Original Article

Relationship of B-vitamins (Vitamin B_{12}, B_6 and Folic Acid) and Coronary Artery Disease in Pakistan

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Abstract

Background: Coronary artery disease is one of the most lethal diseases in Pakistani population and vitamin deficiency is common in this country.

Aim: Present study was carried out to find a probable relationship between vitamin B_{12}, B_6 and Folic acid and coronary artery disease in Pakistani population.

Methodology: A cross sectional study was carried out. A total of 40, angiographically diagnosed male patients of coronary artery disease, between 30 – 45 years of age were taken as cases. A same number of age, sex and socioeconomically matched normal healthy male subjects with normal carotid doppler study were taken as controls. Fasting venous blood was obtained from all the cases and controls in E.D.T.A vacutainers. Plasma was analyzed for vitamin B_{12} and folic acid levels using competitive protein binding radioassay and for vitamin B_6 (PLP, a co-enzymic form of vitamin B_6) using radioenzymatic assay.

Results: Mean plasma vitamin B_{12} concentration in coronary artery disease patients was found to be lower than the mean for controls (150.25 ± 135.98 pmol/L vs 204 ± 92.26 pmol/L). Plasma folic acid levels were also lower in patients than in the controls (12.11 ± 17.35 nmol/L vs 28.47 ± 37.23 nmol/L). The levels of vitamin B_6 were also low in patients than in controls (8.26 ± 6.18 nmol/L vs 11.82 ± 9.24 nmol/L).The results also showed an inverse relation between the vitamin B_{12}, B_6 and folic acid levels and the extent of coronary artery disease as shown by the number of coronary blood vessels blocked.

Conclusion: There is an inverse relationship between the plasma vitamin levels and coronary artery disease. It may therefore be concluded that the deficiency of B-vitamins (B_{12}, B_6 and folic acid) may be aggravating the risk of coronary artery disease through an interplay with the classical risk factors of coronary artery disease.

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Introduction

Coronary artery disease is one of the major diseases causing mortality and morbidity in South Asians[1] including Pakistanis.[2] There are many risk factors linked to the development of this disease. Some of them are modifiable like lifestyle[3] and cigarette smoking[4] while others are not modifiable like genetic makeup. Beside other modifiable risk factors results of some researchers have shown vitamin B deficiency especially of vitamin B_6, B_{12} and folic acid as important

modifiable risk factors[5] for coronary artery disease. These vitamins play an important role in the metabolism of homocysteine[6] which is an independent risk factor for coronary artery disease.[7]

Vitamin B_{12} is found in animal products like fish, poultary, meat milk and milk products. Beside other functions of vitamin B_{12} like red cell formation, DNA synthesis and neurologic functions, an important function of this vitamin is to act as a co-factor for methionine synthase which catalyzes the conversion of homocysteine to methionine,[8] needed for the formation of S-adenosyl methionine which is a methyl donor for DNA, RNA, proteins and lipids. A deficiency of this vitamin, may thus lead to a rise in plasma homocysteine level leading to atherosclerosis and finally the development of coronary artery disease.

Vitamin B_6 is found in beans, poultary, fish,some fruits like oranges and some green leafy vegetables. This vitamin is an important co-factor in the metabolism of homocysteine which if present in excess of normal, may lead to atheroma and coronary artery disease. It has been found that a rise of only 1 μmol/L of homocyteine above normal is equivalent to 20 mg/dl rise of cholesterol above normal. Thus a deficiency of vitamin B_6 may cause coronary artery disease. Moreover a supplementation with B_6, reduces cardiovascular disease as shown by the result of research by Rimm EB et al.[9]

Folic acid is found in beans, whole grains, cereals, vegetables and fruits. This is destroyed if the food is over cooked. Folic acid has many important functions in body like red cell formation. In addition to other functions in body, it also plays a role in metabolism of homocysteine in the remethylation pathway,[10] and thus prevents coronary artery disease. Many researchers have been carried out to see the effect of vitamin supplementation on homocysteine and coronary artery disease. Results of these researches have shown a decrease in homocyteine levels by supplementation with vitamin B_{12}, B_6 and Folic acid,[11] but their effect in actually reducing the risk of coronary artery disease is controversial. Some have shown a positive relationship,[12] while others have shown no effect of vitamin supplementation.[13] However most of the subjects, included in studies showing negative results, had a normal baseline homocysteine level. Randomized trials are needed to see if supplementation with B-vitamins improves outcome in patients with high homocysteine levels.

