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## CLINICAL CORRELATION OF SERUM HOMOCYSTEINE LEVEL WITH LIPID PROFILE IN CORONARY ARTERY DISEASE PATIENTS

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**ABSTRACT:** Majority of patients who experience a Coronary Heart disease event have one or more of the conventional risk factors for atherosclerosis and so do many people who have not yet experienced such an event. Thus predictive models based on conventional risk factors have lower than the desired accuracy, providing a stimulus to search for new factors to predict accurately the risk of CAD. In this regard newer risk factors like homocysteine, Lp (a), insulin resistances are the important ones and are called as 'novel risk factors'. The study was undertaken to find the prediction of CAD risk by homocysteine in comparison with other conventional risk factors. The data obtained suggests a very high sensitivity, specificity and accuracy with above 90% positive prediction value for homocysteine in CAD patients when compared to commonest conventional risk factors. Treatment of hyperhomocysteinemia is cost effective in a developing country like India so early diagnosis of hyperhomocysteinemia can reduce morbidity and mortality of patient with coronary artery disease.
**KEYWORDS:** Homocysteine, coronary artery disease, hyperlipidemia, hypertension, diabetes.

**INTRODUCTION:** Today, it is widely accepted that an elevated level of Homocysteine (>15 µmol/L) is an independent risk factor for cardiovascular disease. Recent studies have also demonstrated a strong correlation between elevated HCY levels and diseases such as diabetes, Alzheimer's, osteoporosis, and renal failure. More and more research data is beginning to merge into a consensus that HCY is an important indicator for overall health status. Professor Per-Magne Ueland, a leading scientist in HCY research from the University of Bergen/Haukeland Hospital, Norway, states: "Hcy is in fact a health measure. There is an extraordinary connection between the quantity of Hcy and the patient's general state of health. The HCY value is an indicator for both health and non-health factors such as exercise, smoking, coffee drinking, cholesterol, vitamins, etc."

**MATERIAL & METHODS:** This case control study was conducted at Department of Pathology & Department of Medicine, M. G. M. Medical college & M. Y. Hospital Indore, with 40 patients admitted to the intensive coronary care unit of Department of Medicine, M. Y. Hospital, Indore between May 2011 to September 2011, with the recent diagnosis of acute myocardial infarction as well as known cases of coronary artery disease with past history of myocardial infarction. Cases were selected on the basis of simple random sampling method with exclusion of Patients with hepatic, renal, thyroid diseases and alcoholics. Age and sex matched healthy controls numbering 10 were taken with no prior history of coronary artery disease. After drawing the blood, immediately, samples were Centrifuged at 1000g for 10 minutes and serum was separated As synthesis of homocysteine continues in red blood cells even after drawing.

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**Method and Equipment used:** ADVIA-Centaur Homocysteine assay is a competitive immunoassay using direct chemiluminometric technology. The different forms of homocysteine in the patient sample are reduced to free HCY by the Reducing Reagent. Free HCY is then converted to S-adenosyl homocysteine (SAH) by the enzyme Reagent. Converted SAH from the patient sample competes with SAH covalently coupled to paramagnetic particles in the Solid Phase for a limited amount of acridium ester-labelled anti-SAH in the lite Reagent. Normal range of Homocysteine is 3.7-14.9 μmol/L, whereas the assay range of instrument is 0.5-65.0 μmol/L. The sensitivity of the Homocysteine assay was calculated to be <0.50 μmol/L which corresponds to the upper limit of the 95% confidence interval. Cross - reactivity was found for compounds whose chemical structure or concurrent usage may potentially interfere with the Homocysteine assay.

**RESULTS:** A study consisting of 40 CAD patients and 10 age and sex matched controls was undertaken to investigate the relationship of homocysteine with CAD, lipid profile & other risk factors.

