Original Research Article

# Study of correlation between serum vit-B12 and homocysteine levels with ischemic heart disease

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## Abstract

**Background:** Vitamin B12 reduces the risk of coronary heart disease and stroke by quenching homocysteine. Hyperhomocysteinemia, is emerging as a strong risk factor for cardiovascular events. **Aim:** To find out the correlation between serum vit-B12 and homocysteine levels with ischemic heart disease. **Material and Methods:** A total of 100 patients of Ischemic Heart Disease (IHD) were included as cases and 150 normal healthy populations was selected as controls. Quantitative determination of total L-homocysteine in human serum or plasma was done by using Chemiluminescent Microparticle Immunoassay (CIMA) technology and vitamin B12 assay estimation was done by competitive binding immunoenzymatic assay. **Results:** Out of 100 IHD patients, 59 had low vitamin B12 levels and 69 out of 150 individuals in control group had low vitamin B12 levels ($p<0.05$). The number of individuals with high homocysteine levels was 75 out of 100 in patient group and 79 out of 150 in control group. 25 cases were with normal vit B12 levels and high homocysteine and 22 controls with normal vit B12 levels and high homocysteine levels. Hyperhomocysteinemia was found even amongst those with normal vit B12 levels ($p>0.05$). **Conclusion:** Low vit B12 and high homocysteine levels are significantly associated with IHD.

**Key Words:** Ischemic heart disease, serum vit-B12, homocysteine, correlation

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Received Date: 29/09/2019 Revised Date: 30/10/2019 Accepted Date: 23/11/2019
DOI: https://doi.org/10.26611/10211236

| Access this article online | |
|---|---|
| Quick Response Code: | Website: www.medpulse.in |
| | Accessed Date: 09 December 2019 |

## INTRODUCTION

Despite considerable understanding of the cause of cardiovascular disease, the established risk factors cannot fully explain its occurrence. Homocysteine has emerged after 25 years of research as the "new cholesterol", and researchers estimate that it is a major risk factor in 10 to 40 percent of heart attacks and strokes in the United States.1 Under normal circumstances, this amino acid is a short-lived by product of methionine metabolism, but a diet short on B vitamins prevents its breakdown. Hyperhomocysteinemia, is emerging as a strong risk factor for cardiovascular events, atherosclerotic vascular disease in the coronary, cerebral and peripheral vessels and for arterial and venous thromboembolism.[1] Vitamin B12 reduces the risk of coronary heart disease and stroke by quenching homocysteine, an amino acid in the blood that attacks blood vessel walls and promotes cardiovascular disease.[2] Years ago, vitamin B12 deficiency was typically diagnosed by looking at red-blood cell abnormalities under a microscope. Very high doses of folic acid can mask these blood abnormalities, allowing neurological damage from B12 deficiency to continue unnoticed. The advent of simple assays has changed vitamin B12 and homocysteine measurement from a research tool to a standard and routine clinical test. Strict vegetarians and occasional non vegetarians

How to cite this article: Shilpa Shaunak Sule, N C Edul, R S Wadia. Study of correlation between serum vit-B12 and homocysteine levels with ischemic heart disease. *MedPulse International Journal of Medicine.* December 2019; 12(3): 91-95.
https://www.medpulse.in/Medicine/

routinely suffer from Vit-B12 deficiency.[3] Thus, Vit-B12, folate and homocysteine levels may be associated with ischaemic heart disease. This study was conducted to find out the correlation between serum vit-B12 and homocysteine levels with ischemic heart disease.

## MATERIAL AND METHODS

**Type of study**

Prospective case control study

**Source of data**

The data for the study was collected from the inpatients and outpatients of the hospital, who fulfilled the inclusion and exclusion criteria. Patients of Ischemic Heart Disease (IHD) admitted in Department of Medicine were included. Normal healthy population was selected from people who came for routine health checkup and staff members of the hospital.

Group I (Cases): 100 cases of Ischaemic Heart disease

Group II (Controls): 150 normal healthy people

**Inclusion criteria (Group I-cases)**

- 100 randomly selected patients who came to OPD / IPD of our hospital and diagnosed as Ischaemic Heart Disease.

**Exclusion criteria (Group I-cases)**

- Patients < 12 years
- Patients on Haemodialysis
- Patients with renal transplant
- Patients on drugs such as methotrexate, theophylline, metformin and niacin
- Patients with other renal, liver or major systemic disorder.

**Inclusion criteria (Group II-controls)**

- Lab staff of our hospital
- People who came for routine health check-up in our hospital
- Resident doctors and consultants of our hospital

**Exclusion criteria (Group II-controls)**

- DM/HTN
- vitamin supplement
- Any apparent disease

After obtaining informed consent they were evaluated through a structured proforma designed especially for this study. For every patient detailed history including personal and family history were taken. Each patient was subjected to thorough general examination and systemic examination. The lab investigations done in each patient were Serum B12, Homocysteine, Lipid profile and fasting (F) and postprandial (PP) blood sugar level (BSL).

