Analytical Investigation of Li^+, Na^+, K^+, Ca^{2+} and Mg^{2+} Levels in Human Blood Serum of Cholistan Desert Population

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Summary: Hot, arid and sandy Cholistan desert living people have poor food habit due to their low socioeconomic status. Thirty individual's blood samples were analysed by atomic absorption spectrophotometer. Average levels were for Li^+ (9.79 ± 2), Na^+ (3113 ± 2), K^+ (156 ± 3), Ca^{2+} (109 ± 2) and Mg^{2+} (35 ± 9). Li^+ and Ca^{2+} were observed higher than the normal person levels, Na^+ and K^+ within the normal range and variable levels for Mg^{2+} were also found. A strong positive correlation was observed by pairs like Na^+-K^+ (r = 0.465), Na^+-Ca^{2+} (r = 0.376) and K^+-Ca^{2+} (r = 0.360) and negative correlation was observed by pairs like Li^+-Na^+ (r = -0.091), Li^+-K^+ (r = -0.036) and K^+-Mg^{2+} (r = -0.059). All levels have showed negative *skewness* except for Mg^{2+} and positive *Kurtosis* for Na^+ and K^+.

Introduction

Blood volume in the human body varies according to size of an individual and accounts for about 7 percent of the body weight. Its major function is to transport all the vital substances necessary to maintain bodily life processes. The composition of blood is 90 percent water and 10 percent dissolved substance [1, 2].

Among other constituents, the human blood serum also contains macro inorganic elements like Ca^{2+}, Mg^{2+}, K^+, Na^+ and Fe^{3+} and micro inorganic elements like Li^+, Zn^{2+}, Hg^{2+} and Mo^{6+} trace elements. These elements are required for maintaining the electrolyte, acid-base balance and osmotic equilibrium system in extra and intracellular fluids of human body. Some of these metal ions also play a significant role as catalyst and cofactors in various biochemical processes such as electron transfer, oxygen transport and enzymatic reactions. The concentration of these essential trace elements is constantly regulated and therefore, remains stable by such biochemical and biological processes. This stability and maintenance is utmost essential for the normal functioning of many organs. Any appreciable shift in the concentration of NaCl and $NaHCO_3$ causes disturbance in the electrolytic balance system and ultimate death of the cells, similarly, changes in the concentration of K^+, Ca^{2+}, and Mg^{2+} effect intercellular membrane of the cells [3-9].

It is interesting to note that concentration of one element is highly dependent on the concentration of others. Somehow if it changes, it disturbs the whole balance system resulting improper functioning of organs. Li^+ is among the same element. Previous studies [10-14] have shown that when the manic depressive patients were treated with oral dose of lithium carbonate (450-1200 mg/ day), serum Mg^{2+} and K^+ levels in the blood were increased and this increase remained at higher level for at least 90 days.

Desert living people have different food habits than any other part of the world. So a study was conducted to monitor the levels of Li^+, Na^+, K^+, Ca^{2+} and Mg^{2+} in blood serum samples of healthy adult individuals who were donating blood to their diseased patients admitted in Bahawal Victoria Hospital (BVH), Bahawalpur and further more to correlate relationship of these elements with each other. A healthy eating plan of different food items based on 2000 calories per day is also given in Table-1 for comparison and discussion [15, 16].

Results and Discussion

Small daily dose of Li_2CO_3 provides an effective treatment for manic-depressive psychoses (Schizopherenia). In all samples (Table-2) the average level of Li^+ found is 9.79 ppm ± 2 and

Table-1: Healthy eating plan for different nutritive elements.