**Age and Sex Distribution:** Among 40 cases, 31 were males and 09 were females. Among the 10 controls, 07 were males and 03 were females. In this study most of the cases were in the age group of 51 to 60 years. Mean age of cases was 51.7 years and the sex ratio between male and female was 3.3:1.

| Age (years) | Cases | | Controls | |
|---|---|---|---|---|
| | **Males** | **Females** | **Males** | **Females** |
| 31-40 | 05 | 01 | 02 | 02 |
| 41-50 | 09 | 03 | 02 | 01 |
| 51-60 | 09 | 05 | 01 | 0 |
| 61-70 | 7 | 0 | 02 | 0 |
| >70 | 1 | 0 | 0 | 0 |
| **Total** | **31** | **09** | **07** | **03** |
| Table 1: Age distribution with sex | | | | |

Half of the patients had plasma t-homocysteine on the higher side (above 15 μmol/L). A single healthy control had hyperhomocysteinemia inspite of the absence of traditional risk factors.

| t-Homocysteine (μmol/L) | Total Cases (n=40) | Controls (n=10) |
|---|---|---|
| 5 -14. 99 (Normal) | 21 | 09 |
| 15 -29. 99 (Moderate) | 16 | 01 |
| 30 -100 (Intermediate) | 03 | 0 |
| >100 (Severe) | 0 | 0 |
| Table 2: Fasting plasma t-Homocysteine in Patients and Controls | | |

Homocysteine levels and lipid profile: Serum homocysteine levels were found to be significantly elevated in cases with dyslipidemia, when compared to cases without dyslipidemia. However, the homocysteine levels in cases without dyslipidemia were significantly higher than in controls.

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| Lipid Profile | No. of cases | Percentage of cases | Hyperhomocysteinemia (>15µmol/L) cases |
|---|---|---|---|
| Total Cholesterol (<200mg/dl - Normal) | 27 | 67.5% | 10 |
| Total Cholesterol (>200mg/dl - Raised) | 13 | 32.5% | 09 |
| S. Triglycerides (<100 mg/dl - Normal) | 02 | 05% | 06 |
| S. Triglycerides (>100 mg/dl - Raised) | 38 | 95% | 13 |
| S. LDL-C (<100mg/dl - Normal) | 16 | 40 % | 05 |
| S. LDL-C ( >100mg/dl - Raised) | 24 | 60% | 14 |
| S. HDL-C (>40mg/dl - Normal) | 32 | 80% | 14 |
| S. HDL-C (<40mg/dl - Decreased) | 8 | 20% | 05 |
| Both Total CHL + Triglyceride Raised | 13 | 32. 5% | 09 |
| Table 3: Distribution of cases of deranged lipid profile and hyper homocysteinemia | | | |

**Traditional Risk Factors:** The presences of traditional risk factors were also recorded in all patients and control group and were subsequently analysed in association with the new risk factor. The association of homocysteine with hypertension, smoking, & diabetes in the patient groups are shown in following table.

| Traditional risk factors | Total patients n=40 | No. of patients with Homocysteine | | Control n=10 | No. of patients with Homocysteine >15 µmol/L |
|---|---|---|---|---|---|
| | | >15 µmol/L | <15 µmol/L | | |
| Hypertensives | 18 | 10 | 08 | 03(30%) | 0 |
| Nonhypertensives | 22 | 09 | 13 | 07(70%) | 1(10%) |
| Table 4: Fasting plasma t-Homocysteine in patient and controls with Hypertension | | | | | |

P-value is > 0.05 & hence insignificant.

| Traditional risk factors | Total patients n=40 | No. of patients with Homocysteine | | Controls n=10 | No. of patients with Homocysteine >15 µmol/L |
|---|---|---|---|---|---|
| | | >15 µmol/L | <15 µmol/L | | |
| Diabetics | 05(12.5%) | 04(80%) | 01(20%) | 01(10%) | 0 |
| Non-Diabetics | 35(87.5%) | 15(37.5%) | 20(62.5%) | 09(90%) | 1(10%) |
| Table 5: Fasting plasma t-Homocysteine in patient and controls with Diabetes | | | | | |

p-value is >0.05 & hence insignificant.