**Definitions**

1. Patients having BP >140/90 on 2 occasions and those who were already on antihypertensive medication were considered to be hypertensive.
2. Patients with Fasting BSL>126mg/dl and Postprandial BSL>200mg/dl and those who were already on anti-diabetic treatment were considered diabetic.
3. Vit. B12 level <210pg/ml was considered as having vit B12 deficiency. Values >1500pg/ml were not measurable with our instruments.
4. Homocysteine levels </=15µmol/l were considered normal and >15µmol/l were considered high.
5. People who consumed non-vegetarian diet atleast thrice a week were considered non-vegetarians.

**Homocysteine estimation**

Homocysteine assay is a one-step immunoassay for the quantitative determination of total L-homocysteine in human serum or plasma using Chemiluminescent Microparticle Immunoassay (CIMA) technology, with flexible assay protocols, referred to as Chemiflex.

**Vitamin B12 estimation**

Vitamin B12 assay is a competitive binding immunoenzymatic assay.

**Statistical analysis**

SPSS for windows (version 21.0, SPSS Inc., Chicago, IL, USA) was employed for data analysis. $P < 0.05$ was considered as significant. Fisher's exact test was used to determine if there are nonrandom associations between two categorical variables.

## RESULTS

In our study, 59 patients of IHD out of 100 had low vitamin B12 levels and 69 out of 150 individuals in control group had low vitamin B12 levels. By using Fisher's exact test p-value <0.05, therefore there is a significant difference between vit B12 levels of cases and controls. Thus we found that low serum vitamin B12 levels were significantly associated with IHD in our study.

**Table 1:** Distribution of patients with respect to serum-B12 in group 1 and 2

| B12 level | Groups | | p-value |
|---|---|---|---|
| | Group 1 (cases) | Group 2 (controls) | |
| < 210 | 59 (59.00%) | 69 (46.00%) | 0.005 |
| 210 – 1500 | 38 (38.00%) | 81 (54.00%) | |
| > 1500 | 3 (3.00%) | 0 | |
| **Total** | **100** | **150** | |

The number of individuals with high homocysteine levels was 75 out of 100 in patient group and 79 out of 150 in control group. By using chi-square test p-value <0.05, therefore there is a significant difference between homocysteine levels of cases and controls. Thus we found that higher homocysteine levels were significantly associated with IHD in our study.

**Table 2:** Distribution of patients with respect to homocysteine in group 1 and 2

| Homocysteine level | Groups | | p-value |
|---|---|---|---|
| | Group 1 (cases) | Group 2 (cases) | |
| ≤ 15 | 25 (25.00%) | 71 (47.33%) | < 0.001 |
| > 15 | 75 (75.00%) | 79 (52.67%) | |
| **Total** | **100** | **150** | |

We had divided the cases in 2 groups depending upon the age. The young group consisted of individuals of or below 50 yrs of age and old group consisted of patients above 50 yrs of age. In our study we found that low B12 and high homocysteine levels are uniformly distributed among both the age groups. By using Fisher's exact test p-value > 0.05, therefore there is no significant difference between vit B12 levels of both the age groups among the cases.

**Table 3:** Distribution of cases with respect to vit. B12 levels in both the age groups

| Group code | B12 | Age | | p-value |
|---|---|---|---|---|
| | | ≤ 50 | > 50 | |
| Group 1 cases | < 210 | 26 | 33 | 0.862 |
| | 210 - 1500 | 15 | 23 | |
| | > 1500 | 1 | 2 | |
| | **Total** | **42** | **58** | |

The mean Homocysteine was higher in our population, 24.48 µmol/l in cases group and 21.17 µmol/l in control group. The mean B12 in our population was 298 pg/ml in cases group and 288 pg/ml in controls. In our study, 59 out of 100 cases and 69 out of 150 controls i.e. 128 of the total 250 individuals (51.2%) had low vit B12 levels (below 210 pg/ml). High homocysteine levels were found in 75 out of 100 cases and 79 out of 150 controls i.e., 154 out of total 250 individuals (61.6%).

**Table 4:** Mean homocysteine and vit B12 levels in our population

| | | Serum B12 | Homocysteine |
|---|---|---|---|
| Group I (cases) | Mean | 298.39 | 24.48 |
| | SD | 349.98 | 13.84 |
| Group II (Control) | Mean | 282.16 | 19.02 |
| | SD | 225.81 | 10.23 |
| Overall | Mean | 288.61 | 21.17 |
| | SD | 281.25 | 12.07 |

In our study, we had 25 cases with normal vit B12 levels and high homocysteine and 22 controls with normal vit B12 levels and high homocysteine levels. Thus we found hyperhomocysteinemia even amongst those with normal vit B12 levels. By using Fisher's exact test p-value >0.05, thus there is no statistically significant correlation between vit B12 and homocysteine levels in cases. By using Chi-square test p-value >0.05, therefore there is no statistically significant correlation between vit. B12 and homocysteine levels in controls.

