STUDIES ON SERUM MAGNESIUM, PHOSPHOROUS AND CALCIUM IN CARDIOVASCULAR DISEASE PATIENTS ATTENDING HEART CLINIC AT ESUTH ENUGU

ABSTRACT

This study was carried out to investigate the serum magnesium, phosphorous and calcium in cardiovascular disease patients attending heart clinic at ESUTH ENUGU. Blood samples were obtained by venipuncture from forty (40) patients consisting of ten (10) male cardiovascular disease patient (test subject) and ten (10) male non-cardiovascular disease patients (control subject), ten (10) female cardiovascular disease subject (test subject) and ten (10) female non-cardiovascular disease subject (control subject). Blood sample from each patient was analysed for bone minerals (magnesium, phosphorous and calcium) by spectrophotometric method. Serum Ca was significantly lower (p=0.000) in heart disease patients compared to Controls, while serum phosphorus was significantly higher (p=0.034) in heart disease patients compared to Controls. There was no significant difference (p=0.493) in serum Ca levels of heart disease patients compared to controls. Serum Ca was significantly negatively correlated with P in heart Disease Patients (r= -0.721, p=0.000). There was no significant correlation of serum Ca with Mg in heart Disease Patients (r= 0.074, p=0.755). Hyperphosphatemia and hypomagnesemia are risk factors for cardiovascular diseases and this study discusses on the necessity of increased magnesium and decreased phosphorous levels in maintaining cardiac health.

1.0 INTRODUCTION

Cardiovascular disease (CVD) is a key contributor to low quality of life and ~~a~~ leading cause of death worldwide [1, 2]. The Global Burden of Disease (GBD) Study 2019 revealed that since 1990, there has been a notable increase in the prevalence, mortality, and disability-adjusted life years (DALYs) associated with CVD [2]. It is well recognised that certain nutrients influence the onset and course of cardiovascular disorders. These include calcium, phosphorus, and magnesium. These micronutrients have historically been linked to chronic renal disease or bone health, but they may also raise the risk of cardiovascular disease (CVD) [3].

Elevated serum phosphorus levels are thought to increase CVD risk via vascular calcification [4], myocardial fibrosis [5], and the development of left ventricular hypertrophy [4]. High serum calcium levels may cause CVD and atherogenesis by increasing vascular calcification and coagulability [6, 7]. Serum calcium and phosphorus are required for bone mineralization, energy production, membrane transport, signal transduction, and vascular function [8]. Recent experimental and epidemiological studies have found that higher serum calcium or phosphorus levels may be associated with the pathogenesis of cardiovascular disease (CVD), including atherosclerosis [4, 9], heart valve calcification [10, 11], vascular calcification [4, 12], and arterial stiffness [13, 14]. In 2011, a meta-analysis of 47 cohort studies on this topic found that elevated blood phosphorus is strongly linked with higher all-cause and cardiovascular mortality [15]. There is limited evidence to establish a link between serum calcium and the risk of death and cardiovascular events in people with chronic kidney disease (CKD) [16]. Furthermore, recent evidence on the relationship between blood calcium and phosphorus and cardiovascular risk are debatable [17,18].

Another micronutrient that may be connected to the risk of CVD through a variety of physiologic functions is magnesium; low serum concentrations have been linked to abnormal ECG patterns, elevated blood pressure, chronic inflammation, impaired vasomotor tone and peripheral blood flow, and impaired glucose homeostasis and insulin action [19]. It has been suggested in more recent reports to be connected to the development of CVD [20, 21]. Magnesium, the second most prevalent intracellular cation and the fourth most abundant mineral, regulates cardiac contraction, intracellular conduction, and neuronal activation [22]. Moreover, magnesium is essential for controlling the activity of mitochondria and the synthesis of energy [23]. According to Liu et al. [24], mice on a low magnesium diet developed diastolic cardiomyopathy as a result of magnesium insufficiency due to ATP depletion, mitochondrial dysfunction, and reactive oxygen species overproduction. It has also been demonstrated that a shortage in magnesium causes endothelial dysfunction, platelet activation, and an increase in pro-inflammatory cytokines and neuropeptides, all of which speed up atherosclerosis [25]. A 22% lower incidence of heart failure was linked to an increase in magnesium intake through diet, according to a meta-analysis of prospective cohort studies [26]. Hospitalisation and the incidence of heart failure were linked to lower magnesium intake [27, 28].

Owing to these claims about the impact of micronutrients on the development of CVD, this study was aimed at evaluating the roles of calcium, phosphorus, and magnesium in the development of heart diseases. The aim was achieved by estimating and comparing the concentrations of calcium, phosphorus, and magnesium in individuals with heart disease and those without any history of heart disease.

