EVALUATION OF SERUM LEVELS OF SOME WATER-SOLUBLE VITAMINS B COMPLEXES IN CARDIOVASCULAR DISEASE PATIENTS

ABSTRACT

This study was carried out to investigate the serum levels of water-soluble vitamin B complexes (vitamin B3, vitamin B6, vitamin B9 and vitamin B12) in patients with cardiovascular diseases attending Cardiac Clinic at ESUTH Enugu State. Blood samples were obtained using the venipuncture technique from twenty cardiovascular disease patients, consisting of ten male and ten female patients. They were aged-matched with ten male and ten female controls, respectively. The serum concentrations of vitamins B3, B6, B9 and B12 were estimated using the titration methods for B3 and B6 and spectrophotometric methods for B9 and B12, respectively, and data generated were subjected to statistical analysis. The results showed that the serum concentrations of vitamin B6, B12 and B9 were significantly higher ($p=0.000$, $p=0.000$ and $p=0.004$, respectively) in cardiovascular disease patients compared to controls. Furthermore, there was no significant difference in serum concentration of vitamin B3 ($p=0.274$) in cardiovascular disease patients compared to controls. Similarly, there were no significant differences in serum concentrations of vitamin B3, B6 and B9 ($p=0.810$, $p=0.650$, and $p=0.478$, respectively) in male cardiovascular disease patients compared to the females. On the other hand, the serum concentration of vitamin B12 was significantly lower ($p= 0.012$) in male patients compared to females. The correlation analysis showed that there was a significant positive correlation of vitamin B3 with B6 ($r=0.564$, $p=0.010$), whereas no significant correlation of vitamin B3 with B12 and B9 ($r=0.137$, $p= 0.565$ and $r= -0.392$, $p=0.088$ respectively) in cardiovascular disease patients. These results suggest that higher doses of these vitamins may play a role in reducing cardiovascular disease complications.

Keywords: Heart diseases, Niacin, Pyridoxine, Folic Acid, Cobalamin.

1.0. INTRODUCTION

Cardiovascular disease (CVD) is responsible for a third of all global deaths, and the prevalence of CVD is increasing worldwide [1]. According to recent estimations, 1 to 2% of the global adult population could be affected by heart failure, with a prevalence of approximately 65 million patients worldwide [2]. This figure is expected to increase as the population ages [3]. As the rise in the CVD incidence poses an economic burden and leads to increases in the number of disability-adjusted life years, indicating a diminished quality of life, it is essential to identify and understand the potential risk factors and protective factors that may alleviate the burden of this disease [4].

Micronutrients, especially vitamins, have been reported to play an essential role in the evolution of cardiovascular diseases (CVD) [5]. This is due to their significant involvement in numerous elementary reactions of cellular homeostasis [6]. The use of vitamin supplementations to treat specific diseases spans several years [7, 8]. Specifically, different B vitamins have been observed to show beneficial effects in preventing various cardiovascular diseases [9]. This potential role of B vitamins in reducing cardiovascular risk has been supported by observational studies [10-12]. Although the mechanism of action is unclear, B vitamins may affect cardiovascular outcomes by lowering homocysteine concentrations, which correlate strongly with the risk of coronary disease [13, 14] and stroke [15-17].

Despite the discovery of the potential role of vitamin B complexes in the development and progression of cardiovascular diseases, much research on the topic has not been carried out in Nigeria. Hence, this study was conducted to determine the blood levels of the B vitamins in cardiovascular disease patients attending clinical visits at ESUTH, Enugu, Nigeria.

2.0. Methodology

2.1 Advocacy and mobilisation and pre-survey contact

Ethical approval was obtained from the Ethical/Medical Advisory Committee of ESUTH, Enugu after a proposal detailing the essence of the research was presented on request to the Ethics Committee of ESUTH. Personnel anonymity was maintained. Good laboratory practice and confidentiality of all findings were guaranteed. All volunteers were verbally notified before the sample collection date, and their informed consent was duly obtained.

2.2. Study Design

The period of subjects' enrollment, classification administration of questionnaires, sample collection, estimation of water-soluble vitamins, and data generation in this study lasted from February 2018 to July 2018. The study area is ESUTH in Enugu State of Nigeria. The method of selecting the study participants was random sampling.

2.3. Study population (Subjects)

The study population consisted of twenty cardiac patients (ten male and ten female patients) attending the Cardiac Clinic between February 2018 and April 2018 and who were within the age range of 40 to 60 years. Patients who did not give their informed consent were excluded from the study. Patients presenting with a history of any chronic disease like diabetes mellitus and AIDs were excluded. Demographic data were obtained using the study questionnaires administered to the Cardiac patients. These include age, gender, basic socioeconomic information, medical history, and dietary vitamin and other supplement intake.

