EVALUATION OF SERUM VITAMIN B₁₂ AND FOLIC ACID IN PATIENTS WITH ACUTE MYOCARDIAL INFARCTION AMONG SUDANESE'S PATIENT'S

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Abstract

The Sudanese population has high rate of coronary artery disease (CAD). The potential association between deficiency of vitamin B₁₂ and folic acid, in patients with acute myocardial infarction (AMI), where investigated previously with conflicted results. A case-control study was carried out involving 60 AMI patients (age 39–87 years; 40 men and 20 women) and 40 normal healthy individuals (age 39–84 years; 20 men and 20 women). Fasting venous blood samples were obtained from patients and controls. Serum was analyzed for vitamin B₁₂ and folic acid using radio assays. The mean concentration of serum B₁₂ and folate in AMI patients were found to be significantly lower than in controls. Vitamin B₁₂ and folate deficiency may be considered as a risk factor for CAD development.

Key Words: Cardiovascular Disease, Acute Myocardial Infarction, Vitamin B₁₂, Folate – Sudan.

Introduction

Cardiovascular disease (CVD) is the leading cause of death in both developed and developing countries. A number of studies during the past few years have indicated a protective role of vitamin B₁₂ and folic acid against the development of CAD[1]. More recent reports have shown an association between the deficiencies of vitamin B₁₂ and, folic acid known as a risk factor for myocardial infarction[2]. This has focused attention on vitamin B₁₂ and folate and the important role they might play in protection against the development of CAD[3].

AMI an acute coronary syndrome is nearly always caused by a sudden reduction in coronary blood flow caused by atherosclerosis with thrombosis superimposed, with or without concomitant vasoconstriction[4]. The clinical presentation and outcome depend on the location of the obstruction and the severity and duration of myocardial ischemia[5].

Folic acid is a B vitamin which is vital for the formation of red blood cells. The form of folic acid occurring naturally in food is termed ‘folate’[6]. Folic acid, together with vitamin B₁₂, is necessary to form red blood cells. Vitamin B₁₂ was discovered because of its relationship to the disease pernicious anemia (PA)[7]. PA was a fatal illness before the 1920s. But this changed after Whipple suggested raw liver as a treatment, Those vitamins also help nerves to function properly[8]. Folic acid is also essential in the formation of DNA (genetic material) within everybody cell, allowing each cell to replicate perfectly[9].

Few case reports were published on the deficiency of vitamin B₁₂ and folic acid known as a risk factor for coronary artery syndromes in adult patients [10]. We present 60 case diagnosed with acute myocardial infarction (AMI) subsequently with vitamin B₁₂ and folic acid deficiency. A comparative case control report evaluation has been conducted to identify the differences and
similarities in clinical presentation, laboratory investigation reports, and management.

The objectives of this research were to evaluate the relationship between deficiency of vitamin B12 and folic acid and ischemic heart disease and to investigate whether patients with acute myocardial infarction (AMI) have lower levels of serum B12 or folate compared with healthy individuals.

**Materials, Patients and Methods:**
Subjects were selected from patients admitted to the coronary care unit of Al-Shaab Hospital and Sudan Heart Hospital, Khartoum, Sudan, during the period from March to August 2020. The selection was based on the AMI WHO (World Health Organization) criteria: clinical history of myocardial ischemia, ECG findings, and elevation of biochemical markers. Patients were also assessed for risk factors for CVD, such as hypertension, obesity, and a family history of ischemic heart disease (IHD). All blood samples were obtained at least 3 months following AMI. Similarly, 40 aged and sex-matched healthy individuals were selected as controls and screened for the above risk factors. Informed consent was obtained from all participants and the study was approved by the Ethics Committee of Sudan International University of Science and Technology college of Laboratory Science. Hemoglobin, packed cell volume, Mean cell hemoglobin and Mean cell volume were analyzed from venous blood obtained within 24hr of AMI, using a commercial colorimetric kit (Sysmex KX 21). Serum samples were analyzed for vitamin B12 and folate using radio assays (Cobas- 6000). Statistical analysis values are reported as mean ± S.D. Percentages were compared with the test of proportions using chi-square. Mean values of various groups were compared using Analysis of Variance. The analyses were performed using SPSS software version 9 (SPSS Inc., Chicago, IL, and U.S.A). A P value of less than 0.05 was considered significant.