Food Group	*Daily Servings	Serving Sizes	Examples	Significance
Grain Products	7-8	1 slice bread ½ cup dry cereal ½ cup cooked cereal, rice, or pasta	Whole wheat bread, English muffin, pita bread, bagel; whole grain cereals, grits, oatmeal (prepared without salt)	Major sources of energy and fibre
Vegetables	4-5	1 cup raw leafy vegetable ½ cup cooked vegetable 6 oz vegetable juice	Tomatoes, potatoes, carrots, peas, squash, broccoli, turnip greens, swiss chard, kale, spinach, artichokes, sweet potatoes, beans (all prepared without salt)	Rich sources of potassium, magnesium, and fibre
Fruits	4-5	1 medium piece of fruit ¼ cup dried fruit ½ cup fresh, frozen, or canned fruit 6 oz fruit juice	Apricots, bananas, cantaloupe, dates, oranges, orange juice, figs, grapefruit, grapefruit juice, mangoes, melons, peaches, pineapples, prunes, raisins, strawberries, tangerines	Important sources of potassium, magnesium, and fibre. Juice is not a good fibre source
Low-fat or non-fat dairy foods	2-3	8 oz milk 1 cup yogurt 1 ½ oz cheese	Skim or 1% milk, non-fat or low-fat yogurt, skim or low-fat buttermilk, part skim mozzarella cheese, non-fat cheese (use low salt cheeses if available)	Major sources of calcium and protein
Meats, poultry and fish	2 or less	3 oz cooked meats, poultry or fish	Select only lean cuts; trim away visible fats; broil, roast or boil instead of frying; remove skin from poultry; cook without salt	Rich sources of protein and magnesium
Nuts, seeds, and legumes	Per week 4-5	1 ½ oz or 1/3 cup nuts ½ oz or 2 Tbsp. seeds ½ cup cooked legumes	Unsalted almonds, filberts, mixed nuts, peanuts, walnuts, sunflower seeds, kidney beans, lentils (prepared without salt)	Rich sources of energy, magnesium, potassium, protein, and fibre

*This eating plan is based on 2000 calories per day. The number of servings that one need from each food group may be different, depending on one's daily energy needs.

highest level observed is 13.90 ppm. Most of the individuals have Li^+ above the normal person levels *i.e.* 4.2-8.4 ppm. Moreover Li^+ (Table-3) shows weak positive correlation with Ca^{2+} ($r = 0.112$) and Mg^{2+} ($r = 0.120$) and negative correlation with Na^+ ($r = -0.091$) and K^+ ($r = -0.036$). The real mode of Li^+ action is not well understood but it appears that the medicinal dose maintains the level of Li^+ in the blood and its action may be related to the influence of Li^+ on Na^+/ K^+ balance and (or) Mg^{2+}/ Ca^{2+} balance because Li^+ is chemically related to both pair of elements. Table-2 also shows that when Li^+ level is increased; the level of K^+ and Mg^{2+} is also increased, whereas levels of Na^+ and Ca^{2+} do not alter [10-14].

Average level of Na^+ (Table-2) is 3113 ppm ± 2 and highest observed level is 3227 ppm. All the individuals have Na^+ level within normal range, *i.e.* 3128-3335 ppm, indicating that peoples of the region are free from hypertension related diseases. Na^+ (Table-3) shows strong positive correlation with K^+ ($r = 0.465$) and Ca^{2+} ($r = 0.376$), weak correlation with Mg^{2+} ($r = 0.082$) and negative correlation with Li^+ ($r = -0.091$). Na^+ help the body to maintain electrolytic balance and is associated with increased blood pressure in susceptible individuals. Minimum recommended sodium level in daily adult diet is 500 mg/ day. For a long time it was assumed that cell membrane is impermeable to Na^+ and K^+ but the current theory suggests that because of the smaller size of the Na^+, it can be bound closely to negatively charged sites on the proteins within the cells than can the larger K^+. According to this theory, the loss of Na^+ under unfavorable metabolic conditions is due to the chemical changes in the intracellular proteins, which reduces the number of sites for binding Na^+ [3-9, 17].

Average level of K^+ (Table-2) is 156 ppm ± 3 and highest observed level is 210 ppm. Again K^+ level is within the normal person level *i.e.* 137-195 ppm except the one individual with 210 ppm. K^+ (Table-3) shows strong positive correlation with Na^+ ($r = 0.465$) and Ca^{2+} ($r = 0.360$) and negative correlation with Mg^{2+} ($r = -0.059$) and Li^+ ($r = -0.036$). Balanced K^+ level is necessary for normal heart rhythm, also helps to regulate high blood pressure. Some researchers [3-9] suggest that high

Table-2: Levels of Li^+, Na^+, K^+, Ca^{2+} and Mg^{2+} in human blood serum of Cholistan desert population

