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SERUM LIPID AND LIPOPROTEIN EVALUATION IN PATIENTS WITH ISCHAEMIC HEART DISEASE

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Abstract: Serum lipid profiles were analysed in the study population comprising of 330 healthy persons and 166 patients with ischaemic heart disease. Comparison amongst the study population was done in order to find out the frequency of hyperlipidemias in relation to age and sex, and the susceptibility to this disease was assessed in terms of low density lipoprotein and high density lipoprotein concentrations. The present study strengthens the importance of elevated levels of serum cholesterol, triglycerides, beta-lipoprotein, prebeta-lipoprotein, and low density lipoprotein cholesterol., and a reduced concentration of alpha-lipoprotein and high density lipoprotein cholesterol as risk factors for ischaemic heart disease.

1. Introduction

Ischaemic heart disease (IHD) has become an alarming public health problem. Hyperlipidemia is a common finding in patients with IHD. There is a strong evidence that the risk for IHD rises with increasing levels of plasma total cholesterol.[28] Worldwide epidemiological surveys consistently support this relation.[24,34,37] Three major studies - Framingham Heart Study,[23] the Pooling Project,[31] and an Israeli Prospective Study[12] are representative of most surveys. The relation between plasma triglyceride levels and coronary risk has been a subject of dispute. A positive correlation has been noted in some studies,[14,17] but the pathogenic significance of the variability in triglyceride levels within the normal range remains disputed.

The overall incidence of abnormal lipoproteins was more in myocardial infarcts than in patients with angina. These patients showed significantly lower values of high density lipoprotein (HDL) as compared to the healthy controls. Jenkins *et al*.[22] emphasise that the concentration of various lipoproteins were directly related to the severity of coronary atherosclerosis, HDL having an apparent retarding effect.

Concentration of low density lipoprotein cholesterol (LDLc) are positively correlated with IHD risk, while high density lipoprotein cholesterol (HDLc) is correlated inversely. This inverse association has been well documented by the Framingham Heart Study.[16] The present work has been aimed to study the serum lipid profile of the study population comprising of controls and survivors of IHD, and

comparison amongst the study population in order to find out the frequency of hyperlipidemias in relation to age and sex.

2. Materials and Methods

A total of 330 healthy persons comprising of 215 males and 115 females were selected as controls for the present study. Subjects, born of healthy parents and having healthy brothers and sisters, with a normal ECG and with no known manifestation of any disease or evidence of any metabolic disease or history of cardiovascular disease, were selected as controls.

166 IHD patients from Hyderabad and neighbouring places and from middle to affluent class of various age groups and of either sex were investigated for the serum cholesterol, triglycerides, low density lipoprotein (LDL), very low density lipoprotein (VLDL), high density lipoprotein (HDL), low density lipoprotein cholesterol and high density lipoprotein cholesterol concentrations. These investigations were carried out between the second to the eighth day following the onset of the disease. These cases were selected from the Cardiology Unit of the Nizam's Institute of Medical Sciences, Hyderabad. Out of these, 112 patients suffered from acute myocardial infarction and the balance 54 suffered from angina pectoris. A minimum of two of the following criteria were taken into account for the diagnosis of myocardial infarction (MI): typical history, typical ECG changes, or raised SGOT or LDH levels. The diagnosis of angina pectoris (AP) was based on a typical history with confirmatory ECG changes. The controls as well as the patients were studied under the following age groups: 30-39 years, 40-49 years, 50-59 years and 60-69 years.

Serum samples : Blood samples were collected with the consent and awareness of the individuals involved. These samples were drawn after the subjects had fasted for 12-14 hours and between the second to the eighth day after the onset of the heart disease. Blood was allowed to clot in a test tube at room temperature for 2 hours. The clot was gently freed from the wall of the test tube. The red cells were sedimented by centrifugation at 3000 rpm for 10 minutes. The serum was subsequently stored at 4°C and analysed the same day the blood is drawn.