2.4. Sample Collection and Preparation.

Fresh venous blood (5ml) was collected from each patient by venipuncture using a sterile needle and syringes into clean, sterile, plain plastic tubes, taking care to avoid hemolysis. The samples were allowed to clot, and the serum separated after centrifugation. The serum samples were stored at -20^{o}C before use.

2.5. Determination of vitamin B_3 (Nicotinamide)

Vitamin B_3 was determined using the titrimetric/Titration method described by Kirk and Sowyer [18]. The principle of this method is based on the hydrolysis of vitamin B_3 with acetic acid after warming slightly and further extraction with acetic anhydride. In the presence of perchloric acid, nicotinamide produces a greenish colour. The procedure involved the addition of 0.1ml of serum sample into 2ml of anhydrous glacial acetic acid with an automatic pipette. This mixture was warmed slightly. Then, 5ml of acetic anhydride was added and mixed. This was followed by adding three drops of crystal violet solution as an indicator. The solution was titrated with 0.1M perchloric acid to a greenish-blue colour.

2.6. Determination of Vitamin B_6

Vitamin B_6 was determined using the titrimetric/Titration method described by Kirk and Sowyer [18]. The principle of this method is based on the hydrolysis of vitamin B_6 with acetic acid following slight warming and further extraction with mercury acetate. Vitamin B6 in the sample solution extract produces a bluish colour in the presence of perchloric acid. To determine the concentration of Vitamin B_6, fresh 0.1ml of the sample was dissolved in a mixture of 5ml of anhydrous glacial acetic acid and 6ml of 0.1m mercury II acetate solution. Then, two drops of crystal violet were added as an indicator. It was then titrated with 0.1m perchloric acid to a green colour endpoint.

2.7. Determination of Vitamin B9

Vitamin B_9 was analysed using the spectrophotometric method described by Kirk and Sowyer [18]. This involved the addition of 1ml of sample and blank solution into test tubes. In each test tube, 2 ml of 0.2% solution of phenylhydrazine (in hydrochloric acid and alcohol in a ratio of 1:5 v/v) was added and mixed well. It was heated in a water bath to almost dryness and cooled at room temperature. Then, a 15 ml solution mixture (ammonia and alcohol in a ratio of 1:1) was added to each test tube. The absorbance was read at 635 nm wavelength against blank.

2.8. Determination of vitamin B_{12}

Vitamin B_{12} was analysed using the spectrophotometric method Kirk and Sowyer [18] described. Vitamin B_{12} is extracted with deionised water, which is detected at wavelengths of 261nm and 258nm, respectively.

3.0. RESULTS

3.1 Serum Vitamin B3, B6, B12 and B9 in Cardiovascular Diseases Patients compared with Controls.

Serum Vitamins B6, B12, and B9 were significantly higher ($p=0.000$, $p=0.000$, and $p=0.004$, respectively) in cardiovascular disease patients compared to controls. There was no significant difference in serum Vitamin B3 ($p=0.274$) in cardiovascular disease patients compared to controls (Table 1).

Table 1: Serum Vitamin B3, B6, B12 and B9 in Cardiovascular disease patients compared with controls.

VARIABLES (Mean ± SD	Cardiovascular Disease (n=20)	Controls (n=20)	t-value	p-value
Vit B3(mg/dl)	0.212±0.066	0.260±0.152	-1.127	0.274
Lower 95%C.I	0.181	0.188		
Upper 95%C.I	0.243	0.331		
Vit B6(mg/dl)	6.620±1.299	3.837±1.255	9.316	0.000
Lower 95%C.I	6.012	3.249		
Upper 95%C.I	7.228	4.425		
Vit B12 (mg/dl)	28.425±12.111	16.287±5.291	4.411	0.000
Lower 95%C.I	22.756	13.811		
Upper 95%C.I	34.093	18.763		
Vit B9(mg/dl)	21.821±5.083	17.627±1.445	3.326	0.004
Lower 95%C.I	19.441	16.950		
Upper 95%C.I	24.200	18.304		

3.2 Serum Vitamin B3, B6, B12 and B9 in Male Cardiovascular disease patients compared to female Cardiovascular disease patients.

There were no significant differences in serum Vitamin B3, B6 and B9 (p=0.810, p=0.650, and p=0.478, respectively) in male cardiovascular disease patients compared to female cardiovascular disease patients. Serum vitamin b12 was significantly lower (p= 0.012) in male cardiovascular disease patients compared to female cardiovascular disease patients (table 2).

Table 2: Serum Vitamin B3, B6, B12 and B9 in Male Cardiovascular Diseases Patients with Female Cardiovascular Diseases Patients.

