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Original Research Article

Correlation of serum cholestryl ester transfer protein (CETP) level with lipid profile in young myocardial infarction patients

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ABSTRACT

Introduction: Heart Disease is number one cause of mortality and a silent epidemic among Indians. The risk of coronary artery disease is found to increase with increase in serum Cholesteryl ester transfer protein (CETP) levels. CETP mediates the exchange of lipids between the lipoproteins. Serum CETP levels of young myocardial infarction (MI) patients are measured to see its correlation with lipid profile.

Materials and Methods: This prospective observational case-control study was done on 50 young MI patients and 30 age-matched controls for measuring serum CETP and lipid profile in the biochemistry laboratory of Christian medical college, Ludhiana. The study started from 1st November 2014 till full enrolment. Serum CETP were quantified by Enzyme Linked Immunosorbent Assay (ELISA) method.

Results: The mean serum CETP value observed among cases was $1168.7\pm738.3 \ \mu g/L$ and that among cases was $978.4\pm337.2 \ \mu g/L$ with a p-value was 0.18. The correlation coefficients of serum CETP with total cholesterol, triglyceride, Low density lipoprotein and high density lipoprotein are -0.083, -0.037, -0.020 and 0.050 and similarly among controls were -0.292, 0.086, 0.282, 0.337 respectively.

Conclusion: The serum CETP concentrations were found to be higher in cases as compared to the controls but were not found to be statistically significant, while no correlation was found between serum CETP concentration and lipid profile in young MI cases.

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1. Introduction

Myocardial infarction is an acute and catastrophic event with serious consequences in terms of mortality, morbidity and cost to the society with an estimated burden of 3.6 million young individuals being affected each year.¹ (1). MI is an acute coronary syndrome that occurs during natural course of coronary atherosclerosis. MI remains to pose a major burden on the society despite the major improvements made in its management. Public health estimates show that coronary artery disease (CAD) would rise in the developing nations by 120% and 137% in women and men, respectively by 2020. Of all the developing nations, India is undergoing the most rapid epidemiologic transition from communicable to non-communicable diseases characterized by high burden of atherothrombotic dominated noncommunicable diseases. In India CAD is the leading cause of death accounting for 1.46 million deaths which represented 14% of all-cause mortality in 2004. It is projected that by 2020 ischemic heart disease will result in 2.5 million deaths. Coronary atherosclerosis i.e. formation of waxy plaque in coronaries is the most important cause of myocardial infarct, which is attributed to derangement in lipid profile.²

Cholesteryl Ester Transfer Protein (CETP) is a plasma protein secreted from the liver that has an established role in lipoprotein metabolism. It transports cholesterol from peripheral tissues to liver in a process called as reverse cholesterol transport (RCT). It also transfers cholesteryl esters from High-density lipoprotein (HDL) to Low-density lipoprotein (LDL) & Very low density lipoprotein (VLDL) and transferring triglycerides from VLDL to HDL & LDL.^{3,4}

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Cholesterol when combines with a free fatty acid forms cholesteryl ester (CE). This esterification takes place either in the cell or in the plasma. In cells of liver, intestine and adrenal cortex- the cholesterol reacts with Acyl CoA (fatty acid) to form cholesteryl esters in a reaction catalysed by ACAT, [Acyl- CoA Cholesterol Acyl Transferase] an enzyme found in endoplasmic reticulum of the cells. Whereas, in plasma, cholesteryl ester (CE) synthesis is catalysed by LCAT [Lecithin Cholesterol Acyl Transferase] an enzyme secreted by liver. This esterified cholesterol is transported in blood as integral part of lipoprotein. The fact that CETP redistributes Cholesteryl Esters (CE) from non-atherogenic lipoprotein HDL to potentially atherogenic lipoprotein i.e. LDL and VLDL fraction implies that it may be pro-atherogenic. But, by the virtue of its ability to increase the rate of reverse cholesterol transport in which cholesterol from peripheral tissues is transported to the liver for its elimination in bile, CETP may be considered to be antiatherogenic. A third view shows evidence of concentration dependent anti -inflammatory function of HDL, which adds on to its antiatherogenic property. As CETP is found to reduce the concentration of HDL it may reduce the anti-inflammatory impact of HDL in a process that is ultimately pro-atherogenic. Theoretically CETP may be either pro-atherogenic or antiatherogenic. Hence, the net impact of CETP is uncertain.^{3,4}