Serum total cholesterol was estimated with the modified method of Zlatkis, Zak and Boyle[39] as described by Henly.[21] Triglycerides were estimated by the modified method of Fletcher[10] as described by Foster and Dunn.[11] Lipoproteins were separated by electrophoresis with the method of Noble.[29] LDLc was estimated with the method of Henly[21] after precipitating LDL with the method of Burstein and Samaille.[5] Serum was added to heparin/$CaCl_2$, cyclomixed and set aside for 15 minutes at room temperature. The tube was then spun at 3000 rpm. The supernatant was carefully discarded. The tube was then placed inverted on a filter paper for 10 minutes. Residual supernatant was removed with filter paper. Cholesterol was extracted from the residue by the addition of ferric chloride acetic acid reagent. Sulphuric acid is then added, cyclomixed carefully and set aside for colour development. The colour developed was read in a colorimeter. Blank and standard were also run simultaneously. HDLc was estimated with the method of Henly[21] after

removing HDL with the method of Allen et al.[1] Polyethylene glycol was added to the serum, mixed well and set aside for 10 minutes. The tube was then spun at 3000 rpm. To the supernatant, ferric chloride acetic acid reagent was added, mixed well and set aside for 15 minutes to precipitate proteins and then centrifuged. The supernatant was transferred into another tube and sulphuric acid was added to it, mixed well, set aside for 20 minutes and the colour developed was read colorimetrically. Blank and standard were run simultaneously.

The significance between the means of the lipid levels in the controls and the survivors, and between the subgroups was tested by the t test. Any calculated value which was greater than the tabulated value at 5% was regarded as significant implying significant differences between the means at that probability level.

3. Results

The cholesterol and triglyceride contents of serum were measured in a series of male and female IHD patients. Variations with age were found to exist in all the groups. The cholesterol and triglyceride contents of serum in controls and in IHD cases and the significance of the differences as compared with the controls are presented in Table 1. In males, the mean concentrations of both lipids were greater than control values, particularly in the younger age groups, and the percentage of IHD patients with abnormal values in the older age groups steadily decreased. Among the controls, the cholesterol level increased with each successive decade, the peak incidence being expressed in the 50-59 age group. Serum triglyceride expressed its optimal values in the fifth as well as in the seventh decades. The maximum values of these two lipids in the MI group occurred in the younger age groups and thereafter the levels tended to decrease with increasing age. The condition was similar in the AP group too. In females, the mean serum cocentrations of these two lipid fractions in controls was higher in the 40 years age group and above, i.e. post-menopausal age. High levels of cholesterol was found in the age group 40-49 years in both the MI and AP patients. In the AP group, the serum triglycerides were significantly high in the seventh decade.

Table 2 indicates the serum lipoprotein electrophoretic values in controls and in IHD patients. These values represent the mean relative percentage of lipoprotein bands. LDL and VLDL levels were high and HDL low in the high-risk group when compared to the controls. The data corresponding to the statistical significance of the differences in mean lipoprotein values in the IHD subgroups as compared with the controls and with each other is presented in Table 2[a]. The WHO extension of the original five-type classification system was used and the patients were allocated to one of the six types.

Table 1: Serum cholesterol and triglyceride contents in controls and in IHD subgroups

Age group (years)	Sex	Controls					Myocardial Inf.					Angina Pectoris				
			CHOL		TG			CHOL		TG			CHOL		TG	
		No	M	SD	M	SD	No	M	SD	M	SD	No	M	SD	M	SD
30-39	Males	39	156	29	93	19	8	251.2	23	187.9	58.9	5	259	29.8	213	50.8
40-49	Males	63	195	26	136	23	18	234.0	33	176.4	50.9	11	237	36.1	165	25.7
50-59	Males	97	221	28	129	27	29	234.2	22	171.2	44.5	8	223	23	158	38.9
60-69	Males	16	212	39	138	31	17	231.1	17	144.2	33.2	10	227	22.1	137	16.8
30-39	Females	25	140	23	79	21	6	225.5	25	154.3	49.1	3	240	30.7	150	14.1
40-49	Females	42	165	25	111	17	12	250.3	32	176.2	31.6	6	243	29.6	146	12.6
50-59	Females	41	201	26	126	25	15	226.7	26	164.0	41.2	7	230	26.9	146	15.7
60-69	Females	7	197	31	126	18	7	226.9	12	126.9	13.5	4	226	22.4	173	46.2

