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Elemental profile of blood serum of dengue fever patients from Faisalabad, Pakistan

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Abstract

The present study appraises the elemental profile of blood serum samples of dengue fever patients from Faisalabad, Pakistan. The elements including heavy metals (such as lead, chromium, iron, nickel, copper), zinc, electrolytic minerals (sodium, potassium, calcium) and chloride ions were measured and compared in the serum samples of control group (healthy individuals) and test group (dengue fever patients). The amounts of sodium (P = 0.000), chloride (P = 0.008), calcium (P = 0.000) were varied significantly while that of potassium (P = 0.072) non-significantly between the control and patient serum samples. Similarly, the contents of heavy metals; lead (P = 0.000), nickel (P = 0.001), copper (P = 0.000), zinc (P = 0.000) and iron (P = 0.007) differed significantly whereas that of chromium (P = 0.106) non-significantly between the serum samples of control and Dengue fever patient samples.

Key words: Elemental profile; blood serum; dengue patients

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1. Introduction

Dengue (DEN) is a transmittable disease caused by the DEN virus (DENV), which is the member of the genus *Flavivirus,* in the family *Flaviviridae*. It has a single positive-stranded RNA genome about 11 Kb in length and is principally transmitted to humans by the vector mosquito *Aedes aegypti*. Dengue virus (DENV) has four distinct but closely linked serotypes (DENV-1, DENV-2, DENV-3 and DENV-4); all of them can cause dengue fever (DF) and dengue hemorrhagic fever (DHF). Dengue infection primarily occurs in tropical and sub-tropical regions throughout of the world, mainly in urban and semi-urban areas [1]. The World Health Organization has reported that an estimated 2.5 billion people- two-fifths of the world population live in areas where dengue infection can be transmitted, annually up to 50 million infections arise worldwide, including 500 000 cases of DHF and 25,000 deaths. In South East Asia, the average annual figure of DHF cases has increased from 10,000 in the 1950s to more than 200,000 in the 1990s [2, 3]. Dengue infection is endemic in Pakistan and first report was acknowledged in 1985. An immense dengue infection strike in 2011 and more than 20,000 cases were reported and 300 deaths were caused mostly by DEN-2 and DEN-3 serotypes [4,5].

Symptoms of dengue infection in humans include a mild flu like illness, the conversational "break-bone" fever and dengue hemorrhagic fever (DHF) and dengue shock syndrome (DSS). Dengue fever advances to DHF and DSS generally after a second infection with a dissimilar serotype, which is notified as antibody-dependent enhancement (ADE) of infection [6]. Capillary leakage in dengue hemorrhagic fever makes it more severe than dengue fever [7]. The minerals those are significant, assist and maintain the normal physiological mechanisms, are divided into two groups: as macro elements i.e. calcium, phosphorous, magnesium, sodium, potassium, chlorine and sulfur and trace elements (micronutrients) which consist of metals present in biological fluids at concentrations less than 1 µg/g wet weigh. The majority of the trace metals, include selenium, zinc, copper, cobalt, manganese, molybdenum, chromium, nickel and iron, are essential nutrients for humans and animals [8].

Trace elements deficiencies and infectious diseases are correlated with each other as they frequently coexist and reveal complex interactions. Various trace elements perform antiviral activity by inhibiting virus replication in the host

cells. Many trace elements support to regulate immune responses of the host, acting as antioxidants thus can change the genome of the viruses and as a result new infections may appear [8]. In dengue patients low serum level of zinc are found. Zinc deficiency is known to happen along with an imbalance of Th-1 cell and Th-2 cell resulting in deregulated resistance to infection so can increase the hazard of morbidity and mortality of infection [9]. Electrolyte disturbances take place in dengue infection. Sodium is an essential nutrient in human; regulate blood volume, blood pressure, osmotic equilibrium and pH. Hyponatremia is defined as a serum sodium level <130 mEq/l. Hyponatremia is frequent in DHF, especially in shock patients [10, 11].

Dengue virus infection led to acute neuromuscular weakness due to hypokalemia [12]. In dengue fever along with other infectious disorders, cause of hypokalemia is inadequate dietary intake and breakdown of tissues releases potassium into the extracellular compartment [13]. In persons infected with dengue virus, extracellular calcium plays an imported role in platelet aggregation and for the regulation of the immune response [14]. Therefore, a research work was designed to compare and evaluate specific biochemical parameters in healthy persons and patients of Dengue fever of Faisalabad.

