

## Blood Homocystiene and Lipoprotein (A) Levels, Stress and Faulty Diet as Major Risk Factors for Early Cardiovascular Diseases in Indians

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### Abstract

Cardiovascular diseases (CVD) are one of the major causes of death all around the world. In India, death rate from heart diseases has increased in parallel with the tremendous increase in population which is a major reason why clinical research in this field is extremely important. The present study was undertaken to assess the nutritional status and the risk factors for prediction of CVD in forty middle aged Indian subjects to enable them to take early preventive measures. The study revealed that genetic factors, high Body Mass Index (BMI), high fat and low fibre diets, serum Lipoprotein (a) and Homocystiene were largely responsible for the subjects to be in high risk category. The serum Lipoprotein (a) levels ( $47.7 \pm 2.5$  mg/dl) and Homocystiene levels ( $19.0 \pm 7.8$  mg/dl) were significantly high ( $p \leq 0.01$ ) as compared to normal. A strong two tailed correlation was seen at 0.01 and 0.05 levels between the serum cholesterol and LDL levels of diabetic subjects. Similarly correlation was seen between high fat intake and high BMI of all the forty subjects studied. The results indicate that Indians need to control their saturated fat intakes, take a high fibre and folic acid rich diet, exercise and reduce stress to bring a halt to the rapidly rising incidence of this chronic and serious problem of CVD in India.

**Keywords:** Cardiovascular diseases; Saturated fats; Body Mass Index; Lipoprotein (a); Homocystiene

### Introduction

Cardiovascular disease (CVD) is defined as any serious or abnormal condition of the heart or the blood vessels (arteries, veins). It includes coronary heart disease, stroke, peripheral vascular disease, congenital heart disease, endocarditis, and many other conditions. It includes dysfunctional conditions of the heart, arteries and veins that supply oxygen to vital life-sustaining areas of the body like the brain, the heart itself, and other vital organs.

Cardiovascular disease is the most common cause of death in developed as well as developing countries of the world. It threatens to cripple India's workforce and stunt India's growth if timely and appropriate public health measures are not instituted. Out of CVDs, Coronary Heart Disease (CHD) is the most prevalent cause of premature death. Extensive research is being conducted for the prediction, prevention and treatment of risk factors associated with CVD [1].

It has been estimated that 92% reduction in chronic diseases death rates per year globally could result in saving about 36 million premature deaths by the year 2015 [2].

### Cardiovascular disease risk in india

Epidemiologists in India and International agencies such as the WHO have been sounding an alarm on the rapidly rising burden of CVD for the past 15 years. It is estimated that by 2020, CVD will be the largest cause of disability and death in India. A total of nearly 64 million cases of CVD are likely in the year 2015, of which nearly 61 million could be CHD cases. Deaths from this group of diseases are likely to amount to be a staggering 3.4 million.

The WHO and the World Bank estimate that deaths attributable to CVD have increased in parallel with the expanding population in India, and that CVD now accounts for a large proportion of Disability Adjusted Life Years (DALY) lost [3]. With CVD emerging as a major cause of mortality in India, clinical research in CVD is becoming increasingly important.

### The conventional and emerging risk factors of CVD

The conventional risk factors for CVD include age, gender, socioeconomic status, obesity, diabetes, genetic predisposition, and elevated levels of cholesterol, LDL and Triglycerides, low levels of HDL, blood pressure, ethnic group, physical inactivity, stress, smoking and a faulty diet which is high in calories, cholesterol and saturated fats. Although the importance of the major Cardiovascular risk factors has been strongly substantiated and it is likely that they account for most cases of heart disease, it is also likely that other novel risk factors may account for a substantial proportion of CVD cases. This has initiated a search for alternative risk factors which are also a part of the present research study. Some new markers have been identified. Among the leading new potential culprits are C-reactive protein (CRP), Homocysteine and Lipoprotein (a). Information about how these substances are connected to CVD is still emerging, and researches continue to debate their importance. Recent studies say that fibrinogen concentration has continuous association with the risk of CHD, ischemic stroke, vascular mortality. Associations with ischemic vascular disease depend considerably on conventional risk factors and other markers of inflammation [4]. There is hope that they may help lead to additional prevention and treatment strategies for CVD.