(Cholesterol and triglyceride levels are expressed in mg%)

M = MeanCHOL = Cholesterol

SD = Standard DeviationTG = Triglyceride

Table 2: Serum lipoprotein electrophoretic values in controls and in IHD subgroups

Age group (years)	Sex	Controls							Myocardial Infarction							Angina Pectoris						
			LDL		VLDL		HDL			LDL		VLDL		HDL			LDL		VLDL		HDL	
		No	M	SD	M	SD	M	SD	No	M	SD	M	SD	M	SD	No	M	SD	M	SD	M	SD
30-39	Males	39	44.1	1.8	22.5	2.9	31.8	3.2	8	55.6	6.5	25.9	4.4	20.4	2.8	5	55.9	3.4	27.5	5.2	19.8	2.6
40-49	Males	63	46.2	2.6	23.8	3.1	30.1	3.9	18	54.6	3.9	25.5	4.5	19.8	2.0	11	54.7	4.2	24.2	3.4	20.9	3.0
50-59	Males	97	48.1	4.1	24.6	4.1	29.6	4.2	29	54.9	3.7	26.3	3.8	34.1	4.91	8	51.7	3.7	24.8	3.6	25.9	4.9
60-69	Males	16	48.9	3.7	24.9	3.1	26.8	3.6	17	51.6	2.5	24.6	2.7	26.9	4.2	10	52.2	3.2	23.5	1.7	27.9	3.9
30-39	Females	25	44.4	2.1	20.8	4.2	30.9	3.9	6	52.7	3.1	24.2	3.4	21.9	1.9	3	54.9	3.6	22.6	0.3	19.8	1.6
40-49	Females	42	45.9	2.9	24.2	2.9	31.2	3.1	12	56.1	5.4	24.9	2.1	21.9	3.3	6	55.0	3.7	24.8	2.1	21.8	2.9
50-59	Females	41	46.3	3.2	25.6	3.1	28.7	3.2	15	51.1	4.3	24.8	3.9	26.4	4.1	7	53.1	4.6	23.5	1.0	25.8	4.8
60-69	Females	7	49.1	2.3	25.1	2.3	26.1	1.8	7	50.4	1.4	23.4	0.7	30.1	1.5	4	51.8	2.6	27.0	5.3	28.6	6.1

(Lipoprotein values expressed in relative percent)

M = Mean

SD = Standard Deviation

LDL = Low density lipoprotein

VLDL = Very low density lipoprotein

HDL = High density lipoprotein

Table 2a : Statistical significance of the differences in mean lipoprotein concentrations in the IHD subgroups as compared with the controls and with each other

Age Group (years)	Sex	Myo. infarct & controls						Angina pec. & controls						Myo infarct & Angina pec.					
		LDL		VLDL		HDL		LDL		VLDL		HDL		LDL		VLDL		HDL	
		t	df	t	df	t	df	t	df	t	df	t	df	t	df	t	df	t	df
30-39	Males	*9.3	45	*3.0	45	*9.5	45	*13.1	42	*3.0	42	*8.0	42	0.29	11	0.61	11	0.4	11
40-49	"	*12.0	79	*2.1	79	*12.8	79	*10.6	72	0.44	72	*8.4	72	0.07	27	0.86	27	1.2	27
50-59	"	*8.5	124	*2.1	124	0.9	124	*2.6	103	0.14	103	*2.5	103	*2.1	35	0.01	35	0.45	35
60-69	"	*2.6	31	0.35	31	0.07	31	*2.4	24	1.33	24	0.73	24	0.58	25	0.64	25	0.33	25
30-39	Females	*8.3	29	1.8	29	*5.6	29	*9.2	26	0.46	26	*4.8	26	1.1	7	0.8	7	1.8	7
40-49	"	*10.0	52	0.9	52	0.4	52	*7.58	46	0.54	46	*3.0	46	0.46	16	0.11	16	0.06	16
50-59	"	*5.3	54	0.8	54	*2.5	54	*5.2	46	1.9	46	*2.0	46	0.57	20	0.86	20	0.31	20
60-69	"	1.4	12	*2.1	12	*5.0	12	1.8	9	0.86	9	1.08	9	1.16	9	1.89	9	0.65	9