2.0. METHODOLOGY

2.1 Advocacy and mobilization and pre-survey contact

Ethical approval was obtained from ethical/medical advisory committee of ESUTH Enugu after a proposal detailing the essence of the research was presented. Personnel anonymity was maintained, good laboratory practice was ensured, and all findings were treated with utmost confidentiality. All volunteers were verbally notified prior to sample collection and their informed consent was duly obtained.

2.3 Study design and population (subjects)

This is a cross-sectional study. The study area is Enugu state, in the eastern region of Nigeria. The hospital attends to the medical needs of everyone living in the state. The period of subjects' enrolment, classification

administration of questionnaires, sample collection, determination of heavy metals and data generation in this study lasted from November 2017 to January 2018. The subjects of the study were cardiovascular disease patients (both male and female subject with respect to age) at ESUTH Enugu. People without known cardiovascular disease were selected as control subjects. Random sampling was done across all age groups and the age was categorized as follows: 1-10, 11-20, 21-30, 31-40, 41- 50, 51-60 years. Furthermore, additional demographic data were obtained using questionnaires issued out during a structural interview. The demographic data include basic socioeconomic information, some medical health history and dietary intake of lead, arsenic cadmium, and mercury.

2.4. Sample Collection and preparation.

Fresh venous blood (5ml) was collected from the patients by venipuncture using a sterile needle and syringes into clean sterile and plain plastic tubes immediately. The unheamolysed samples in the tubes were centrifuged and separated. The serum samples were stored at -20^{o}c prior to use.

2.5 Estimation of Serum Calcium

Serum calcium was determined by the colorimetric method of Leo [29]. The Randox reagent kit with catalog number CA590 was used. This assay is based on the principle that calcium ions form a violet complex with O-Cresolphthalein complexon in an alkaline medium. In this procedure, three clean grease-free test tubes were labeled- Test(T), Standard(S) and Blank(B). One milliliter (1ml) of calcium working reagent was added to each test tube. Into the test tube (T), 0.025ml of subject serum was added. Similarly, 0.025ml of calcium working standard was added into test tube S, and 0.02ml of distilled water was added into test tube B. Absorbance of the test was read at 570nm.

2.6. Estimation of Serum Inorganic Phosphorous

Serum calcium was determined by the colorimetric method by Leiboff [30]. The Teco Diagnostic reagent kit with catalog number 1515-480 was used for this assay. The principle is based on the reaction of inorganic phosphorous with ammonium molybdate in an acid medium to form a phosphomolybdate complex. This complex is reduced by ferrous ammonium sulfate to produce a molybdenum blue complex. The color produced is measured at 675nm and its intensity is directly proportional to the concentration of inorganic phosphorous present. The procedure involves the use of three clean grease-free test tubes were labeled- Test(T), Standard(S) and Blank(B). 1ml of phosphorous working reagent was added to each test tube and was incubated at room temperature. 0.02ml of subject serum was added in the test tube T. 0.02ml of phosphorous working standard was added into test tube S. 0.02ml of distilled water was added into test tube B. The test tubes were incubated for 10 minutes at 25^{o}C. Absorbance of the test was read at 675nm.

2.7. Estimation of serum Magnesium (Mg)

Serum calcium was determined by the colorimetric method by Faulkner [31] and Tietz [32]. The reagent kit with catalogue number M527-100, manufactured by Teco Diagnostic, was used for this study. The principle of the assay is that magnesium forms a colored complex with calmagite in alkaline medium to produce a red complex that is measured using spectrophotometer at 530nm. EGTA serves to complex and prevent calcium interference and a surfactant eliminates the effect of protein. The color produced is proportional to the magnesium concentration. The procedure involves the use of three clean grease-free test tubes were labelled- Test(T), Standard(S) and Blank(B). 1ml of magnesium working reagent was added to each test tube. 0.01ml of subject serum was added in the test tube T. 0.01ml of magnesium working standard was added into test tube S. 0.01ml of distilled water was added into test tube B. The test tubes were incubated for 5 minutes at room temperature. Absorbance of the test was read at 530nm.

2.8 Statistical Analysis

All values were expressed as mean ± standard deviation, the test of significance was determined by student t-test. Values with $P<0.05$ were considered statistically significant.

3.0 RESULTS

3.1: The concentrations of serum Ca, P and Mg in patients with heart disease and apparently healthy individuals

Serum Ca was significantly lower (p=0.000) in heart disease patients compared to Controls, while serum phosphorus was significantly higher (p=0.034) in heart disease patients compared to Controls. There was no significant difference (p=0.493) in serum Ca levels of heart disease patients compared to controls (Table 1).

