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Level of Serum Copper in Patients with Dilated Cardiomyopathy (DCM)

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Abstract: ***Background: Copper is an essential trace element that is required for human health. It is incorporated into a variety of proteins and metalloenzymes which perform essential metabolic functions. Dilated cardiomyopathy (DCM) is a condition in which the heart becomes enlarged and cannot pump blood effectively. It represents a leading cause of severe heart failure and heart transplantation in younger adults. Trace elements are being increasingly recognized as essential mediators of the development and progression of cardiomyopathy. Objectives: To evaluate serum copper levels in patients with DCM and in healthy controls, and to find any possible association between serum copper levels and DCM patients. Methodology: A cross - sectional study, consisting of*** **110 subjects out of which 55 subjects diagnosed with DCM and 55 healthy subjects, was carried out in the Department of Biochemistry in collaboration with Department of Cardiology, RIMS, Imphal, Manipur, India from *October 2019 to September 2021*. Serum copper levels were measured using Randox series R_X Imola Autoanalyzer (manufactured 2007, UK). Results: Mean ± SD of copper (Cu) was found to be significantly higher in cases (131.827±44.683 µg/dL) than the controls (94.455±30.861 µg/dL) (p=0.009). *Conclusion:* Serum copper *can be used in combination with other investigations such as ECG, chest X - ray, echocardiography for detecting DCM and could be utilized as an early biomarker for diagnosing DCM.***

Keywords: Copper, Dilated Cardiomyopathy, Biomarker.

1. Introduction

Copper is an essential trace element that is required for human health. It is incorporated into a variety of proteins and metalloenzymes which perform essential metabolic functions. It plays an important role in our metabolism, largely because it allows many critical enzymes to function properly.1 Cardiomyopathy is a group of diseases affecting heart muscles. Dilated cardiomyopathy (DCM), a leading cause of heart failure and heart transplantation in younger adults, is characterized by dilatation and impaired contraction of the left or both ventricles.2, 3 Imbalance of trace elements may cause myocardial metabolic dysfunction and may have a role in aetiology of cardiomyopathy, particularly in idiopathic dilated cardiomyopathy.4 Changes in the serum copper concentration have been reported previously in DCM but the results were controversial. The early and accurate diagnosis of DCM is a desirable goal for management of the disease. The field of biochemical markers of cardiovascular disease is in a dynamic state, with new applications continually appearing and new markers being developed. The relationship between copper and development of human cardiomyopathy is least understood and so far no study has been taken up in this part of the country regarding serum copper as a diagnostic marker in DCM. So, this study was taken up to evaluate serum copper levels in patients with DCM and in healthy controls, and to find any possible association between serum copper levels and DCM patients.

2. Materials and Methods

This study was a cross - sectional study conducted in the Department of Biochemistry in collaboration with Department of Cardiology/Medicine, Regional Institute of Medical Sciences (RIMS), Imphal, Manipur from October 2019 to September 2021. In the present study 110 subjects, of which 55 DCM patients and 55 age matched healthy subjects were enrolled as cases and controls respectively. Patients with hepatic and renal failure, heavy alcohol abuse and intake of supplements containing copper within one week of taking the blood samples were excluded from the study. A written informed consent was obtained from each participant in this study and the study was approved by Research Ethics Board, RIMS, Imphal (Ref. No. A/206/REB - Comm (SP) /RIMS/2015/536/14/2019.5 ml of venous blood was taken from each participant and serum was separated by centrifugation at 2000 rpm for 10 minutes. The serum was stored at - 20°C till analysis. Estimation of serum copper was carried out by Colorimetric method using Randox series R_X Imola Autoanalyzer (manufactured 2007, UK).5 Serum magnesium was also measured by Colorimetric method using Randox series R_X Imola Autoanalyzer (manufactured 2007, UK).6

3. Statistical Analysis

The collected data was analysed using SPSS version 21 for windows (IBM Corp., Armonk, NY, USA). The results were reported as number of cases and controls along with percentages for the categorical variables and mean ± SD (Standard Deviation) for quantitative variables. p value <0.05 was taken as significant.

4. Results

It is evident from table 1, that the majority of dilated cardiomyopathy cases (41.8%) occurred in the age group of >55 years, followed by 34.5% in the age group >45 - 55 years, 18.2% in the age group of >35 - 45 years and 5.4% in the age group of 25 - 35 years. Of the healthy controls, majority of the respondents were in the age group of >45 - 55 years which constitutes 38.2%, followed by 29.1% in the age group of >35 - 45 years, 20% in the age group of >55 years and 12.7% in the age group of 25 - 35 years. The Mean±SD of the age of cases and controls were 54.09±11.48 years and 46.51±9.19 years respectively. Although there were some differences, it was found to be statistically insignificant (p=0.202). So, both the groups were comparable with respect to age.