Lp(a) is essentially an LDL particle with an apoprotein (a) attached to it. The protein moiety comprises of two components, a single copy of apolipoprotein B-100 linked to a single copy of a protein referred to as apolipoprotein. Numerous studies have documented that high plasma Lp(a) concentration is associated with a variety of

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CV disorders, including peripheral vascular disease, cerebrovascular disease and premature coronary disease. Lp(a) is deposited at sites of vascular injury [5,6]. Lp(a) levels >25 mg/dl were associated with CHD [7]. Homocystiene might be an independent risk factor for heart disease. Observational studies have found a stronger correlation of Homocystiene with stroke than with CHD and more in women than in men. Dietary saturated fat intake has been shown to increase LDL-C and therefore has been associated with increased risk of CVD [8].

Acute stress is of critical importance as a potential trigger of acute coronary syndromes and cardiac arrhythmias in vulnerable individuals. Psychologic distress is a continuous variable and evidence suggests a dose response relationship between the severity of psychological distress with bio behavioural correlates as well as cardiovascular disease risk [9].

Hence the present study was undertaken for the purpose of Nutritional Screening of subjects for prediction and assessment of the risk factors for cardiovascular disease with special reference to Lipoprotein (a), Homocystiene levels, stress and dietary patterns in middle aged (30-60 years) Indians.

## Materials and Methods

The present study aimed at assessing the risk factors of cardiovascular diseases for screening the general population (age group 30-60 years) residing in Ajmer city (Rajasthan, India). A total of 40 subjects were selected for the present piece of research, out of which 20 were men and 20 were women. The subjects were selected using convenience sampling method/technique.

### Development of tool/data collection

Two questionnaires were developed to gather the information.

**Questionnaire I-Risk Assessment Questionnaire:** A score - based questionnaire was developed for the purpose of data collection. The questionnaire was divided in two parts i.e. Part A and Part B. Part A of the questionnaire consisted of questions related to the general information, stress related information, genetic history, activity patterns, sleep pattern and smoking and alcohol related information of the subjects, whereas Part B of the questionnaire consisted of the questions related to the biochemical and clinical profile of the subjects.

All the questions had many answers to them, and each answer was allotted a different score according to its severity. More severe risk factors were allotted higher scores, whereas low scores were given to the less severe risk factors. The subjects were asked to circle the score corresponding to the correct answer. Finally, all the scores were added to arrive at the total score. The subjects were then categorized into different risk categories according to the table given below (Table 1).

### Questionnaire II: Dietary Assessment Questionnaire

Information related to the dietary intake and food patterns of the subjects was collected through this questionnaire by a three day recall method (24x3 = 72 hours recall). General information of all the forty subjects was collected. General information consisted of all the personal information of the subject i.e. information regarding socio-economic status, education, type of family, number of family members, occupation etc.

Anthropometry of all the forty subjects was done. It included the following parameters – Weight, height, Waist Hip Ratio and Body Mass Index of the subjects. Different biochemical estimations of all the subjects were done which included - Blood Glucose Fasting, Post

Prandial (P.P), Total Cholesterol, Triglycerides(TG), HDL Cholesterol, LDL Cholesterol, VLDL Cholesterol, LDL/HDL ratio, Lipoprotein(a), and Homocystiene (Immolute 1000 Analyzer) [10-13].

The dietary intake data was assessed by collecting retrospective intake data and summarizing prospective intake data with the goal to determine the nutrient content of the food and the appropriateness of the intake for a particular individual [7].

Two types of information on food intake were collected first about the qualitative aspects of food consumed i.e. kind of food eaten and the second about the food consumed in quantitative terms i.e. how much of food was taken. Dietary information was collected through 24 hour recall method for 3 consecutive days. The 24 hour recall method was clubbed together with the weighment method to collect the dietary information.

The different food items consumed were converted into their raw equivalents, categorised into the respective food groups and the average daily intake of energy, proximate principles, important minerals including sodium and fibre were calculated from the value per 100 gm. of edible portion given in the Food Composition Tables and compared to the RDA given by the ICMR for adults [14,15]. The results were statistically analysed by calculating the mean and standard deviations [16]. The t test statistics was used for determining the difference between the means at 0.01 and 0.05 levels. The test of hypothesis for the existence of a linear relationship between two variables was conducted by determining the two tailed correlation coefficient [16].