Researches to find out any correlation between vitamin B levels and coronary artery disease have been mostly carried out in developed countries with high per capita income, and with high literacy rate. Vitamin B deficiency is therefore less likely to develop in these countries. In under developed countries like Pakistan, with a low per capita income and low literacy rate a deficiency of B-vitamins (B_{12}, B_6f, and folic acid) is expected. The present research was thus, carried out to ascertain the relationship of deficiency of B-vitamins and coronary artery disease in Pakistan.

Materials and Methods:

Subjects

Both cases and controls participated willingly with prior consent to undergo tests and examination. The criteria for inclusion for cases were, males between 30 – 45 years of age, who were documented cases of coronary artery disease, diagnosed on the basis of angiography and for controls were healthy males between 30 – 45 years with no history of any disease and with normal carotid doppler study. The exclusion criteria were all patients taking vitamin B (i.e B_6, B_{12} or Folic acid) preparations for the last 6 months prior to sample collection or patients suffering from malabsorption syndrome, acute infections and an age group > 45 years. In controls in addition to above, any abnormality in carotid doppler study were also excluded. Both cases and controls were age, sex and socioeconomically matched.

A total of 80 subjects with 40 controls and 40 cases, were selected after observing the inclusion and exclusion criteria. Patients were selected from Punjab Institute of Cardiology, Lahore and controls were from general public. A 5 ml sample of venous blood from both cases and controls was taken in E.D.T.A vacutainers. The plasma was separated within half an hour and after dividing it into two alliquotes (one for vitamin B_{12} and Folic acid and other for vitamin B_6 estimation) was stored in dark boxes at -21°C till analysis.

Plasma vitamin B_{12} and Folic acid were estimated using Simul – TRAC – SNB Radioassay kit, Vit B_{12} (^{57}Co) / Folate (^{125}I) and were determined on β-counter by Barthold LBL111.[14] Plasma vitamin B_6 levels were determined using BUHLMANN Vit B_6, pyridoxal – 5 – phosphate[5] H-REA[15] kit on ϒ – counter. Quality control was maintained using standard solutions and sera with both low and high values provided in the kit. Statistical analysis was done using SPSS version 15.0. The students't test was applied and coefficient of cor-

Table 1: Comparison of Plasma Vitamin B_{12}, Folic Acid and Vitamin B_6 in controls Vs Cases. Mean ± SD is given.

Parameters	Controls (40)	Cases (40)	P-Value
Plasma Vitamin B_{12} (pmol/L)	204 ± 92.26	150.25 ± 135.98	0.042
Plasma Folic acid (nmol/L)	28.47 ± 37.23	12.11 ± 17.35	0.015
Plasma Vitamin B_6 (nmol/L)	11.82 ± 9.24	8.26 ± 6.18	0.046

$P < 0.05$

relation was calculated. The p value < 0.05 was considered significant.

Results

The comparison of mean values for vitamin B_{12}, B_6 and Folic acid between control group and cases (patients) showed a significant p-value (table 1). Normal reference range of plasma vitamin B_{12} is 118 – 716 pmol/L.[14] Lab analysis of 40 control subjects for vitamin B_{12} showed a mean value of 204 ± 92.26 pmol/L and for cases the value was 150.25 ± 135.98. The mean values were within reference range in both groups, but the patients had lower values and greater standard deviation than controls with a significant p-value of 0.042. The value ranged from 109.2 – 390.17 pmol/L in controls and 116.0 – 457.7 pmol/L in patients showing that the minimum level of B_{12} was much lower in patients. The results also showed that the number of patients with lower values was more than the controls. There was also an inverse relationship between the vitamin B_{12} level and extent of coronary artery disease shown by number of vessels blocked.