2. Materials and Methods

A total number of 30 individuals (15 Healthy Persons, 15 Dengue patients) were included in this research project; we selected the maximum dengue fever patients and healthy individuals having a back ground of industrial locality. Samples were collected from Allied Hospital (a teaching hospital) of Punjab Medical College, Faisalabad, Pakistan. About 5 mL of venous blood was taken from every patients and healthy persons. The blood samples were processed and analyzed in the Bio-Medical Research laboratory, Department of Chemistry & Biochemistry, University of Agriculture Faisalabad, Pakistan. BD syringes were used to draw the blood. About 3 ml of blood was collected in vacuum tubes and remaining 2 mL blood was collected in EDTA containing tube. The 3 mL blood sample was centrifuged at 4500 rpm for fifteen minutes and serum was separated. The serum was then collected in Eppendorf tubes. They were stored at -35℃ in freezer until analysis.

2.1. Analysis of Heavy Metals

All the serum samples were subjected to wet digestion as described by Richards [15] to determine the levels of minerals. Heavy metal concentration in the digested serum samples was determined by atomic absorption spectrophotometer [9].

2.2. Analysis of electrolytes

Sodium, potassium, chloride and calcium analysis was performed on fully automated electrolyte analyzer 9180 (microprocessor-based technology) by the method described by Monds *et al.* [16] from Tahir Diagnostic and Research Laboratories, working on the principal of ion selective electrodes. A volume of 100 µL was added in serum cups. Then all samples analyzed simultaneously. The sample adjusted to the probe of equipment and it sips approximately 50 µL serum and displays result after 45 seconds.

2.3. Statistical analysis

The data obtained was analyzed by ANOVA to determine the significance of results using SPSS version 11.5 (SPSS Inc., Chicago, IL, USA). Mean ± SD concentrations of biochemical parameters in healthy control and serum of dengue patients are presented [17].

3. Results and Discussion

3.1. Heavy metals and Electrolytes

The levels of sodium, chloride, calcium, zinc, lead and chromium of dengue patients were compared with the group of healthy individuals. The findings of our research demonstrate that the patterns of lead level in the serum of healthy controls and in serum of dengue patients were not same. Statistical analysis demonstrate that levels of lead in serum (Fig.1) of dengue patients increased significantly (P = 0.000) as compared to that of healthy individuals (control group). Significant serum levels of lead in dengue patients are presented first time in the present research. The patterns of zinc level in the serum of healthy controls and in serum of dengue patients were not same. Statistical analysis demonstrate that levels of zinc in serum (Fig.1) of dengue patients decreased significantly (P = 0.000) as compared to that of healthy individuals (control group). Findings of present research work correlate with the results of Widagdo *et al.*, [13] and Laoprasopwattana *et al.* [18]. Moreover, Laoprasopwattana *et al.*, [18] stated that dengue virus infection-associated with diarrhea is due to an increased number of inflammatory cytokines, which directly affects and leads to zinc loss through the gastrointestinal tract during the toxic phase of DVI [21]. Zinc deficiencies can result in inefficient clearing of infections by creating an imbalance of pro- and anti-inflammatory cytokines, and induction of cell death via apoptosis [13].

Sodium is thus important in neuron function and osmoregulation between cells and the extracellular fluid; the distribution of sodium ions are mediated in all animals by Na^+/K^+-ATPase [19]. Sodium level in the serum of healthy controls and in serum of dengue patients were not same. Statistical analysis demonstrate that levels of sodium (Na) in serum (Fig. 2; Table 1) of dengue patients decreased significantly (P = 0.000) as compared to that of healthy individuals (control group). Hyponatremia is commonly found in association with dengue fever, dengue hemorrhagic and shock patients. Dengue patients are 9.7 times more likely to have clinically significant hyponatremia (Na <130mmol/L) than patients with similar febrile illnesses as demonstrated by the findings of Mekmullica *et al.* [11].

The findings of this research demonstrate that levels of calcium in serum (Figure 4.3; Table 4.3) of dengue patients decreased significantly (P = 0.000) as compared to that of healthy individuals (control group). The observed significant values of calcium in dengue patients correlate with findings of (Castilla-Guerra *et al.*, [20]. Castilla-Guerra *et al.*, [20] also stated that acute hypocalcemia is primary cause of increased neuromuscular excitability and tetany is infrequently associated with dengue virus infection. Calcium deficiency may also result from appetite loss, specifically a decreased intake of dairy products and these are main dietary source of calcium.