## Results and Discussion

The present study was conducted to assess the risk factors of CVD for screening the general middle aged population (30-60 years) residing in Ajmer City. The subjects were divided into two groups namely Group I & Group II. Group I consisting of subjects from 40-50 years & Group II consisting of subjects from 50-60 years.

Anthropometric data of all the subjects was gathered. Mean height amongst men in Group I was found to be  $5.61 \pm 0.32$  ft. whereas in Group II it was  $5.63 \pm 0.12$  ft. In women, mean height was found to be  $5.22 \pm 0.22$  ft. and  $5.30 \pm 0.23^{ns}$  ft. in Group I and Group II respectively.

Body weight in all the subjects was found to be greater and towards the higher side. In women, weight was found to be significantly higher (Group II), as compared to women in Group I (Table 2).

Mean weight of women was found to be greater than men in Group II. Waist circumference & Hip circumference of women was greater than those of men in both the age groups. Studies suggest that the CVD burden is exceptionally high in participants classified as overweight or obese. The relationship between risk factors of CVD and obesity may be considered to be similar in all racial/ethnic and sex groups [17].

High BMI has frequently been associated with increased risk of CVD. The results showed that average BMI of men in Group I was  $25.82 \text{ kg/m}^2 \pm 5.7$ , whereas in Group II, it was  $25.68 \text{ kg/m}^2 \pm 2.17$ . The values in both the age groups did not show a significant difference. The average BMI of Group II women were found to be greater than those in Group I. In Group I average BMI was  $24.87 \text{ kg/m}^2 \pm 3.68$ , whereas in

Low risk	88-100	Less than 1
Moderate risk	101-220	1-2.9
High risk	221-350	3-4.9
Very high risk	351 & above	5+

Table 1: Total Cardiovascular Risk.

	Men			Women		
	40-50 yrs. (n=10)	50-60 yrs. (n=10)	p-value	40-50 yrs. (n=10)	50-60 yrs. (n=10)	p-value
Height (ft.)	5.61 ± 0.32	5.53 <sup>ns</sup> ± 0.12	0.185	5.22 ± 0.22	5.30 <sup>ns</sup> ± 0.23	0.795
Weight (lkg)	74.55 ± 12.15	75.60 <sup>ns</sup> ± 8.00	0.228	62.50 ± 16.29	78.85 <sup>**</sup> ± 11.05	4.066
Waist circumference (inches)	36.85 ± 2.50	39.10 <sup>*</sup> ± 12.40	2.05	37.5 ± -5.85	40.95 <sup>ns</sup> ± -5.79	1.325
Hip circumference (inches)	39.35 ± -4.05	39.85 <sup>ns</sup> ± 3.05	0.31	40.85 ± 14.98	43.20 <sup>ns</sup> ± 7.98	0.86

**Table 2:** Anthropometric Data of the Subjects selected for Risk Assessment.

	Men		Women	
	40-50 yrs. (n=10)	50-60 yrs. (n=10)	40-50 yrs. (n=10)	50-60 yrs. (n=10)
Normal Range (Kg/m <sup>2</sup> )	20-25	20-25	20-25	20-25
Mean BMI (Kg/m <sup>2</sup> )	25.8 <sup>ns</sup> ± 5.7	25.68 <sup>ns</sup> ± 2.17	24.87 ± 3.68	27A5 <sup>**</sup> ± -2.92
p-value	0.64	IA	0.158	3.75

Values are Mean ± SD  
<sup>\*</sup>Significant at 0.01 level  
 NS = Not Significant

**Table 3:** Body Mass Index of the Subjects Selected for Risk Assessment.

BMI ranges (kg/m <sup>2</sup> )	40-50 years (n=20)		50-60 years (n=20)	
	Men (n=10)	Women (n=10)	Men (n=10)	Women (n=10)
<20 (Thin)	0%	0%	0%	0%
20-25 (Normal)	60%	50%	50%	20%
25-30 (Grade I Obesity)	20%	40%	50%	60%
>30 Grade II (Obesity)	20%	10%	0%	20%

**Table 4:** Percentage of Subjects under Different Categories of BMI. BMI of none of the subjects was less than 20 in either of the age groups.