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| Traditional risk factors | Total patients n=40 | No. of patients with Homocysteine | | Control n=10 | No. of patients with Homocysteine >15 µmol/L |
|---|---|---|---|---|---|
| | | >15 µmol/L | <15 µmol/L | | |
| Smokers | 19(47.5%) | 12 | 07 | 04(40%) | 0 |
| Non Smokers | 21(52.5%) | 07 | 14 | 06(60%) | 1(10%) |
| Table 6: Fasting plasma t-Homocysteine in patient and controls with Smokers | | | | | |

P-value is > 0. 05 & hence insignificant

**DISCUSSION:** Age and sex distribution - In our study the mean age of patients was 51. 7 ± 9. 27 years, In Puri A. et al. [1] it was 41.37 ± 4.48 years.

| Study | Puri A. et al. | Present study |
|---|---|---|
| Mean age (years) | 41.37±4.48 | 51.7±9.27 |
| Table 7: Comparing age distributions | | |

The sex distribution in this study was 77.50% males and 22.50% females, comparable to Robinson et al.[2] (1996) 58% Males, 42% Females, Gurpal et al.[3] (2002) 84% Males, 16% Females, Puri A.et al.[1] (2003) 82.35% Males, 22.50% Females, The study was predominantly male oriented, as CAD affects males more commonly than females. It was found in this study, that IHD was higher in males (31/40) when compared to females (09/40), and more commonly encountered in the fifth & sixth decade, which correlates well with many other studies done both within the country & abroad. This can be attributed to protective effects of estrogens in premenopausal females.

**Homocysteine Levels:** In our study we classified the serum Hyperhomocysteine, as denoted by Kang et al into three groups: (a) Moderate 15-29.9 µmol/L (b) Intermediate 30-100 µmol/L (c) Severe >100 µmol/L. Out of 40 CAD cases, 16(40%) cases had moderate hyperhomocysteinemia i. e. levels less than 30µmol/L, whereas 03 cases had intermediate hyperhomocysteinemia. None of the cases had severe hyperhomocysteinemia >100µmol/L. A single healthy control had moderate hyperhomocysteinemia inspite of the absence of traditional risk factors. In our study, the mean homocysteine levels above which an increased risk of developing IHD was found was 16.60 µmol/L which is comparable to Gupta M et al.[4] & Harish Rao B et al[5] who have reported Mean homocysteine levels as 16.57 & 18.59 in cases (our study: 16.60) & 11.47 & 11.69 in controls (our study: 10.67). The mean levels of homocysteine in patients (16.60±12.08) were almost 1.5 times that of controls (10.67±4.53). In the total patient group (n=40), 14(35%) had homocysteine levels greater than 16.60 µmol/L (mean levels).

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| Study | Cases | Inference |
|---|---|---|
| Chamber et al[6] | 764 | Homocysteine levels were almost twice in cases when compared to controls |
| Kuldeep et al[7] | 75 | Homocysteine levels were almost twice in cases when compared to controls |
| Present study | 40 | Homocysteine levels were almost 1.5 in cases when compared to controls |
| Table 8: Comparing Hcy levels in cases & controls in different studies | | |

Graham et al[8] in large European study ECAP showed that HCY levels were significantly higher in patients than controls. Gills et al in 2000 (3rd NHANES Study) also concluded that a 2 fold increase in MI occurred in patients with a mean concentration of HCY >15 μmol/L.

**Correlation of homocysteine levels with sex:** In our study significant differences in homocysteine levels were found in males and females. Some studies done in India and abroad have shown that males have higher homocysteine levels as compared to females the reason being more muscle mass in males since the formation of muscle mass is associated with simultaneous formation of homocysteine in connection with creatine/ creatinine synthesis.

Secondly higher oestrogen status is associated with a decreased mean serum tHcy concentration, and this may explain the male–female difference in tHcy concentration. Their fore pre-menopausal women are at a much lower risk of developing high homocysteine levels. In our study the mean levels of homocysteine in males (18.88±14.70) μmol/L were >1.5 times that of females (10.94±7.05) μmol/L & p -value at 0.032 was found to be significant.