VARIABLE (Mean ± SD	Male Cardiovascular Disease Patients (n= 10)	Female Cardiovascular Disease Patients (n=10)	t-value	p-value
Vit B3 (mg/dl)	0.2075±0.076	0.2172±0.061	-0.248	0.810
Lower 95%C.I	0.1528	0.1735		
Upper 95%C.I	0.2620	0.2609		
Vit B6(mg/dl)	6.498±1.731	6.744±0.753	-0.469	0.650
Lower 95%C.I	5.266	5.205		
Upper 95%C.I	7.729	7.283		
Vit B12 (mg/dl)	22.234±7.303	34.624±13.079	-3.155	0.012
Lower 95%C.I	17.009	25.267		
Upper 95%C.I	27.458	43.980		
Vit B9mg/dl)	20.822±4.231	22.818±5.869	-0.741	0.478
Lower 95%C.I	17.794	18.619		
Upper 95%C.I	23.949	27.016		

3.3 Correlation of serum vitamin B3 with B6, B12 and B9 in Cardiovascular disease Patients.

There was a significant positive correlation of vitamin B3 with B6 (r=0.564, p=0.010) in cardiovascular disease patients. There was no significant correlation of vitamin B3 with B12 and B9 (r=0.137, p= 0.565 and r= -0.392, p=0.088, respectively) in cardiovascular disease patients (Table 3).

Table 3: Correlation of serum vitamin B3 with B6, B12 and B9 in cardiovascular disease patients

Dependent variables	n	r-value	p-value
Vit B6	20	0.564	0.010
Vit B12	20	0.137	0.565
Vit B9	20	-0.392	0.088

4.0. DISCUSSION

In this present study, which was conducted to determine the blood levels of the B vitamins in cardiovascular disease patients, it was observed that the concentrations of vitamins B6, B12, and B9 in serum were significantly higher in cardiovascular disease patients than in the control group. This may be because of the consumption of vitamin supplements taken by patients with cardiovascular diseases. Studies have shown that some water-soluble vitamins are deficient in different types of cardiovascular diseases [19-25]. As a result, the intake of vitamins is recommended for cardiovascular diseases. In this case, since the study participants are already attending clinical appointments, they might have been placed on vitamin-rich medications and diets, which might have probably increased the concentrations of those vitamins.

Furthermore, there was no significant difference in serum vitamin B3 in cardiovascular disease patients compared to the control group. This may be because vitamin B complexes, including vitamin B3, are water-soluble vitamins. As a result, they are dissolved in water and are readily absorbed in the tissue for immediate use, while the excess is usually excreted in urine [26]. Also, there were no significant differences in serum vitamin B3, B6 and B9 in male cardiovascular disease patients compared to female cardiovascular disease patients. This may result from the absorption and excretion rate of these vitamins [26]. This may suggest that the gender factor may not affect this vitamin B metabolism in Cardiac disease.

In this study, serum vitamin B12 was significantly lower in male cardiovascular disease patients than in female patients. Vitamin B12 plays a vital role in protein and amino acid metabolism. Homocysteine is an essential amino acid derived from the conversion of methionine to cysteine. It is increased in men compared to premenopausal women; this difference is also present in postmenopausal women [27]. The relative increase in serum homocysteine level in males as compared with females may be the reason for the reduction in the serum vitamin B12 level of male patients as compared with the female patients with cardiovascular diseases due to the critical role played by vitamin B12 as a cofactor in homocysteine metabolism.

A significant positive correlation was found between vitamin B3 and B6 in heart disease patients. This is because the two vitamins are vital in reducing the risk factors leading to cardiovascular diseases. There was no significant correlation of vitamin B3 with B12 and B9 in Cardiovascular Disease Patients. This is

because, as much as the three vitamins play essential roles in reducing risk factors for cardiovascular diseases, they affect different metabolic pathways. Vitamin B3 reduces secondary outcomes associated with atherosclerosis, such as low-density lipoprotein cholesterol (LDL), very low-density lipoprotein cholesterol (VLDL-C), and triglycerides (TG). Still, it increases high-density lipoprotein cholesterol (HDL). Furthermore, studies have shown that vitamins B12 and B9 help reduce serum homocysteine levels [28]. However, further research on this is crucial to validate these propositions.

Conclusion

This study has shown that there could be a correlation between water-soluble vitamins and the risk of the development of cardiovascular diseases. That is to say, vitamin supplementation might decrease the progression of cardiovascular diseases. However, further research should be carried out to authenticate these findings. Future research should consider increasing the sample size, conducting an observational study over a long period, and in vivo studies using animal models.

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