* = significant at 5% level

df = degrees of freedom

IHD = Ischaemic heart disease

LDL = Low density lipoprotein

VLDL = Very low density lipoprotein

HDL = High density lipoprotein

The frequency of the different types of lipoproteinemias in IHD patients is presented in table 3. The commonest abnormality was type IIa, which was observed in 31 (18.6%) cases. The next frequent condition was type IV, seen in 19(11.5%) cases, followed by type IIb observed in 6(3.6%) cases.

Table 3:Frequency of the different types of lipoproteinemias in IHD patients

Manifestation of IHD	Type of lipopro-teinemia	Males (No.)	Females (No.)	Frequency Total No.	Percentage
Myocardial infarction	IIa	12	6	18	10.8
	IIb	3	2	5	3.0
	IV	11	3	14	8.5
Angina pectoris	IIa	8	5	13	7.8
	IIb	1	nil	1	0.6
	IV	4	1	5	3.0
			TOTAL	56	33.7

IHD = Ischaemic heart disease
Total number of IHD cases studied = 166
Hyperlipidemic IHD cases = 56

The mean levels of LDLc and HDLc in controls and in the IHD survivors are presented in figures 1 and 2 respectively.

56 of the 166 IHD patients studied were hyperlipidemic. Not all were hyperlipidemic. Table 4 shows the frequency of hyperlipidemic states in IHD patients. The highest frequency of hyperlipidemia was observed in both sexes in the 40-49 years age group. In the hyperlipidemic IHD patients studied it was observed that their serum HDLc levels were low and LDLc levels were high when compared to the controls. The frequency of hyperlipidemias in relation to age and sex in IHD survivors is presented in figure 3.

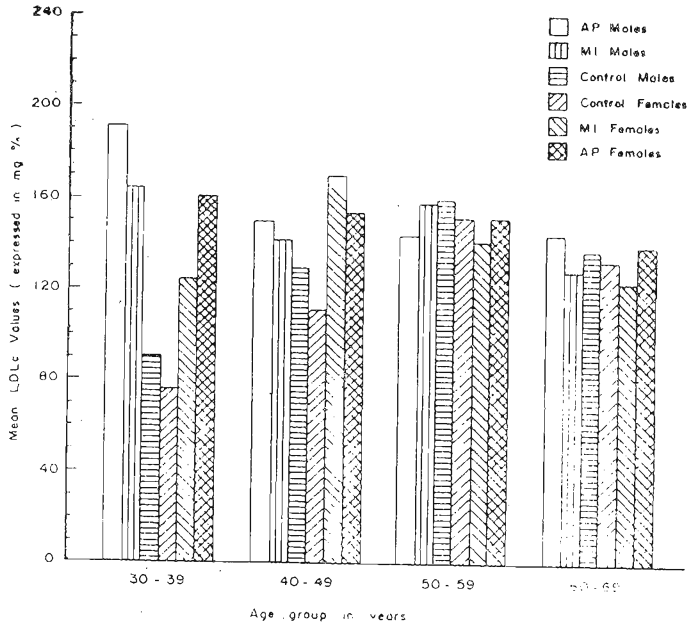

Figure 1: Mean LDL - Cholesterol Values in Controls and in Survivors.
(LDLc = Low density lipoprotein cholesterol, MI = Myocardial infarction, AP = Angina pectoris)