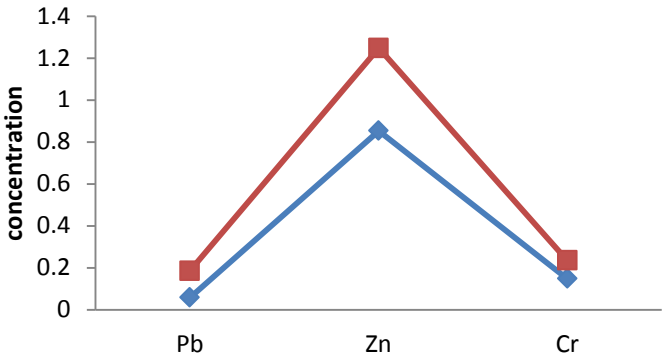

Fig. 1. Diagrammatic representation of heavy metals in control (Healthy individuals) and test (Dengue fever patients)

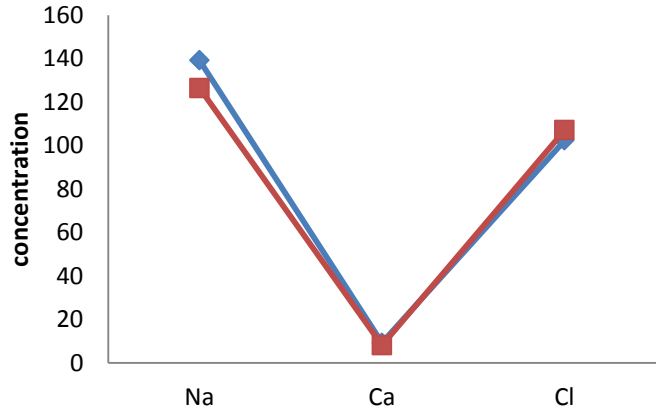

Fig. 2. Diagrammatic representation of serum electrolytes in control (Healthy individuals) and test (Dengue fever Patient)

Table 1. Biochemical parameters in serum of control (healthy individuals) and test (Dengue Fever patients)

Parameters	Control Mean ± SD	Test Mean ± SD	p-value
SODIUM (mEq/L)	139.27±3.43	126.47±9.26	0.000*
CHLORIDE (mEq/L)	102.13 ±4.85	107.07±4.67	0.008*
CALCIUM (mg/dL)	9.2200 ±0.6816	8.0933±0.5378	0.000*
LEAD (mg/L)	0.05847±0.0282	0.1276±0.0127	0.000*
ZINC (mg/L)	0.8537±0.2245	0.3940±0.1731	0.000*
CHROMIUM (mg/L)	0.14800±0.1404	0.0874±0.0069	0.106^{NS}

* Significant (P- value ≤ 0.05), NS (Non-Significant) (P-Value ≥ 0.05)

Chromium levels in the serum of healthy controls and in serum of dengue patients were not significantly different. Statistical approach demonstrate that levels of chromium in serum (Fig.2; Table 1) of dengue patients decreased non-significantly (P = 0.106) as compared to that of healthy individuals (control group). The effect of chromium compromises the immune response of the host [21]. Shrivastava *et al.* [22] showed in their study that the adverse effects of dengue virus infection, especially on platelets and leucocytes, were abolished by pretreatment of mice with Chromium picolinate monohydrate (CrP).

The patterns of chloride level in the serum of healthy controls and in serum of dengue fever patients were not same. Statistical analysis demonstrate that levels of chloride in serum (Fig. 2; Table 1) of dengue patients increased significantly (P = 0.008) as compared to that of healthy individuals (control group).

4. Conclusion

The elemental profile of blood serum samples of dengue fever patients from Faisalabad, Pakistan was appraised to explore the variation in the elements including heavy metals such as lead, chromium, iron, nickel, copper

zinc and electrolytic minerals (sodium, potassium, calcium) and chloride ions in control group (healthy individuals) and test group (dengue fever patients). The amounts of sodium, chloride and calcium varied significantly while that of potassium non-significantly between the control and patient serum samples. Similarly, the contents of heavy metals; lead copper, zinc and iron differed significantly whereas that of chromium non-significantly between the serum samples of control and dengue fever patient sample.

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