**Results:**
Sixty patients, aged 39–87 years, with a confirmed diagnosis of AMI were included in this study. The distribution of the patients in correlated to gender was (60%) of the patients were males and (40%) were females. The majority of the patients were adults.

The mean results of vit B12 was significantly lower among AMI case group (169.9± 64.2) than control group (551.2±154.0) with p.value (0.00) in table (1). The mean of folic acid was significantly lower among AMI case group (2.7± 1.2) than control group (10.7±3.5) with p.value (0.00).

The mean concentration of vit b12 and folate were found significantly lower than in controls with results ((169.9± 64.2).

| Sample | Vit B12 | Folic Acid |
|--------|---------|------------|
|        | Mean    | Std. Deviation | P.value | Mean    | Std. Deviation | P.value |
| Case   | 169.9   | 64.2       | 0.00     | 2.7     | 1.2          | 0.00    |
| Control| 551.2   | 154.0      |          | 10.7    | 3.5          |        |

P. value <0.05 is consider significant  
SD= stander deviation

There were significant correlation between Hb, MCV, MCH and RBC in study population when correlate with control group with p. value (0.00&0.00&0.00&0.00) respectively in table (2).

| Sample | Hb | MCV | MCH | RBC |
|--------|----|-----|-----|-----|
|        | Mean | Std. Deviation | Mean    | Std. Deviation | p. value |
| Case   | 9.8  | 1.6  | 12.8 | 1.3       | 0.00     |
| Control| 112  | 10.0 | 87   | 4.0       | 0.00     |
| Case   | 37.4 | 3.3  | 27.5 | 1.8       | 0.00     |
| Control| 2.66 | 0.64 | 4.62 | 0.51      | 0.00     |

P. value <0.05 is consider significant  
SD= stander deviation

The results showed insignificant correlation between serum B12, folate and gender with P. value (0.6, 0.1) respectively. On other hands, the control group show that insignificant association between the folic acid and B12 with p. value (0.6) for vit B12 and (0.8) for folic acid.

| Sample | Case | Control |
|--------|------|---------|
| Hb     | Mean | Std. Deviation | Mean    | Std. Deviation | p. value |
| MCV    | 112  | 10.0 | 87   | 4.0       | 0.00     |
| MCH    | 37.4 | 3.3  | 27.5 | 1.8       | 0.00     |
| RBC    | 2.66 | 0.64 | 4.62 | 0.51      | 0.00     |
Table 3: Result of vit B12 and folic acid with gender of case and control:

| Gender | Case | Control |
|--------|------|---------|
|        | Vit B12 | Folic Acid | Vit B12 | Folic Acid |
|        | Mean | Std. Deviation | Mean | Std. Deviation | Mean | Std. Deviation | Mean | Std. Deviation |
| M      | 180.9 | 70.3 | 2.9 | 1.4 | 563.1 | 135.1 | 10.6 | 3.5 |
| F      | 147.9 | 43.6 | 2.4 | 0.9 | 539.4 | 173.5 | 10.7 | 3.6 |
| P. value | 0.06 | 0.1 | 0.6 | 0.8 |

P. value <0.05 is consider significant
SD= stander deviation

The RBCs and Hb with their parameter in case group and control group in association with all ages in table (4) was found to be significant with p. value (0.00) in all ages .On the other hand, vit B12 and folic acid were found in case and control group significant in all ages with p. value (0.003) & (0.14) respectively.