Animal studies with the evidence supporting both a pro-atherogenic and an antiatherogenic role of CETP have been reported depending on the metabolic settings and on the species being investigated.³⁻⁶ The ILLUSTRATE Trial (Investigation of lipid level management using coronary ultrasound to assess reduction of atherosclerosis by CETP inhibition and HDL raising) done on humans has shown that inhibition of CETP has favourable effects on lipid profile, by raising HDL (high density lipoprotein) by 72% and lowering LDL (low density lipoprotein) by 25%. A hypothesis was formed that pharmacological inhibition of CETP retards the development of atherosclerosis.⁷⁻⁹ Most of the studies of CETP on human subjects have revealed that the incidence of cardiovascular disease (CVD) is directly related to plasma CETP levels and hence it is atherogenic, even though the evidence of antiatherogenic role of CETP has also been reported under different lipid environment. 10-12

The human CETP gene has been mapped to chromosome. 16. It was found that a subset of Japanese population with genetic deficiency of CETP had markedly elevated HDL cholesterol.¹³ There is paucity of studies correlating serum CETP & lipid profile in m yocardial Infarction patients. Hence, this study strives to know if there is a correlation of raised CETP levels with changes in lipid profile in myocardial infarction patients.

2. Materials and Methods

A prospective observational case-control study was conducted in the Department of Biochemistry on the diagnosed young myocardial infarction patients admitted to Department of Cardiology at Christian Medical College and Hospital, Ludhiana. After due approval from IEC (Institutional Ethics Committee), a total of 80 individuals were enrolled for the study, of which 50 were cases and 30 were healthy matched controls. The study period started in November 2014 and ended in August 2015. Young patients (age \leq 45 years) with diagnosed myocardial infarction, according to WHO criteria,14 admitted to Coronary C are U nit (CCU) were included in the study. Consent in written form was taken from patients fulfilling the criteria, then findings in terms of history, biochemical test parameters (blood sugar, CK, CKMB, troponin T) ECG, ECHO, angiography were noted. Blood samples were collected within 24 hours of the infarct event, but after an informed & written consent.

Blood sample were brought to the Department of Biochemistry, where the serum was separated from it after proper clot formation and centrifugation. Lipid profile was performed as routine on these serum samples from the patients and controls on the HITACHI modular p-800 autoanalyser (Roche Diagnostics, Germany). Serum samples were then stored in the deep freezer (- 4° C) until further processing for serum CETP level s which were determined using commercially available CETP ELISA assay kit of BIOSPES, obtained from INFOBIO, New Delhi.

2.1. Statistical analysis

F or comparison of means of biochemical values student's t-test was used whereas, the proportions was compared using χ^2 (Chi-Square) test. Linear and logistic regression analysis was used to determine the correlation of lipid profile parameters with serum CETP levels and to study the association of Acute Coronary Syndrome (ACS) with serum CETP concentrations. The data was analysed using Statistical package for social sciences (SPSS) statistics for windows version 21.0.

3. Results

The lipid profile and serum CETP concentrations of cases and controls were obtained and compared. The Table 1 depicts the mean and median of the lipid profile parameters-Total Cholesterol (TC), Low density lipoprotein (LDL), Triglyceride (TG) and High density lipoprotein (HDL) among cases and cont rols. The corresponding p-Value is also shown.

The mean serum CETP observed among cases was 1168.7 \pm 738.3 μ g/L and that among controls was 978.4 \pm 337.2 μ g/L along with p-value of 0.18.

The correlation coefficients of serum CETP with total cholesterol (TC), triglycerides (TG), Low density lipoprotein (LDL) and High density lipoprotein (HD L) is depicted inTables 2, 3, 4 and 5 respectively.

4. Discussion

The consequences of the myocardial infarction when affecting the young individuals are usually tragic and devastating for the family. This subgroup of young patients with MI, represent those who come to medical attention owing to symptomatic disease and may just represent the "tip of iceberg" when considering the symptomatic and subclinical disease together.¹⁵ The strong inverse relation of CAD with HDL- cholesterol suggests that increments in HDL makes an independent contribution to reduction in CAD risk.¹⁶ Kurasawa et al. (1985) suggested that the rate of cholesteryl ester transfer is proportional to HDL concentration in the serum within the ranges of physiological concentration but under the conditions of low cholesteryl transfer rate, cholesteryl ester generated by the action of LCAT enzyme may accumulate in HDL if free cholesterol is continuously provided.¹⁷ Koizumi et al. in the same year (1985) were the first to show that it was the genetic deficiency of CETP that is the most important and frequent cause of hyperalphalipoproteinemia (elevated HDL levels) in the Japanese.¹⁸ In 1990, Yamashita et al. investigated the molecular mechanism of CETP deficiency in 4 unrelated families who were CETP deficient, with HDL levels exceeding 150 mg/dl.¹⁹

Studies on association of common variants in human CETP gene and the cardiovascular diseases have yielded contradictory results despite consistent association with HDL-c levels.^{20,21} Similarly, only few studies have been published on association of plasma CETP levels and CHD outcomes, and they also do not provide a consistent result.^{22–30} The question of whether CETP is pro- or antiatherogenic, is still open to debate.