Group II average BMI was found to be greater i.e. 27.45 kg/m<sup>2</sup> ± 2.92 (Table 3). Looking at the data, it can be said that all the subjects were at a high risk of developing CVDs.

In the age group of 40-50 years, 60% of the men subjects had BMI between 20-25. Twenty per cent of them had BMI between 25-30 whereas the rest 20% had their BMI above 30 kg/m<sup>2</sup> (Table 3).

In women, half of the subjects i.e. 50% were in the normal category i.e. BMI between 20-25. 40% of them were in the category of 25-30, whereas the rest 10% had their BMI above 30.

In the age of 50-60 years, 50% of the men subjects were under normal category whereas in women only 20% were under this category (Table 4).

The rest of the 50% of the men subjects had their BMI between 25-30. 60% of the women subjects were under Grade I obesity, whereas the rest 20% were under Grade II obesity. A number of studies have documented the association between obesity and CVD risk factors. There is an increased burden of CVD risk factors and exceptionally high prevalence of sub-clinical vascular diseases in people classified as over-weight or obese [17].

Lack of physical activity is another risk factor in development of CVD & thus a major public health issue. Table 4 clearly indicates that a majority of the subjects were performing only sedentary to moderate exercise and that also only once a week. Only 2.5% were involved in moderate exercise (4-5 times/week). Only 17.5% of the total subjects were involved in moderate exercise (>5 times/week). Fifteen per cent of the subjects did not do any sort of exercise even once a week. Physical activity or fitness clearly reduces the risk of CVD with a magnitude of risk reduction comparable to that of not smoking (Table 5).

Stress bears a direct link with heart problems. Stress could be due to several reasons, but studies show that, if modified, the risk could be reduced. Results showed that 95% of the total subjects were suffering from some or the other kinds of stress. Myocardial infarction and sudden cardiac death can be triggered by emotional distress (Table 6).

Majority of the subjects were stressed due to change in their financial state. Other important causes of stress were relationship disputes, change in work responsibilities, change in social habits, personal difficulties at work etc. The main causes of stress were hard to define and pinpoint because the potential causes of stress were highly varied & based on the individual. Financial stress was found to be a sadly widespread experience.

The mean nutrient intake of subjects was studied. The total amount of Energy, Protein, Fat, Fiber and Sodium in the diet of the subjects was calculated and compared with RDA (Table 7).

The fat intake in both men and women subjects was found to be very high. High dietary fat intake has been shown to increase LDL-C and intake has been associated with increased risk of CVD. In humans, saturated fat increases LDL-C in comparison with all the other nutrients [8].

Fiber intake was also found to be very low in all the subjects, including men and women, which can add to the risk of developing heart problems. The values were 6.6 ± 30.0 gms in men, whereas it was 7.7 ± 2.7 gms in women. Increasing dietary fiber intake is associated with a reduction in classical & novel cardio vascular risk factors [18].

Sodium intake of subjects was calculated since high sodium intake bears a direct link with heart problems. Reducing dietary salt intake can lower blood pressure and the risk of CVD [19]. Data also revealed

S.no	Characteristics	Percentage (%) (n=40)
1	<b>Exercise Habits</b>	
	Sedentary moderate exercise <once a week	55
	Moderate exercise (once/week)	10
	Moderate exercise (2-3 times/week)	-
	Moderate exercise (4-5 times/week)	2.5
	Moderate exercise (>5 times/week)	17.5
2	<b>Smoking</b>	
	Never Smoked	95
	Current Smoker (<20 Cigarettes/day)	5
3	<b>Alcohol</b>	
	Non drinker	82.5
	Average 1 drinks daily	10
	Average 1 drinks daily	2.5

**Table 5:** Exercise Habits, Smoking & Consumption of Alcohol related Information of the Subjects selected for Risk Assessment.