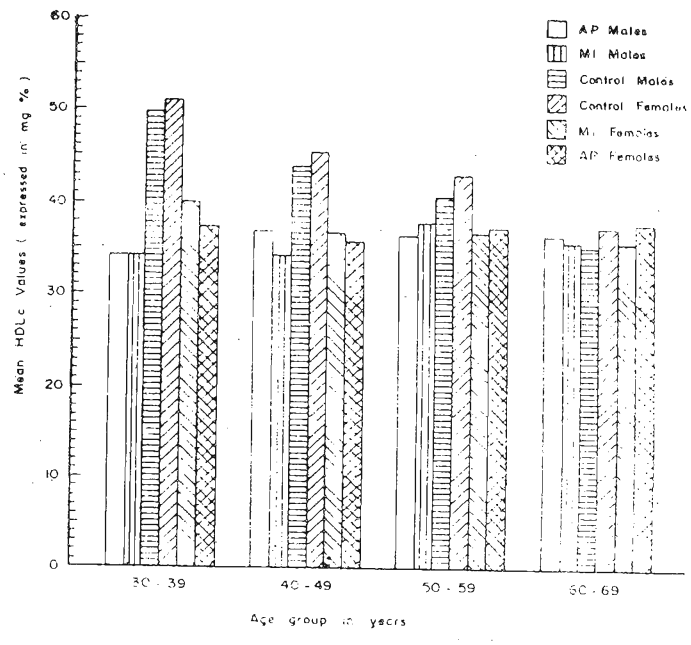

Figure 2 : Mean HDL - Cholesterol Values in Controls and in Survivors.
(HDLc = High density lipoprotein cholesterol, MI = Myocardial infarction), AP = Angina pectoris)

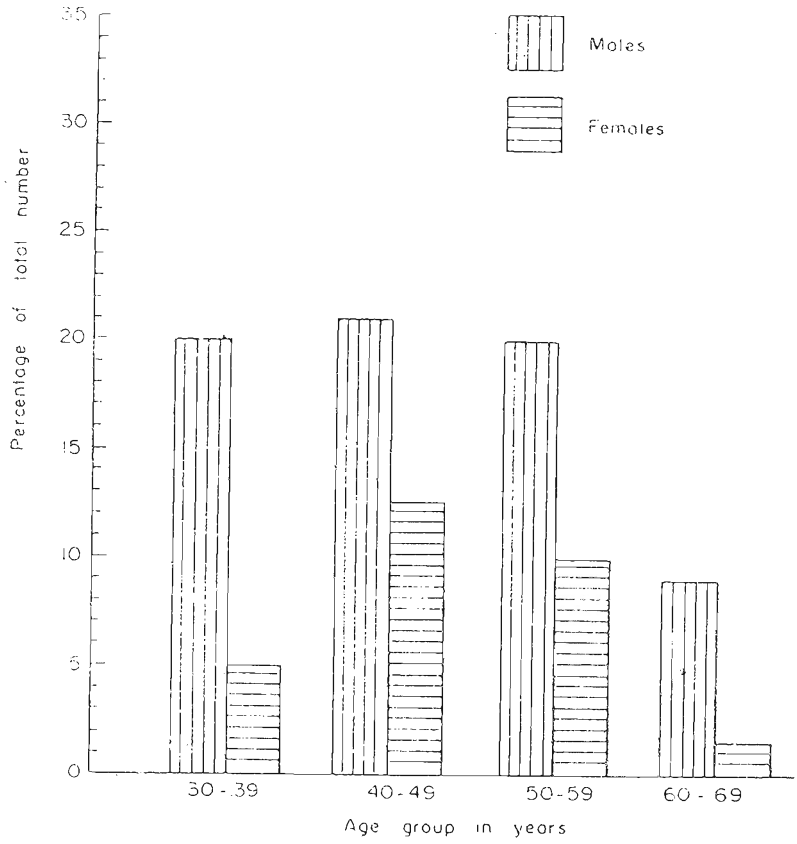

Figure 3: Frequency of hyperlipidaemias in relation to age and sex in Survivors.

Table 4: Frequency of hyperlipidemic states in IHD patients

Manifestation of IHD	Sex	No.	Hypercholesterolemia (per cent)	Hypertriglyceridemia (per cent)	Combined hyperlipidemia (per cent)
Mycocardial infarction	Males	26	46	42	11.5
Angina pectoris	Males	13	53	30	7.0
Myocardial infarction	Females	11	54	27	18
Angina pectoris	Females	6	83	16	nil

IHD = Ischaemic heart disease

4. Discussion

Coronary atherosclerosis and its clinical sequelae represent a multicausal process of enormous magnitude. Lipids and lipoproteins are implicated in coronary atherosclerosis and its major symptomatic manifestations. Hyperlipidemic condition is a frequent finding in IHD patients.