Serum CETP levels in present study group among cases (n=50) was 1168.73 \pm 738.3 μ g/L but comparatively higher levels are noted in most of the other s tudies by Zhuang et al. (2001), Boekholdt et al. (2004), Cho et al. (2009) and Ritsch et al. (2010) as shown in Table 6. Among the normal healthy control group (n=30) serum CETP concentration in our study group was 978.42 \pm 337.19 μ g/L, for Zhuang et al. was 1400.0 \pm 1370.0 μ g/L, for Boekholdt et al. was 3800.0 \pm 2100.0 μ g/L and for Cho et al. was 2300.0 \pm 300.0 μ g/L in.^{23–26} The reason for low levels among present study group could be that this particular group of population has lower endogenous levels of serum CETP, lower levels are noted in the control group as well compared with other study population groups.

The finding of no correlation between serum CETP levels and lipid profile parameters in young MI patients and healthy controls in present study (Chart 1 / Tables 3,

4, 5 and 6) are consistent with that of Zhuang et al.²³ The highest levels of serum CETP in cases were noted among three patients - levels being 4364, 3397.5 and 3050 μ g/L and their total cholesterol levels and triglycerides were noted to be in the lowest quartiles - the levels of total cholesterol being 124, 169 and 123 mg/dl and that for triglycerides being 107,121 and 113 mg/dl respectively. In line with this, the patient having highest levels of total cholesterol (328 mg/dl) and highest levels of triglyceride (461 mg/dl) was found to have normal levels of serum CETP. Similarly among controls, the individual with highest level of serum CETP 1762.5 μ g/L also had total cholesterol level in the lowest quartile – the level being 135 mg/dl. Among the cases, LDL levels as well as HDL levels were also found to be in the lower quartile among the same patients with highest levels of serum CETP and their corresponding LDL levels were 87, 114 and 63 mg/dl and HDL levels were 20, 30 and 37 mg/dl respectively. Similarly among controls, the individual with highest levels of LDL (173 mg/dl) and highest levels of HDL (71 mg/dl) was found to have normal levels of serum CETP.

In similar studies like the nested case-control study by Boekholdt et al. elevated CETP levels were associated with increased risk of future CAD but only in individuals with raised triglycerides.²⁴ Borggreve et al. suggests high plasma CETP may favour reduced risk of CVD in patients with low triglycerides.²⁷ On the other hand, study by Cho et al. shows greatly elevated CETP levels in MI patients.²⁵ Contrary to this, Ritsch et al. suggested that low endogenous CETP levels are associated with increased cardiovascular mortality.²⁶ The relationship of serum CETP with CAD has ever been a question since the discovery of CETP deficiency as a cause of elevated HDL levels, and surprisingly, it remains unanswered.

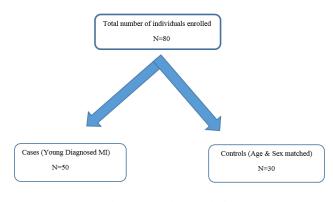


Chart 1: Study Population

5. Conclusion

Myocardial infarction has been recognized among younger age group more frequently in recent years. Considering the social responsibilities that a young individual has towards

Table 1: Comparative lipid profile of cases and controls

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Lipid Profile (mg/dl)	ТС	LDL	TG	HDL
Cases				
Mean	179.1 ± 48.8	112.1 ± 40.2	143.2 ± 76.9	41.7 ± 10.2
Median	174.5	108	125	40
Controls				
Mean	173.7 ± 35.6	107.4 ± 28.5	149.3 ± 67.5	42.9 ± 11.1
Median	165	105.5	155	40.5
p- Value	0.599	0.582	0.719	0.593

Values represent Mean \pm SD (Standard deviation)

p-value <0.05 is considered to be statistically significant

Table 2: Correlation of serum CETP with total cholesterol

Category	Total Cholesterol	Serum CETP levels in μ g/L	p-value	Correlation coefficient
	< 199 mg/dl (n=38)	1232.27 ± 810.13		
Cases (n=50)	200-300 mg/dl (n=11)	901.96 ± 349.18	0.568	-0.083
	>300 mg/dl (n=1)	1685 ± 0		
	< 199 mg/dl (n=22)	914.96 ± 316.34		
Controls (n=30)	200-300 mg/dl (n=8)	1152.94 ± 351.24	0.117	-0.292
	>300 mg/dl (n=0)	0 ± 0		