S. No.	Cause/Types of stress	Score	Percentage
			(%) (n=40)
1	Death of family member	20	10
2	Divorce/ Separation	20	2.5
3	Thiess/Surgery	20	12.5
4	Marriage in family	20	2.5
5	Dismissal from work	10	5
6	Illness in family	10	30
7	Moving to new place	8	2.5
8	Relationship disputes	5	27.5
9	Change in financial state	5	60
10	Change in occupation	3	17.5
11	Change in work responsibility	3	57.5
12	Mortgage	3	17.3
13	Family events, wedding, birth days	3	40
14	Son/Daughter leaving home	3	27.5
15	Personal difficulties at work	3	37.5
16	Change in residence	2	2.5
17	Change in social habits	2	37.5
18	Change in routine	2	20
19	Holidays	2	15
20	Minor violation of laws	2	2.5

**Table 6:** Stress related Information of the Subjects. Selected for Risk Assessment.

a low intake of omega 3 fatty acids in the diet and the Poly unsaturated to saturated fatty acid ratio was also low indicating that subjects were at a high risk of developing CVD (Table 8).

The fasting biochemical profile of all the forty subjects selected for risk assessment was analyzed. The subjects were tested for Fasting & PP Blood Sugar and the complete lipid profile which included serum cholesterol, triglycerides, HDL, LDL and VLDL levels. Besides these the subjects were also screened for two of the new biochemical emerging risk factors which included lipoprotein (a) and homocysteine levels. Lp(a) is a recent evolutionary risk factor [20].

It works by recruiting inflammatory cells for oxidative damage and contributes LDL to sides for plaque formation. The fasting blood sugar levels of the subjects selected for risk assessment was  $107.8 \pm 50.2$  mg/dl. The borderline sugar levels (both Fasting and PP) indicate the fact that the subjects were at a greater risk for developing Impaired Glucose Tolerance and CVD. In patients with elevated sugar levels higher

chance of thrombogenesis, diminished fibrinolytic activity, increased platelet aggregation and raised concentration of fibrinogen have been reported, all of which increase CVD risk [21]. Hyperlipidemia is also frequently associated in subjects with hyperglycemia [22-24].

The serum cholesterol levels of these subjects were also found to be high ( $197.8 \pm 36.5$  mg/dl). These values also showed a highly significant correlation when compared with the normal blood cholesterol values. Similarly, the LDL-C values of the subjects were found to be on the higher side of the normal range. The LDL levels were  $83.3 \pm 18.6$  mg/dl ( $p \leq 0.01$ ). The high values of both serum cholesterol and serum LDL can be attributed to a high fat diet, as was observed in the subjects, dietary saturated fat intake and low fiber, low omega 3/omega 6 ratios and a low P/S ratio in the diet [8]. LDL and cholesterol have a strong association. The serum TG levels were also found to be significantly higher ( $124.3 \pm 49.9$  mg/dl) when compared to the normal range of serum TG values. TG could be high because the blood sugar levels of the subjects were also found to be high [25].

The high TG levels could also be attributed to high fat content of the diet specially the saturated fat content of the subjects. Similar results of impact of dietary saturated fatty acids in directly raising the serum TG levels have been reported by earlier researchers [26,27].

Studies have revealed strong inverse relation between HDL-C and coronary events. Cigarette smoking, obesity and post-menopausal status are associated with low HDL-C whereas moderate alcohol consumption and aerobic exercise raise the HDL-C. HDL-C is also influenced by family history [28].

In the present study the HDL level of the subjects was found to be  $48.3 \pm 8.5$  mg/dl. Although these levels are in the normal range of 35-80 mg/dl yet they were found to be lower than the desirable HDL values. Low levels of HDL-C (less than 40 mg/dl) are an independent risk factor of CVD and raising the level of HDL-C is a major treatment strategy for regressing atherosclerosis and enhancing CVD risk reduction [29]. This can be achieved by pharmacological & non-pharmacological life style measures [30].

The Lp(a) levels were found to be significantly high ( $p < 0.01$ ) when compared to the normal values. The Lp(a) levels of the subjects was  $43.7 \pm 25$  mg/dl. These high levels could be attributed to high intake of total fat and saturated fat by the subjects as has been reported. However, it can be said that although the Lp(a) concentrations are genetically determined, they are completely related to the risk of CVD and myocardial infarction [6,31]. Indians have been shown to have genetically higher levels of Lp(a) [32].

