkably low. Therefore, we hypothesized that the major factor that effect on the cavitations of a stone is their organic content. Cavitation was not significant in the periphery areas, predominantly in ureteric stones and bladder stones. It indicated that the organic matter do not attach to stones during the latter period of the stone growth and only the inorganic processes dominate.

The variation of elemental composition within the stones was determined by spot analysis from nucleus to the peripheral area. Calcium was the most abundant cation in all regions and several elements were detected in the nucleus area compared to the peripheral area of each stone. Higher variation of elements in nuclear area of renal stones indicates that formation of renal stone is complex process and involves large number of compounds. But after formation of nucleus, peripheral area shows limited variation in chemistry. These significant variations of chemical composition in two zones indicate that formations of these zones are governed by two different chemical processes. Results of petrologic study are also conformed the observation. Further, it can be suggested that nuclear formation is predominantly an organic process and peripheral zone formation is predominantly an inorganic process.

In our studies it was shown that the nucleus of calculi are different in each anatomical location[35]. Nucleus of pelvicalyceal system and upper ureteric calculi shows higher concentration of organic material and lower concentration of inorganic crystals through plane polarized light microscope and other crystal structure determination procedures. Calculi in bladder and lower ureter showed initial dislodgement of nucleus with higher amount of crystallinity. Optical studies revealed that peripheral areas of stones

recovered from pelvicalyceal system and upper ureter has lower degree of crystallinity and higher level of organic matter and cavitation while lower ureter and bladder shows higher degree of crystallinity with lower level of organic matter and cavitation. Binocular microscopic observations of renal stones showed that there are two distinguishable areas. One is with dark coloured materials in the center of nuclei. The other is light coloured materials in the peripheral zones. These two areas showed different physical and optical properties. Different characteristic features in these two areas may contribute the final clinical outcome of the shock wave therapy. Petrographic studies under plane polarized light showed that the crystallinity is characteristically high in peripheral areas of renal stones than that of the center nuclei. However, crystallinity is highly variable according to the location of the renal stones. Calculi recovered from pelvicalyceal system and upper ureter show the lowest crystallinity with about 25% of crystalline materials. Stones from the middle and lower ureter are distinguished by the presence of 40% of crystalline materials.

Stones recovered from bladder had the highest and well crystalline materials which cover more than 60% of them. Amorphous materials which should be organic matters are enriched in the center of the nuclei area and this is a common feature for any type stone form the urinary tract. However, cumulative organic matter is highest in general in stones recovered from pelvicalyceal system and upper ureter. Stones from bladder showed the lowest amounts of organic matter. Contents of Organic Matter Thermo gravimetric analysis of selected dry samples all three anatomical locations shows distinctive relationship between the amounts of organic and inorganic matter (minerals) and anatomical locations.

Summary

Minerals and electrolytes play an important role in body functions. Human body has the capacity to maintain the homeostatsis in healthy individuals. However, with unfavorable environmental factors including diet, polluted air and water, the capacity of homeostasis is exceeded resulting in many health problems. Excess serum levels of these minerals result in their precipitating in organs or tissues and cause cellular damage at different organelle levels. It is important to detect these trends of the disturbed body homeostatsis at early stages as it may be possible to halt further progress and full blown diseases.

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