Table (4): Result of vit B12, folic acid Hb, MCV, MCH, RBC with age of case and control:

| Age   | Case | Control |
|-------|------|---------|
|       | 40-60 | 61-80 | 40-60 | 61-80 |
|       | Mean | Std. Deviation | Mean | Std. Deviation | Mean | Std. Deviation | Mean | Std. Deviation |
| Vit B12 | 223.5 | 62.7 | 155.1 | 56.8 | 570.2 | 163.4 | 537.2 | 148.7 | 0.003 |
| Folic Acid | 3.3 | 1.1 | 2.6 | 1.3 | 9.7 | 2.9 | 11.4 | 3.8 | 0.14 |
| Hb     | 11.1 | 1.5 | 9.4 | 1.4 | 13.0 | 1.5 | 12.7 | 1.2 | 0.00 |
| MCV    | 103.8 | 7.3 | 114.6 | 9.7 | 86.4 | 4.1 | 86.8 | 4.8 | 0.00 |
| MCH    | 34.7 | 2.5 | 38.1 | 3.2 | 27.3 | 1.7 | 27.6 | 2.0 | 0.00 |
| RBC    | 3.2 | 0.6 | 2.5 | 0.6 | 4.7 | 0.6 | 4.6 | 0.5 | 0.00 |

P. value <0.05 is consider significant
SD= stander deviation

Discussion
Mortality due to CAD is a global problem[11]. Despite the lack of accurate data on the mortality rate from CAD in Sudan, AMI was reported to be one of the leading causes of death. Therefore, and due to the high prevalence of CAD risk factors we investigated the effect of vitamin B12 and folate deficiencies in AMI patients.

The present study shows a significant association between vitamin B12 and folate deficiencies and the development of CAD. This may suggest that deficiency in those vitamins may play a critical role in the development of MI, and this results are in agreement with those reports by [12] in Jordon which show that there was significant association with deficiencies of vitamin B12 and folate in development of AMI .On the other hand, Hb, MCV, MCH and RBC levels in AMI patients were significant different from those in controls (Table 2). The result of Hb, MCV, MCH, RBC in both males and females which showed significant decrease in Hb & RBCs count and significant increase in MCV and MCH.

The mean of vit B12 was insignificantly among gender of AMI patients group (180.9± 70.3) for male and (147.9±43.6) in female but The mean of folic acid was significantly among AMI patients group (2.9± 1.4) in males and (2.4±0.9) in female with p.value (0.1) in table (3).On other hands, the control group show that insignificant association between the folic acid and B12 with p. value (0.6) for vit B12 and (0.8) for folic acid, these findings were significantly lower levels of folate in gender group are in agreement with those reported by [13], who demonstrated a significant decrease in the levels of folate in males smokers. Free radicals from cigarette smoking, inadequate consumption of vegetables and fruit, and increased excretion of folate might have participated in decreased body folate [14] [15] [16].

The result of Hb, MCV, MCH, RBC in both males and females was significant decrease in Hb &RBCs count and significant increase in MCV and MCH with p. value (0.00&0.00&0.00&0.00) respectively. Furthermore, one of the significant differences between our case and all the published case reports was the finding of hemoglobin level [7-10] g/dl. The arithmetic means of hemoglobin levels was found in patients reported in all the case reports to be 10.85 g/DL. This calculated mean hemoglobin level was comparatively lower than the controls group, which mean presence of macrocytic hypo
chromic anemia, and our findings of significantly lower levels of Hb, RBCs and significant increase in MCV and MCH and raise of macrocytic hypochromic anemia in patients group are agreement with those reported in India[17].

On the other hands, the result of vit B12 and folic acid with age of case and control was found significant also significant in folic acid case and control group with p.value(0.003) & (0.14) respectively .These finding in the present study showed that the negative correlation between folic acid in all ages, since the degree of correlation between CAD and vitamin B12 and folate deficiencies in all ages may differ from population to population depending on genetic factors and gene environment interaction. It has been reported that the genetic background of the Finnish differs from that of other countries [18].

**Conclusion:**

- Based on these findings, we may propose that vitamin B12 and folate deficiencies could aggravate the risk of CAD development.
- There was a deficiency in vitamin B12 and folate in case study group. The deficiency in those vitamins was even more prominent in the AMI patient group. This may explain the rate of CAD among Sudanese and might imply the protective role of those vitamins against the development of CAD.
- In such individual with family history, it is necessary to check the vitamin B12 & folic acid levels at regular interval to avoid CAD complications.