The Homocystiene levels of the subjects were found to be  $19 \pm 7.8$  mg/dl. ( $p \leq 0.01$ ) when compared to the normal values. Observational studies have indicated a strong correlation between hyper homocystienemia and CVD (Table 9) [33-35].

Diabetes has been known to be a major risk factor for CVDs. In the present study, out of forty subjects selected for risk assessment for CVD, five were diabetic subjects. In these subjects a strong correlation was seen between diabetes and fasting blood sugar levels. (0.05 level of significance and also a significant correlation (0.01 level of significance) between diabetes and LDL and total cholesterol levels (Table 10).

The total risk scores were calculated as per the score sheet of the cardiovascular risk assessment questionnaire. Data suggests that 27.5% of the subjects were at a very high risk and 42.5% of the subjects were at a high risk of developing cardiovascular disease. Only 5% of the total subjects were at low or no risk of developing this disease.

		Energy	Protien	Fat	Fiber	Sodium	P/S	W3 Fatty
		(Kcal)	(gm)	(gm)	(gm)	(mg)	Ratio	Acid
Men (n=20)	RDA	2320	60	25	25	2092	-	-
	Mean Intake	2119**	6L8	70.2**	6.6**	415.9**	13	2
	S.D.	35.8	2A5	5.1	30	66.2	1.2	0.66
	p-value	25.1	128	39.63	2.74	11122	-	-
Women (n=20)	RDA	1900	55	20	25	1902	-	-
	Mean Intake	1901.9 <sup>ns</sup>	55.6 <sup>ns</sup>	78.8**	7.7	394.2**	1.2	1.9
	S.D.	487.8	73.3	60.9	2.7	2469	0.77	0.54
	p-value	0.02	0.04	432	28.66	2731	-	-

Values are Mean ± SD

\*\*Significant at 0.01 level

**Table 7:** Mean Nutrient intake of the subjects selected for Risk Assessment and their Comparison with RDA.

	Sugar (Fasting) (mg%)	Sugar (PP) (mg%)	LP(a) (mg/dl)	Hcy (µmol/L)	Cholesterol (mg/dl)	TG (mg/dl)	HDL (mg/dl)	LDL (mg/dl)	VLDL (mg/dl)
Normal values	60-110	<145	0-30	5-15	<200	50-160	35-80	0-100	10-50
Mean±SD (n=40)	107.8 ± 50.2**	131.08 ± 67.1**	43.7 ± 25**	19.0 ± 7.8**	197.8 ± 36.5**	124.3 ± 49.9**	48.3 ± 8.5**	83.3 ± 18.6**	26.2 ± 2.62**

Values are Mean ± SD

\*\*Significant at 0.01 level

**Table 8:** Biochemical data of the Subjects Selected for Risk Assessment.

	Sugar (Fasting)	Sugar (PP)	Hcy	Lp(a)	TG	VLDL	HDL	LDL	Cholesterol
Sugar (n=5) Fasting blood	1.000	0.999**	-0.70	0.56	0.73	0.72	0.52	0.89*	0.88*
Sugar (n=5) PP blood	0.999**	1	-0.69	0.54	0.74	0.74	0.5	0.89*	0.88*

\*\*Correlation is significant at the 0.01 level (2-tailed).

\*Correlation is significant at the 0.05 level (2-tailed).

<sup>ns</sup> = non significant

**Table 9:** Correlation between diabetes and Lipid Profile of the Subjects Selected for Risk Assessment.

Category	Percentage (%) (n=40)
Very High Risk	27.5
High Risk	42.5
Moderate risk	25
Low risk	5

**Table 10:** Total Risk of Subjects Selected for Risk Assessment.

In conclusion it can be said that an early screening can go a long way in prevention from CVD in high risk subjects. A low fat, low cholesterol, low calorie and high fibre and folic acid rich diet along with a regular exercise regime will definitely help in reducing the incidence of this alarming epidemic of Cardio vascular diseases in India.

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