The reported frequency of lipid abnormalities in IHD is variable. In the present study, hypercholesterolemia was found to be predominant over hypertriglyceridemia and combined hyperlipidemia. This great preponderance of raised cholesterol was also noted by Gustafson *et al.*[19] and Leren *et al.*[26] This may or may not be a universal finding, but comparable findings have also been reported by Goldstein *et al.*[14]

The significance of hypertriglyceridemia in IHD has long been a subject of controversy. Increased triglyceride levels were intepreted to be an independent risk factor for IHD by Carlson *et al.*[6], but Brown *et al.*[3] contradicted this point of view. The present study does not resolve the issue of whether hypertriglyceridemia may be additive to the unarguable role of hypercholesterolemia. There are no grounds for the assumption that one or other form of hyperlipidemia is uniquely associated with atherosclerotic heart disease.

Serum cholesterol and triglyceride levels were higher in the survivors of IHD when compared to the controls. Elevated levels of cholesterol confirm the reports of Chaudhari *et al.*.[8] Rise in the levels of triglyceride in survivors was similar to the reports of Schroff *et al.* [35] and Chaudhari *et al.*.[8]

The overall incidence of abnormal lipoprotein was more in MI than in angina. Sharma *et al.*[36] also reported higher incidence of abnormal lipoproteinemic states in MI as compared to the AP group. Jenkins *et al.*[22] observed a strong inverse association between the concentration of HDL and coronary atherosclerosis score. They also emphasised that concentration of various lipoproteins were directly related to the severity of coronary atherosclerosis, HDL having an apparent retarding effect.

LDLc levels were elevated in IHD patients when compared to the controls. This finding confirms the report of Rao *et al.*.[32] Decreased levels of HDLc in IHD was reported by many clinical studies (Carlson *et al.*, and Brunner *et al.*).[7,4] The present study confirms the finding of Western studies that the presence of IHD is correlated negatively with HDLc and positively with LDLc levels (Enger *et al.*, and Goldbourt *et al.*).[9,13]

Hypercholesterolemia was found to be predominant over hypertriglyceridemia and combined hyperlipidemia. In the present study hyperlipidemia was found in 33.7% of the 166 IHD patients. Patterson and Slack[30] reported the frequency of hyperlipidemias in MI patients as 25%. Goldstein *et al.*[15] reported the frequency of hypercholesterolemia, hypertriglyceridemia and combined hyperlipidemia as 7.6%, 15.6% and 7.8% respectively. Kumar *et al.*[25] reported the frequency of hypercholesterolemia as 18% in the MI patients. They studied lipid profiles in male

subjects only. Harshwardhan *et al.*[20] reported the frequency of hypercholesterolemia in MI patients as 50% and combined hyperlipidemia as 8.3%. Frequency of hypertriglyceridemia was not reported in most of the Indian studies. The frequency of combined hyperlipidemia was low as compared to the studies of Harshwardhan *et al.*.[20]

Gupta *et al.*[18] reported the frequency of types IIa, IIb and IV hyperlipoproteinemia in 20 cases of MI as 15%, 10% and 25% respectively. Lewis *et al.*[27] found types IIa, IIb and IV as 14%, 18% and 31% respectively in 143 IHD patients. The frequency of IIa was high and types IIb and IV were low when compared to the reports of Gupta *et al.*[18] and Lewis *et al.*.[27]

Future prospects for further risk definition will involve the protein constituents of the lipoproteins. Apo-B has a central role in the absorption of cholesterol and triglyceride and of their transport in the body. The plasma levels of Lp(a) and apo-B are under strong genetic control, and increased circulating apo-B and Lp(a) concentrations are additive as risk factors for premature IHD (Armstrong *et al.*, Rhoads *et al.*, and Utermann *et al.*).[2,33,38]

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