Values represent Mean \pm SD (Standard deviation) p-value <0.05 is considered to be statistically significant

Table 3: Correlation of serum CETP with triglyceride

Category	Triglyceride levels	Serum CETP levels in μ g/L	p-value	Correlation coefficient
	< 150 mg/dl (n=31)	1258.31 ± 893.44		
Cases (n=50)	150-300 mg/dl (n=17)	999.5 ± 356.3	0.796	-0.037
	>300 mg/dl (n=2)	1218.75 ± 171.47		
	< 150 mg/dl (n=14)	946.79 ± 366.17		
Controls (n=30)	150-300 mg/dl (n=15)	981.3 ± 314	0.651	0.086
	>300 mg/dl (n=1)	1378 ± 0		
× /	>300 mg/dl (n=2) < 150 mg/dl (n=14) 150-300 mg/dl (n=15)	$\begin{array}{c} 1218.75 \pm 171.47 \\ 946.79 \pm 366.17 \\ 981.3 \pm 314 \end{array}$		

Values represent Mean \pm SD (Standard deviation)

p-value < 0.05 is considered to be statistically significant

Table 4: Correlation of serum CETP with low density lipoprotein

Category	LDL levels	Serum CETP levels in μ g/L	p-value	Correlation coefficient
Cases (n=50)	< 130 mg/dl (n=35) > 130 mg/dl (n=15)	$\begin{array}{c} 1209.33 \pm 842.58 \\ 1074 \pm 415.57 \end{array}$	0.893	-0.020
Controls (n=30)	< 130 mg/dl (n=23) > 130 mg/dl (n=7)	$\begin{array}{c} 918.76 \pm 326.43 \\ 1174.43 \pm 316.82 \end{array}$	0.131	0.282

Values represent Mean \pm SD (Standard deviation)

p-value < 0.05 is considered to be statistically significant

Table 5: Correlation of serum CETP with high density lipop	protein
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Category	HDL levels	Serum CETP levels in μ g/L	p-value	Correlation coefficient
	< 40 mg/dl (n=24)	1287.96 ± 979.18		
Cases (n=50)	40-60 mg/dl (n=24)	1046.69 ± 408.04	0.732	0.050
	>60 mg/dl (n=2)	1202.5 ± 406.59		
	< 40 mg/dl (n=14)	851.11 ± 302.8		
Controls (n=30)	40-60 mg/dl (n=13)	1105.5 ± 368.38	0.068	0.337
	>60 mg/dl (n=3)	1021.83 ± 131.18		

Values represent Mean \pm SD (Standard deviation)

p-value < 0.05 is considered to be statistically significant

CETP levels in cases (µg/L)	Present study (n=50)	Zhuang et al. (n=117)	Boekholdt et al. (n=735)	Kyung-Hyun et al. (n=10)	Ritsch et al. (n=2560)
	1168.73 ± 738.3	${}^{1980.0\pm}_{1680.0}$	4000.0 ± 2200.0	4400.0 ± 400.0	1115.0 ± 295.0

Table 6: Serum CETP levels among cases in various studies

Values represent Mean \pm SD (Standard deviation)

the society at large and the associated long term disability of the premature CAD, there is a need to find more potential risk factors associated with heart diseases in the young that in turn may help in designing preventive programs. Experimental evidence have suggested athero-protective as well as pro-atherogenic role of CETP. The findings of this study can be summarized as:

- 1. The lipid profile parameters were not found to be deranged in majority of the young myocardial infarction cases.
- 2. The average serum CETP level among young patients of myocardial infarction was 1168.73 μ g/L, whereas that among age & sex matched healthy controls was 978.4 μ g/L in this population sub-group (statistically insignificant).
- 3. The serum CETP levels were not significantly different between the young myocardial infarct patients and the control group.
- 4. The levels of serum CETP were not found to be significantly correlating with changes in total cholesterol and HDL concentration among the young myocardial infarction patients.

Thus, based on the findings obtained in the present study it can be concluded that the serum CETP levels are not significantly raised among young myocardial infarction patients of age \leq 45 years in our population as compared to healthy controls and that the levels of CETP cannot be significantly correlated with changes in lipid profile in this young age group. But this being a very controlled study with a small sample size more studies are needed to find correlation with other factors for actually correlating CETP with MI or to find any drugs for its inhibition to raise the HDL and other cardio protective factors in the young population.

6. Source of funding

None.

7. Conflicts of interest

None

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