Normal reference range of plasma folic acid is 3.41 – 38.45 nmol/L.[14] In current study the mean value of folic acid in control group was 28.47 ± 37.23 nmol/L. In cases the value was 12.11 ± 17.35 nmol/L. The value although within the normal reference range in both groups was much lower in cases than in controls with a significant p-value of 0.015.There was also an inverse relationship between the folic acid level and the extent of coronary artery disease as indicated by the number of coronary arteries blocked.

Normal reference range for vitamin B_6 according to the method used in the current study was 9.6 – 23.75 nmol/L.[15] The mean plasma vitamin B_6 level for 40 controls was 11.82 ± 9.24 nmol/L and in cases was 8.26 ± 6.18 nmol/L. The p-value was less than 0.05 and was significant (table 1). Moreover an inverse relationship was found between the vitamin B_6 level and the number of coronary arteries blocked, showing the extent of coronary artery disease.

Discussion

According to the most careful estimates based on scientific studies, nearly 100,000 individuals suffered from acute myocardial infarction in the year 2002.[16] Pakistan is facing a high challenge in combating coronary artery disease. The levels of vitamin B_{12}, B_6 and folic acid were studied in patients with coronary artery disease as well as in healthy individuals in present research to investigate the role of nutritional deficit in terms of vitamin B_{12}, B_6 and folic acid and its relationship to coronary artery disease.

The present study showed significantly low levels of vitamin B_{12}, B_6 and folic acid in patients of coronary artery disease as compared to controls. These B vitamins are needed in the metabolism of certain compounds present in the blood which if not metabolized may have harmful effects on blood vessels leading to atheroma formation and eventually causing coronary artery disease. One such compound is homocysteine. The deficiency of B-vitamins may lead to hyperhomocystenemia and thus coronary artery disease as hyperhomocystenemia is an independent risk factor for coronary artery disease.[17] The results of present study showing a low level of B-vitamins (B_{12}, B_6 and folic acid) in patients of coronary artery disease are in agreement with the results of Genser et al.[18] The inverse relationship of B-vitamins with coronary artery disease, as indicated in the present study is also supported by randomized controlled trials indicating that combination of vitamin B_{12} and folic acid supplements decrease the risk of coronary artery disease, probably through their effect on homocysteine.[19] Current study also indicated an inverse relationship between the extent of disease assessed by the number of coronary arteries involved and the level of B-vitamins. Similar relationship has been reported by Kilmer and Mc Cully[8].

It thus appears that in addition to other risk factors vitamin B_{12}, B_6 and folic acid deficiency may also be an important risk factor for development of coronary artery disease. In order to have a broader and better insight for the implementation of the results of current

study there is a need for designing a large scale study. This would help to recognize a modifiable risk factor for coronary artery disease, thereby helping to fight against this high risk disease with a high rate of morbidity and mortality.

Conclusion

The results of present study suggested that significant deficiencies of vitamin B_{12}, B_6 and folic acid could act synergistically in association with other classical risk factors, thereby further aggravating the risk of coronary artery disease. The results of current study also indicated that there is a prevalence of deficiency of vitamins B_{12}, B_6 and folic acid in all the subjects with a greater deficiency in patients of coronary artery disease than in healthy individuals. This is consistent with their important role in preventing the development of coronary artery disease. Thus in essence this small scale study representing only a small proportion of Pakistani population showed the great need for the improvement of vitamin B status in the people of this country to decrease the incidence of coronary artery disease. The measures taken to improve the vitamin status would thereby decrease the risk of this lethal disease.

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