Sample code	Li^+ (ppm)	Na^+ (ppm)	K^+ (ppm)	Ca^{2+} (ppm)	Mg^{2+} (ppm)
*Normal person levels	4.2 - 8.4	3128 - 3335	137 - 195	88 - 104	20 -30
BS–01	6.00	3221	145	120	48
BS–02	5.50	3219	193	96	24
BS–03	6.80	3220	146	121	49
BS–04	6.85	2853	163	112	29
BS–05	8.00	3218	129	80	34
BS–06	8.40	3222	164	122	24
BS–07	9.00	3223	129	121	30
BS–08	11.05	3225	178	80	25
BS–09	10.25	3224	165	121	34
BS–10	10.90	3217	210	123	48
BS–11	11.20	2509	80	80	34
BS–12	11.50	2853	144	81	24
BS–13	13.90	3216	193	125	48
BS–14	12.85	3225	163	124	24
BS–15	10.35	3214	98	136	39
BS–16	11.20	2853	193	112	29
BS–17	11.50	3217	144	119	25
BS–18	13.85	3218	163	124	27
BS–19	10.85	3219	178	136	39
BS–20	10.90	3216	129	80	48
BS–21	10.20	3227	193	118	24
BS–22	6.80	2853	163	115	34
BS–23	10.25	3218	145	117	48
BS–24	6.80	3217	143	96	24
BS–25	9.00	3216	129	80	34
BS–26	6.85	3226	193	96	37
BS–27	11.20	2531	84	80	34
BS–28	13.90	3222	178	112	49
BS–29	10.90	2875	145	118	48
BS–30	6.80	3220	193	116	29
Average	9.79	3113	156	109	35
SD	± 2	± 2	± 3	± 2	± 9
Skewness	-0.10	-1.85	-0.61	-0.59	0.41
Kurtosis	-0.90	2.46	0.12	-1.05	-1.32

*Normal person average levels set by different European laboratories.

Table-3: Linear correlation coefficient matrix for Li^+, Na^+, K^+, Ca^{2+} and Mg^{2+} in human blood serum of Cholistan desert population (n = 30).

	Li^+	Na^+	K^+	Ca^{2+}
Na^+	-0.091			
K^+	-0.036	0.465		
Ca^{2+}	0.112	0.376	0.360	
Mg^{2+}	0.120	0.082	- 0.059	0.197

Values > 0.3 or < - 0.3 are significant at P< 0.01

potassium diets reduce the rise in blood pressure related to high sodium intake. Minimum recommended level of K^+ in daily adult diet is 200 mg/ day. One of the biological problems under the most active investigations is the mechanism by which the cells maintain high concentration of K^+ and Mg^{2+} and at the same time exclude Na^+ and Ca^{2+}. Unequal distribution of these electrolytes across the cell membrane is highly characteristic of living tissues, which in turn is responsible for the electrical properties of cells and conduction of impulses in nerve and muscle tissues. Outside and inside the cell membrane is like poles of a battery, across which a voltage or potential exists. A stimulus acts by disturbing the equilibrium at the membrane so that the potential suddenly decreases which is called depolarization. The change in potential at one point affects the adjacent areas on the membrane and causes them to depolarize. In consequence, a wave of depolarization spreads in each direction and the normal electrolyte concentrations are rapidly restored and the membrane potential returns to its normal level [3-9, 14-17].

Average level of Ca^{2+} (Table-2) is 109 ppm ± 2 and highest observed level is 136 ppm. Most of the samples have Ca^{2+} level above the normal person levels *i.e.* 88-104 ppm. Ca^{2+} (Table-3) shows strong positive correlation with Na^+ (r = 0.376) and K^+ (r = 0.360) and weak correlation with Li^+ (r = 0.112) and Mg^{2+} (r = 0.197). Calcium is essential to bone and tooth development, blood clotting, muscle contraction, nerve transmission and reduces heart diseases [18]. Ca^{2+} is needed to be combined with Mg^{2+} in the diet at a ratio of 2: 1. Minimum recommended level of Ca^{2+} in daily adult diet is 1000-1200 mg/ day [10-17].