Table 1: Age distribution of the respondents stratified by cases and controls

Age in years	Cases (n=55) n (%)	Controls (n=55) n (%)	p - value
25 - 35	3 (5.4)	7 (12.7)	0.202
>35 - 45	10 (18.2)	16 (29.1)	
>45 - 55	19 (34.5)	21 (38.2)	
>55	23 (41.8)	11 (20.0)	
Mean±SD	54.09±11.48	46.51±9.19	

Table 2 shows male predominance in cases (60%) and also in controls (50.9%), whereas in females the percentage distribution in cases was 40% and 49.1% in controls. Chi - square test was applied and the difference observed was found to be statistically insignificant (p=0.443) and so there was no difference between the groups regarding gender. So, both the groups were comparable.

Table 2: Distribution of the respondents by gender stratified by cases and controls

Gender	Cases (n=55) n (%)	Controls (n=55) n (%)	p - value
Male	33 (60%)	28 (50.9%)	0.443
Female	22 (40%)	27 (49.1%)	

Table 3 shows the Mean ± SD of Cu in cases and controls were 131.827±44.683 µg/dL and 94.455±30.861 µg/dL respectively with p=0.009 which is highly significant. Mean ± SD of Mg in cases and controls were 1.427±0.431 and 2.256±0.339; p=0.129 which were similar in both the cases and controls groups

Table 3: Mean ± SD of other biochemical parameters in cases and controls

Biochemical parameters	Mean±SD		p value*
	Cases (n=55)	Controls (n=55)	
Cu (µg/dL)	131.827±44.683	94.455±30.861	0.009
Mg (mg/dL)	1.427±0.431	2.256±0.339	0.129

*Independent sample T test

Table 4 shows that among the male cases, Mean ± SD of serum copper was 131.386±45.850 µg/dL which was higher than controls (87.425±25.016 µg/dL). The difference was found to be statistically significant (p=0.000). Among female cases, Mean ± SD of serum copper was 125.959±45.011 µg/dL which was also higher than the controls (103.836±37.481 µg/dL) but was statistically not significant (p=0.084). Therefore, in males, the Mean ± SD of serum copper was significantly higher in cases as compared to controls.

Table 4: Distribution of serum copper (Cu) stratified by cases, controls and sex:

Gender	Cu (µg/dL) (Mean ± SD)		p value*
	Cases (n=55)	Controls (n=55)	
Male	131.386±45.850	87.425±25.016	0.000
Female	125.959±45.011	103.836±37.481	0.084

*Paired samples T test

5. Discussion

It was evident from Table 1 that dilated cardiomyopathy (DCM) patients showed predominance in the age group above 55 years, which comprised of 41.8%; followed by the age group of >45 - 55 years which comprised of 34.5%, 18.2% in the age group of >35 - 45 years and 5.4% in the age group of 25 - 35 years. Of the healthy controls, majority of the respondents were in the age group of >45 - 55 years which constitutes 38.2%, followed by 29.1% in the age group of >35 - 45 years, 20% in the age group of >55 years and 12.7% in the age group of 25 - 35 years. The Mean±SD of the age of cases and controls were 54.09±11.48 years and 46.51±9.19 years respectively. DCM occurs most often in adults ages 20 to 60 years. DCM often has a long latency period and patients can be clinically asymptomatic in the early stages of cardiomyopathy. But as the condition advances, signs and symptoms usually appear, leading to the diagnosis of the condition. Thus, the prevalence of DCM in our study is highest in the age group of above 55 years of age.

Among the DCM case group (Table 2), the total number of males was 33 (60%) and females was 22 (40%). Among the controls, 28 (50.9%) and 27 (49.1%) were males and females respectively. Men have more risk when compared with women. These findings were similar to the study done by Jain A et al.[7] It has been reported that sex differences occur in DCM for all causes including familial/genetic, with more males developing disease than females. It may be due to the hormone testosterone. The elevated testosterone levels in men increase cardiac inflammation and fibrosis, leading to DCM and heart failure.