**Traditional Risk Factors associated with CAD & fasting serum Homocysteine levels:**
Deranged lipid profile (with both Total CHL+Triglycerides raised) was present in 32.5 % of cases as compared to controls. In our study two subgroups were formed in the patient group which included patients who were having an abnormal lipid profile, compared to those patients with a normal lipid profile. Out of total 40 cases: In 27 cases (67.5%) serum total cholesterol was raised, In 38 cases (95%) serum triglycerides were raised, In 16 (40%) cases serum LDL- cholesterol was raised & In 08 cases (20%) serum HDL- cholesterol was decreased. Both Total CHL+Triglyceride were raised in 13 cases (32.5%).

| Studies | Dyslipidemia |
|---|---|
| Gurpal et al[3] (2002) | 38 % |
| Puri A. et al.[1] (2003) | 58.8% |
| Present study (2011) | 32.50 % |
| Table 9: Incidence of dyslipidemia in different studies | |

Hyperlipidemia was present in 38% of cases in present study compared to 38% of Gurpal A. et al.[3] Elevated serum cholesterol is associated with increased risk of CAD. Specifically a 10%

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increase in serum cholesterol is associated with a 20 to 30 % increased risk of CAD. A low HDL and high triglycerides along with high LDL-C can occur alone or in combination and multiply the risk of CAD. In the present study the hyper homocysteinemia and hyperlipidemia correlated well and the mean homocysteine levels were significantly elevated in hyperlipidemic patients. Statistically significant correlation was found with total cholesterol, serum triglycerides, serum LDL- cholesterol & Homocysteine in our study. Amongst the patients who had elevated total cholesterol (>200 mg/dl) the mean Hcy values were 25.60±16.93 µmol/L as compared to 12.26±5.17 µmol/L, in those with normal cholesterol.

The p–value was significant at 0.016 (<0.05).Similarly amongst the patients who had elevated total triglycerides (>100 mg/dl) the mean Hcy values were 16.79±12.37 µmol/L as compared to 12.26±5.17 µmol/L in those with normal triglycerides. The results were higher but statistically insignificant at p -value 0.229 (>0.05).Amongst the patients who had elevated LDL cholesterol (>100 mg/dl) the mean Hcy values were 20.03±14.04 µmol/L as compared to 11.44±5.51 µmol/L in those with normal LDL cholesterol. The p – value was significant at 0.011 (<0.05). Amongst the patients who had decreased HDL cholesterol (<40 mg/dl) the mean Hcy values were 17.09±13.21 µmol/L as compared to 14.63±5.98 µmol/L in those with normal HDL cholesterol. The p–value was insignificant at 0.0442 (>0.05).

| Parameter | Kuldeep Singh et al [7] 2008 | | Present study | |
|---|---|---|---|---|
| | Total Cases ( n=75) | Controls ( n=75) | Total Cases ( n=40) | Controls ( n=10) |
| Total Cholesterol (mg/dl) | 276.29±2.32 | 192.02±0.11 | 181.50±31.92 | 159±11.93 |
| Triglycerides (mg/dl) | 229.88±13.89 | 123.14±0.05 | 159.63±50.20 | 114±16.08 |
| HDL-C (mg/dl) | 24.10±3.71 | 52.17±5.96 | 46.24±4.87 | 47.10±4.35 |
| LDL-C (mg/dl) | 206.20±10.12 | 115.26±9.19 | 104.76±30.27 | 88±13.89 |
| Table 10: Comparing fasting serum lipid profile of Kuldeep Singh et al & Present study | | | | |

The study shows that serum homocysteine levels were significantly elevated in cases with dyslipidemia, when compared to cases without dyslipidemia.

| Traditional risk factors | Patients (%) | |
|---|---|---|
| | Puri A. et al[1] | Present study |
| Dyslipidemics | 58.8 | 38 |
| Hypertensives | 54.8 | 45 |
| Smokers | 52.92 | 47.5 |
| Diabetics | 21.56 | 12.5 |
| Table 11: Comparisons of various traditional risk factors | | |

In the present study smoking was the most common traditional risk factor compared to Puri A. et al.[1], in whom hyperlipidemia was the most common risk factor present in 58.8% of total patients. Hypertension was the next common risk factor followed by dyslipidemia and diabetes in the

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present study comparable to Puri A. et al,[1] in whom also diabetes was the least common. Mean fasting plasma t-Homocysteine in hypertensive patients was 15.82±11.05 when compared to non-hypertensive patients in whom it was found to be 17.23±13.09.