Average level of Mg^{2+} (Table-2) is 35 ppm ± 9 and highest observed level is 49 ppm. 50 % samples have Mg^{2+} above the normal person level *i.e.* 20-30 ppm. Mg^{2+} (Table-3) shows weak positive correlation with Li^+ (r = 0.120), Na^+ (r = 0.082) and Ca^{2+} (r = 0.197) and negative correlation with K^+ (r = -0.059). Mg^{2+} is involved in neuromuscular activity of heart, energy metabolism, protein synthesis and protects against heart disease; maintains normal heart rhythm; necessary for proper calcium and vitamin C metabolism. Some investigators [19, 20], thought that serum Ca^{2+} and Mg^{2+} levels may be used as an index for the activity of leukemia. Minimum recommended level of magnesium in daily adult diet is 310-420 mg/ day. An increase in Mg^{2+} intake has been suggested to lower blood pressure and increase in arties elasticity [19, 20].

Experimental

Methods and Techniques

All chemicals used were of analytical reagent grade procured from E.Merck and BDH. Freshly prepared bidistilled water was used for the preparation of all solutions. Ca^{2+} and Mg^{2+} were determined by complexometric titration with disodium salt of ethylenediamine tetraacetic acid (EDTA). Flame photometer model 'Corning-40', was used for the determination of Na^+ and K^+. Li^+ was found below the detection limits of flame photometer and was determined by atomic absorption spectrophotometer model Hitachi A-1800. Absorption line 283.3 nm, acetylene 2.4 liters/ minute, air 22.5 liters/ minutes, slit 0.2 mm, 4 inch simple slot burner; lamp current 5 milli amps, digital display adjusted too give 20.0 absorption for a standard solution μg/ ml. The instrument determines to a level 0.2 ppm.

Thirty different male adult's (aged between 25 to 35 years) human blood samples were obtained from Pathology department of BVH, which is a teaching hospital of Quaid-e-Azam Medical College, Bahawalpur, in accordance to procedure reported elsewhere [21-23].

Preparation of serum solution – Method 1

Blood obtained was allowed to clot. After 15 minutes a colourless fluid, serum was formed which was separated by simple decanting. To this serum solution (50 μl) in a test tube was added bidistilled water (10 ml) and used as such for the determination of Ca^{2+} and Mg^{2+} [8, 22, 23].

Determination of Ca^{2+} and Mg^{2+} by complexometric titration

5 ml serum solution of method-1 was taken in a conical flask with the addition of 3 ml of 10 % KOH solution (1.78 M) and then added 2 – 3 drops of muroxide as an indicator, titrated as swiftly as possible against disodium salt of EDTA solution (0.01M) till violet blue colour appeared which marked the end point. Then the amount in ppm was calculated [21].

Preparation of serum solution – Method 2

5 ml serum solution obtained by method 1 was taken in a china dish with the addition of concentrated H_2SO_4 (2.5 ml). Then this solution was digested with HNO_3/ $HClO_4$, heated slowly near to dryness; diluted with bidistilled water (5 ml) and heated again to dryness. Then the solid obtained was dissolved in H_2O and volume was made up to 25 ml for flame photometer study of Na^+ and K^+ and atomic absorption spectrophotometer study of Li^- [8, 22, 23].

Preparation of stock standard solution

Stock standard solutions of Li^+, Na^+, K^+, Ca^{2+} and Mg^{2+} (100 ppm) were prepared by taking their respective chloride salts. Mitler PM 460 electric balance was used for all weightings. Working standard solutions (5, 10, 15, 20 and 25 ppm) were prepared by their further dilution with freshly prepared bidistilled water.

Statistical analysis

The data was statistically analysed by using SPSS 12 and STATSICA (StatSoft 1999) softwares on P-IV system. The concentrations of elements in canal water samples were correlated by linear correlation coefficient matrix (*pearson*).

Conclusions

Eating food rich in potassium appears to protect some people from developing high blood pressure and provides support for nutritional guidelines to increase dietary potassium to prevent hypertension. Foods that are high in potassium include: bananas, catfish, orange juice, spinach, dried fruit, dried beans, potatoes, and milk. Many people with low calcium intake have higher rates of high blood pressure. While it has not been proven that calcium supplements may prevent high blood pressure, it may be important for those with low calcium intakes. Current recommendations suggest a daily calcium intake of 1200 mg per day for adults. Low fat and non-fat dairy products like milk, yogurt, and cheese are rich sources of calcium. A diet low in magnesium may increase blood pressure. However, a healthy diet should provide enough magnesium. Good sources of magnesium include: whole grain breads and cereals, green leafy vegetables, and beans. Table 1 may be helpful for healthy way of eating.

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