It was evident from Table 3 that Mean ± SD of copper (Cu) was found to be significantly higher in cases

(131.827±44.683 µg/dL) than the controls (94.455±30.861 µg/dL) (p=0.009). This finding is consistent with the study done by Topuzoglu G et al, [8] Saleh BO et al[9] and Oster O.1[0] Ahmad M et al[11] and da Cunha S et al[12] also found that serum copper was higher in DCM cases when compared with normal individuals, however no significant difference between the two groups were observed. The mechanisms underlying the association between serum copper levels and DCM are still not fully understood. Several reports[8, 13] documented significant increased of serum Cu concentrations in patients with heart failure (HF), both IDC (idiopathic dilated cardiomyopathy) and ICM (ischemic cardiomyopathy), than in controls. These authors suggested that high serum Cu concentrations could be related to chronic infections or inflammation. Increased serum copper concentrations are found in most acute and chronic bacterial and viral infections in humans and in leukemia, Hodgkin's disease, various anemias, hemochromatosis, collagenosis, liver diseases, myocardial infarction and diseases like rheumatoid arthritis accompanied by chronic inflammatory processes.1[4] High copper concentrations in serum of patients with DCM may be the result of chronic infections or inflammation.1[0] It has been hypothesized that idiopathic dilated cardiomyopathy is a virus - induced disease.[15, 16] It has been documented that common viral infection (Coxsackie virus) can cause the accumulation of trace elements in the mouse heart. Blood copper level correlates strongly with the marker of inflammation C - reactive protein (CRP) in humans, yet substantially increasing copper intake does not increase CRP.[17] This suggests that elevated blood copper (Cu) is likely a sign of inflammation, rather than its cause. According to Frustaci A et al, [18] a cardiac viral infection might impair cellular biomembranes that could in turn increase the input or reduce the output of certain trace elements, determining toxic intracellular concentrations. Therefore, myocardial accumulation of trace elements in idiopathic dilated cardiomyopathy should be mostly intracellular, which causes myocardial cell degeneration and dysfunction.1[8] In some studies, serum Cu concentrations are found to be highly significantly inversely correlated with the ejection fraction and cardiac index. These parameters, which characterize cardiac output, are obviously negatively affected by the pathophysiological process that increases the copper concentrations in serum.1[0] Trace elements such as Cu and Zn are known to have a key role in myocardial metabolism. Copper can act as both an antioxidant and a pro - oxidant. Previous studies have shown that increased content of copper (Cu) may elevate the oxidative stress leading to cardiac functional disorders.[8, 19] Shokrzadeh M et al[13] proposed that a raised serum Cu might have a role in the development of heart failure and interventions such as administrations of Cu chelators to relieve the symptoms or to decrease the progression of heart failure was needed to be examined. Thus, higher serum copper level in DCM patients may inform the role of copper in cardiomyopathy or serum copper level may increase secondarily as a result of cardiomyopathy.

Mean±SD of serum magnesium (Table 3) was lower in cases (1.427±0.431) as compared to controls (2.256±0.339) but is statistically not significant (p=0.129). This finding is similar to the finding by Topuzoglu G et al.[8]

In this study (Table 4) serum copper levels were also analysed based on the stratification by cases, controls and gender. Mean ± SD of serum copper in male cases was (131.386±45.850µg/dL) which is significantly higher than the male controls (87.425±25.016µg/dL) with (p=0.000). Among females, Mean ± SD of serum copper in cases was (125.959±45.011µGg/dL) which was higher than the controls (103.836±37.481µg/dL) but was statistically insignificant (p=0.084). Therefore, in males, the Mean ± SD of serum copper was significantly higher in cases as compared to controls; but no significant difference was observed in female groups though the level was higher in cases than the controls. In normal individuals, serum copper level is usually higher in female compared to male. But in DCM, serum copper level rises and it is higher in males than females, which may be due to hormone testosterone.

The major impact of a combination strategy based on rapid diagnosis on first presentation will be to improve initial risk stratification and clinical management decisions. In its early stages, DCM may not have any symptoms and are found to have the condition during routine investigation. So, most patients of DCM present with heart failure if diagnosis is missed in the early stage. Therefore, early recognition of complication is of utmost importance because appropriate intervention in a timely manner can be lifesaving.

It is opined in many previous studies that serum copper levels are higher in DCM patients compared to healthy controls which is similar to the observations of our study. Thus, serum copper can be used in combination with other investigations such as ECG, chest X - ray, echocardiography for detecting DCM and could be utilized as an early marker for diagnosing DCM.

Our study has certain limitations. Firstly, serial measurements of serum copper could not be done. Single blood sampling and testing was available for each patient, which precluded an evaluation of the time dependence and peak level of the markers. Secondly, correlation between serum copper and cardiac outcomes could not be evaluated. Moreover, as the sample size is small and the duration of the study is short, it is recommended that larger study with more sample size, with serial measurement of copper and also follow up studies will be useful to come to a definitive conclusion.

6. Conclusion

A multi - biomarker approach is needed to enhance the diagnostic accuracy and appropriate management of DCM. Thus, it can be concluded that serum copper can be used in association with other investigations as an early marker for the diagnosis of DCM.

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