**Table 5:** Correlation between vit B12 levels and homocysteine levels

| Group code | B12 | Homocysteie | | p-value |
|---|---|---|---|---|
| | | ≤ 15 | > 15 | |
| Group 1 (cases) | < 210 | 9 | 50 | 0.779 |
| | 210 - 1500 | 14 | 24 | |
| | > 1500 | 2 | 1 | |
| | Total | 25 | 75 | |
| Group 2 (controls) | < 210 | 12 | 57 | 0.088 |
| | 210 - 1500 | 59 | 22 | |
| | **Total** | **71** | **79** | |

## DISCUSSION

In our study, 59 patients of IHD out of 100 had low vitamin B12 levels and 69 out of 150 individuals in control group had low vitamin B12 levels. This showed a significant difference in vit B12 levels between the cases and controls (p value-0.005). The number of individuals with high homocysteine levels was 75 out of 100 in patient group and 79 out of 150 in control group. This showed a significant difference the homocysteine levels of cases and controls (p value-0.001). Thus in our study low vit B12 levels and hyperhomocysteinemia were significantly associated with the incidence of IHD. This proves our hypothesis that low vit B12 levels and hyperhomocysteinemia are a significant risk factor for IHD. Our finding that low vitamin B12 levels are a risk factor for IHD are similar to findings by Kumar *et al*.4 Hyperhomocysteinemia has been shown to be associated with increased risk of IHD in many studies. Stampfer *et al* found that high plasma homocysteine levels are a risk factor for myocardial infarction in a prospective study in US physicians.5 Arnesen *et al*so have shown high total serum homocysteine levels correlate with coronary heart disease.[6] Alfthan *et al* also in their study have shown hyperhomocysteinemia is associated with increased risk of cardiovascular mortality.[7] In our study, we found that low B12 and high homocysteine levels are uniformly distributed among both the age groups. We did not find a statistically significant difference between vit B12 and homocysteine levels among the young and old age groups with IHD (p value-0.862 and 0.64 respectively). More than half of our population ha hyperhomocysteinemia and is deficient in vitamin B12, comparable with the Indian study of Refsum *et al*8 and Kumar *et al*.[4] Our results showed that low serum vitamin B12 concentrations and hyperhomocysteinaemia are extremely common in patients with cardiovascular disease as well as in our normal population. Hyperhomocysteinaemia has been associated with cardiovascular disease in several meta-analyses.[9] Our population is deficient for vitamin B12 and has high homocysteine levels. There is a significant "healthy" population with hyperhomocysteinemia and low B12 levels but no cardiovascular disease. In contrast to the west, Indian studies examining the prevalence of hyperhomocysteinemia in the community have reported a much higher incidence. This has been shown in studies in Indian rural and urban population by Yagnik *et al*.1[0] Refsum *et al* have demonstrated hyperhomocysteinemia and low vit B12 levels in Asian Indians.[8] The mean homocysteine levels too are quiet high, varying from 19.5 to 23.2 µmol/L.8,10 In view of these high levels, it is felt that hyperhomocysteinemia can be considered to be an important cardiovascular risk factor in Indians. It is easy to appreciate the reasons for the high incidence of hyperhomocysteinemia in Indians based on the metabolism of homocysteine. Amongst Indians, a dietary deficiency of homocysteine lowering B vitamins is often present. Milk contains small amounts of vitamin B12 but it is, to a large extent, destroyed by boiling. As Indians are often vegetarian, it predisposes them to vitamin B12 and Folate deficiency. A second important factor that predisposes Indians to hyperhomocysteinemia is a genetic defect in the enzymes that metabolize homocysteine, especially MTFHR.[11,12] Vit B12 is required to metabolise homocysteine. So it is expected that individuals with high homocysteine levels should have low vit B12 levels. In our study we found that there was correlation between homocysteine and vit B12 levels, but it was not statistically significant in cases (p value-0.779) and in controls (p value-0.088). In our study we had 25 cases with normal vit B12 levels and high homocysteine and 22 controls with normal vit B12 levels and high homocysteine levels. Thus, we found hyperhomocysteinemia even amongst those with normal vit B12 levels. These findings are similar to study by Savage *et al*13 which shows that hyperhomocysteinemia can exist with apparently normal B12 values and hence the measurement of homocysteine levels and methylmalonyl CoA are better markers of vit B12 deficiency. This is because there can be a relative vit B12 deficiency as there are other factors involved in homocysteine metabolism. Vitamin B6, and folic acid are required for the metabolism of homocysteine in addition to vit B12.[14] The deficiency of these vitamins is also largely subclinical. And a genetic defect in the metabolism of homocysteine in MTHFR gene is also commonly found among Indians, which predisposes them to hyperhomocysteinemia even in presence of adequate vit B12 levels.[12]

In addition to this many individuals who are found to have vit B12 levels in the normal range still have vit B12 levels on the lower side of normal in our study. This could lead to a relative deficiency of vit B12 levels. Hence, the individuals with apparently normal vit B12 levels would have hyperhomocysteinemia.

## CONCLUSION

Low vit B12 and high homocysteine levels have been shown to be a significant risk factor for IHD by many studies. We carried out a prospective case control study to find out if such correlation exists in our population. On the basis of our observation we conclude that low vit B12 and high homocysteine levels are significantly associated with IHD.

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Source of Support: None Declared
Conflict of Interest: None Declared