| Traditional risk factors | Kumar et al[9] (2011) (n=30) | | Present Study (n=40) | |
|---|---|---|---|---|
| | N (%) | Mean±SD | N (%) | Mean ± SD |
| Hypertensives | 11 (36.7%) | 26.4±4.5 | 18 (45%) | 15.82±11.05 |
| Smokers | 10 (33.3%) | 32.1±3.5 | 19 (47.5%) | 20.99±5.98 |
| Diabetics | 9 (30%) | 23.6±5.6 | 05 (12.5%) | 17.29±5.24 |
| Table 12: Mean homocysteine (µmol/L) with respect to conventional risk factors in different Indian studies | | | | |

In our study we did not find significant association of homocysteine with hypertension & diabetes. These results coincide with the results of Kumar et al.[9] They did not find significant association of homocysteine with hypertension & diabetes. Smoking was the risk factor which was present both in cases (47.5%) & controls (40 %). Mean homocysteine levels in smoker patients was 20.99±5.98 when compared to non-smoker patients in whom it was found to be 13.57±0.42. Although the p-value was 0.096 (>0.05) i. e. insignificant but the mean homocysteine levels among smokers were higher than non-smokers. In our study 07 cases had multiple risk factors like hypertension, diabetes & smoking. Mean fasting plasma t-Homocysteine in those cases with multiple risk factors was 17.42±7.0 when compared to patients in whom only one of the three risk factors was present was found to be 17.03±14.82.

**CONCLUSION:** Homocysteine levels were found to be raised in the coronary artery disease patients and there was statistically significant difference in plasma homocysteine levels between patients with coronary artery disease and controls. Our study also shows that the homocysteine levels were raised in the coronary artery disease patients with traditional risk factors (Hyperlipidemia, hypertension, diabetes, smoking,) as compared to the controls. Our study although validating the view that increased homocysteine levels are associated with coronary artery disease needs further verification in larger prospective case controls studies. In conclusion, homocysteine levels were increased in patients with coronary artery disease, which shows that it is an independent risk factor for coronary artery disease & needs to be included in the routine investigative panel for evaluating patients at risk of developing CAD.

**REFERENCES:**
1. Puri A, Gupta OK, Dwivedi RN, Bharadwaj RPS, Narain VS, Singh S. Homocysteine and lipid levels in young patients with coronary artery disease. JAPI 2003; 51: 681-685.
2. Robinson K, Arheart K, Refsum H, Brattstrom L, Boeus G, Ueland P, Rubba P, Palma Reis R, Maieady R, Oaly L, Witteman J, Graham I, Low circulating folate and Vitamin 86 concentrations: Risk factors for stroke, peripheral vascular disease, and coronary artery disease: European COMAC Group, Circulation 1998; 97: 437-443.
3. Gurpaal Singh, Arora JS, et al. Myocardial infarction in young population, IHJ 2002; 54: 587-596.

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4. Gupta M, Sharma P, Garg G, Kaur K, Bedi GK, Vij A. Plasma homocysteine: an independent or an interactive risk factor for coronary artery disease Clinica Chimica Acta 2005; 352: 121-125.
5. Harish Rao B, V. Govindaraju and C. N. Manjunath. Risk prediction – homocysteine in coronary heart disease, Indian Journal of Clinical Biochemistry, 2007 / 22 (1) 18-21.
6. Chambers J, Kooner JS et al, Plasma homocysteine and risk of CAD in UK Indian Asian and European men, The Lancet 2000, 355: 523-527.
7. Kuldip Singh and Amandeep Singh Changes in plasma fibrinogen, homocysteine and lipid profile in coronary artery disease patients of north Indian (Punjab) population Biomedical Research 2008; 19 (2): 125-128.
8. Grahan IM, Dalay LE, Refsum HM et al, Plasma homocysteine as a risk factor for vascular disease, JAMA, 1997; 277: 1775-81.
9. Kumar A, Khan et al. The prevalence of hyperhomocysteinemia and its correlation with conventional risk factors in young patients with myocardial infarction in a terti-ary care centre of India. Biomedical Research 2011; 22 (2): 225-229.

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