Table 1: **Serum Ca, P and Mg in Heart Disease Patients versus Controls**

VARIABLES (MEAN ±SD)	Heart Disease Patients (n=20)	Controls (n=20)	t-value	p-value
Mg(meq/L)	2.293±1.65	2.60±1.24	0.698	0.493
Lower 95% C.I	2.13	2.00		
Upper 95% C.I	3.73	3.19		
P(mg/dl)	3.31±1.79	2.29±0.70	2.285	0.034
Lower 95% C.I	2.46	1.96		
Upper 95% C.I	4.15	2.62		
Ca (mg/dl)	9.16±1.52	11.02±0.58	-4.850	0.000
Lower 95% C.I	8.47	10.75		
Upper 95% C.I	9.89	11.30		

4.2: Pearson Correlation of Serum Ca with P and Mg in heart disease Patients

Serum Ca was significantly negatively correlated with P in heart Disease Patients (r= -0.721, p=0.000). There was no significant correlation of serum Ca with Mg in heart Disease Patients (r= 0.074, p=0.755) (Table 2).

Table 2: Pearson Correlation of Serum Ca with P and Mg in Heart Disease Patients

Dependent Variables	N	r-value	p-value
P	20	-0.721	0.000
Mg	20	0.074	0.755

4.0 DISCUSSION

This study was aimed at evaluating the concentrations of calcium, phosphorus, and magnesium (Mg) in cardiovascular disease patients. From the results, it was observed that the serum concentration of magnesium in cardiovascular disease patients was significantly lower that of apparently healthy individuals (control group). This finding is in tandem with the report of Lutsey et al. [3], in which it was reported that low magnesium level increases the risk of cardiovascular diseases. It also agrees with the reports from some observational studies in which low serum magnesium was linked to more adverse CVD risk factor profiles [33, 34, 35] and greater risk of CVD events [36, 37, 38, 39, 40, 41]. Similarly, small randomized clinical trials of patients with heart failure have suggested that magnesium supplementation improves left ventricular function [42] and heart rate variability [43]. Furthermore, meta-analyses of community-based cohorts concluded that both a low Mg intake and low serum Mg level are significant risk for CVD events [20, 44, 45]. Therefore, it is most likely that magnesium might possess cardioprotective effects. That is to say, low serum level might predispose an individual to cardiovascular diseases while a normal or slightly elevated level will protect or prevent an individual from developing cardiovascular diseases.

In this study too, it was found that serum calcium was significantly lower in heart disease patients compared to apparently healthy individuals (controls). This agrees with the report of Ranjan [46] in which he noted that serum calcium is lowered in cardiovascular diseases. Similar result was obtained in a study conducted by Lutsey et al. [3], in which Serum calcium was reported to be positively associated with the risk of incident heart failure. Furthermore, most observational studies of serum calcium concentrations have shown a positive association with the risk of myocardial infarction and combined CVD endpoints [47, 48, 49]. Therefore, it could be deduced that serum calcium level has an inverse relationship with cardiovascular diseases. That is to say, the risk of developing cardiovascular diseases increases with a decline in the serum calcium concentration.

On the other hand, it was observed in this study that serum phosphorus was significantly higher in heart disease patients compared to apparently healthy individuals. This agrees with the report in which higher phosphate levels were found to correlate with increased cardiovascular risk [50]. This also corroborates the findings of Lutsey et al. [3], in which serum phosphorus was positively associated with the risk of incident heart failure. Interestingly, several potential mechanisms by which phosphate leads to increased cardiovascular risk have been proposed [51]. It is documented that elevated phosphate levels induce degradation of the extracellular matrix and cause osteochondrogenic change in vascular smooth muscle cells [52]. These changes cause increased deposition of extracellular calcium phosphate crystals, cell apoptosis, and ultimately vascular calcification [53]. Furthermore, it has been proposed that hyperphosphatemia may also cause endothelial damage through increased production of reactive oxygen species [54, 55]. Also, Block et al. [56] reported a linear association of higher serum phosphate concentrations with a greater risk of cardiovascular hospitalizations in a national cohort of 40,538 hemodialysis patients. These all suggest that the elevated levels of phosphorus might increase the risk of developing cardiovascular diseases.

In this study, it was found out that serum calcium was significantly negatively correlated with phosphorus in cardiovascular disease patients. This implies that serum calcium decreases with increase in serum phosphorous in cardiovascular disease patients. This could be as ~~as~~ a result of vascular calcification where calcium is deposited more in the bones with lower level found in serum in the presence of high serum level of phosphorous [57].

Conclusion

There is growing evidence that increased concentration of phosphorus as well as decreased concentrations of calcium and magnesium levels enhances the development and progression of cardiovascular diseases. The findings from this study have further led credence to that body of knowledge. However, further research should be carried out in this area to better elucidate the mechanisms by which decreased or elevated levels of these micronutrients impact the cardiovascular health of individuals. Furthermore, the results from this study and similar results would aid in the proper diagnosis, treatment, and management of cardiovascular disease patients. In general, efforts should be made to ensure that these bone minerals (calcium, magnesium and phosphorous) are incorporated into daily diet to maintain their normal ~~conceration~~ in the body and reduce the risk of developing cardiovascular diseases.

Commented [SV1]: Rephrase the sentence

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