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**References:**

1. Losonczy, K. G., Harris, T. B. and Havlik, R. J. (1996) Vitamin E and vitamin C supplement use and risk of all-cause and coronary heart disease mortality in older persons: the established populations for epidemiological studies of the elderly. Am. J. Clin. Nutr. 64, 190–196.
2. Verhoef, P., Stampfer, M. J., Buring, J. E., Gaziano, J. M., Allen, R. H., Stabler, S. P., Reynolds, R. D. Kok, F. J., Hennekens, C. H., Willett, W. C. (1996) Homocysteine metabolism and risk of myocardial infarction: relation with vitamins B6, B12 and of No. 1 late. Am. J. Epidemiol., 143, 845–859
3. Nishtar, S. (1999) the role of vitamins as risk modifying agents in coronary artery disease. Pak. J. Cardiol., 10, 5–7.
4. Davies MJ. The pathophysiology of acute coronary syndromes. Heart 2000; 83:361–6.
5. Bertrand ME, Simoons ML, and Fox KA et al. Management of acute coronary syndromes: acute coronary syndromes without persistent ST segment elevation. Recommendations of the Task Force of the European Society of Cardiology. Eur Heart J 2000; 221:1406–32.
6. Devalia, V. et al. (2014). Guidelines for the diagnosis and treatment of cobalamin and folate disorders. British Journal of Hematology, 496-513.
7. Solomon E, Berlin H, Berlin R, Brante G. (2005) Oral treatment of pernicious anemia with high doses of vitamin B12 without intrinsic factor. Acta Med Scand 1968; 184:247–58.
8. Grasbeck R. Biochemistry and clinical chemistry of vitamin B12 transport and the related diseases. Clin Biochem 1984; 17:99–107.
9. Rathod S, Sayyed A, Mahale J, Gaikwad V. Young myocardial infarction: Secondary to vitamin B12 deficiency. Int J Recent Trends Sci Technol 2015; 15(3):521-3.
10. Ticagrelor for the Treatment of Acute Coronary Syndromes Guidance and Guidelines NICE. Nice.Org. UK; 2011. Available from: Appendix I: http://www.gradeworkingingoup.org/index.htm).
11. Facila, L., Nunez, J. E., Bertomeu-Gonzalez V., Sanchis, J., Bodi, V., Chorro, F. J., Llacer, A. and Chorro, F. J. (2005) Early determination of homocysteine levels in acute coronary syndromes, is it an independent prognostic factor? Int. J. Cardiol., 100, 275–279.
12. Mohamad Khakid Nusier and Qasim Abdel El-Dwairib Effects of Vitamin B12 and Folic Acid on Hyperhomocysteinemia in Patients with Acute Myocardial Infarction, Jordan University of Science and Technology School of Medicine, p.o. Box 3030 Irbid 22110, Jordan.
13. Mansoor, M. A., Bergmark, C., Svardal, A. M., Lonning, P. E. and Ueland, P. M. (1995) Redox status and protein binding of plasma homocysteine and other aminothiols in patients with early-onset peripheral vascular disease. Homocysteine and peripheral vascular disease. Arterioscler. Thromb. Vasc. Biol., 15, 232–240.
14. Subar, A. F., Harlan, L. C. and Mattson, M. E. (1990) Food and nutrient intake differences
between smokers and non-smokers in the US. Am. J. Public Health, 80, 1323–1329.

15. Pryor, W. A. and Stone, K. (1993) Oxidants in cigarette smoke. Radicals, hydrogen peroxide, peroxynitrate, and peroxynitrite. Ann. N.Y. Acad. Sci., 686, 12–28.

16. McKay, J. A., Williams, E. A. and Mathers, J. C. (2004) Folate and DNA methylation during in utero development and aging. Biochem. Soc. Trans., 32, 1006–1007.

17. Sastry, B. K., Indira, N., Anand, B., Kedarnath, Prabha, B. S. and Raju, B. S. (2001) a case control study of plasma homocysteine levels in South Indians with and without coronary artery disease. Indian. Heart. J., 53, 749–753.

18. Knekt, P., Reunanen, A., Alftan, G., Heliovaara, M., Rissanen, H., Marniemi, J., Aromaa, A. (2001) Hyperhomocystinemia: a risk factor or a consequence of coronary heart disease? Arch. Intern. Med., 161, 1589–1594.