Indian Journal of Fundamental and Applied Life Sciences ISSN: 2231-6345 (Online)
An Open Access, Online International Journal Available at http://www.cibtech.org/jls.htm
2014 Vol. 4 (2) April-June, pp.247-254/Siyadat Ali
Research Article

EFFECT OF SMOKING ON LECITHIN CHOLESTEROL ACYL TRANSFERASE ACTIVITY, MALONALDEHYDE LEVELS AND LIPID PROFILE IN INDIVIDUALS WITH CORONARY HEART DISEASE

***Mohammed Siyadat Ali**
KBNIMS, Gulbarga, Karnataka, India
**Author for Correspondence*

ABSTRACT
Tobacco smoking has been recognized as a strong risk factor for the development of ischemic heart disease. Results of the study indicate that a significant rise in serum cholesterol, specially the free cholesterol with a decrease in ester fraction and a decrease in the activity of Lecithin cholesterol acyl transferase increase in malonaldehyde levels while HDL-C was normal, there was significant increase of LDL-C and serum triglyceride levels. These findings suggest the predisposition of smokers to coronary heart disease.

Keywords: *Lecithin cholesterol acyltransferase, Malonaldehyde, Cholestrol, HDL, LDL, Serum triglycerides*

INTRODUCTION
Atherosclerosis of coronary artery is found in many cases of ischemic heart disease. The general tendency to deposit Lipids in arterial intima may be determined by the level of blood lipids and blood pressure. However multiple contributions rather than a single etiologic agent may be playing a role.
Timmis GC (1998) reported that Tobacco smoking is a strong risk factor for the development of ischemic heart disease. Cigarette smoking has been reported to encourage formation of atherosclerosis the risk among smokers of younger age group of dying from atherosclerosis is two to three times more than in non smokers.
Astrup & Kjelden (1973) reveals that components of smoke such as tar, carbonmonoxide, nicotine, polycyclic hydrocarbons and wide variety of irritant substances may be responsible for playing role in the pathogenesis of atheroma formation.
Gordar *et al.,* (1977) suggested that a positive relationship in the level of total cholesterol, LDL, VLDL and triglycerides to the risk of coronary heart disease while HDL has been suggested to be protective factor against coronary heart disease.
Der and Gidez (1992) investigated that epidemiologic studies indicate certain genetic or acquired factors increase the risk of atherosclerosis. Risk factors of prime importance are hyperlipedemia, hypertension, cigarette smoke and diabetes. Hyperlipedemia has been reported to be associated with increased incidence of Ischemic heart disease. Hyperlipedemia may be secondary to some well known causes an nephrotic syndrome, hypothyroidism or direct cause of some gene defect and complex inheritance factors. Striking association has been reported with elevated LDL cholesterol and also increased VLDL and triglycerides.
Mosback *et al.,* (1958) reported that elevated blood pressure unequivocally accelerates atherogenesis is and increases the incidence of IHD. Animal experiments in a variety of species have demonstrated unequivocally that lipid induced atherogenesis is markedly accelerated by also producing hypertension.
Schule (1974) suggested that the data from epidemiologic, clinical and postmortem is quite consistent with it in man. The basic factor is the multiplication of intimal cells in response to pressure changes caused by hydrodynamic forces. In atherosclerosis pressure work of heart is also increased due to loss of vessel elasticity and raised systolic blood pressure.
Jameel (2008) studied the Lecithin cholesterol Acyl Transferase activity and Lipid profile in Type 2 Diabetes mellitus. Results of the study indicate derangement of Lipid metabolism in patients with NIDDM. The study reveals that there was increase in serum triglycerides cholesterol, LDL and VLDL levels. HDL cholesterol levels are lowered. Talib *et al.,* (2014) reveals that the smoking associated

oxidant hypothiocyanous acid converts active dimeric endothelial cell nitric oxide synthase into its monomer form decreases enzyme activity and releases $Zn^{2+.}$ This is ascribed to targeting of the critical Zn^{2+} thiol cluster by this thiol-specific oxidant.

MATERIAL AND METHODS

The present study included total 20 patients of coronary heart disease with age between 30 to 70 years and were divided into the following groups.

Control group: Included 20 healthy non smokers.

Group 1: Included 20 patients of coronary heart disease (CHD) with a history of smoking 2 packets a day from the last 15 years.

Informed consent was taken from patients and control subjects. A prestructured and pretested proforma was used to collect the data.

Fasting blood samples are collected in sterile clean and dry bottles. Sera are separated and assays are performed immediately. Estimation of total, free and ester cholesterol LCAT activity and also estimation of HDL, LDL, VLDL, triglycerides and Malonaldehyde are carried out by spectrophotometer.

Estimation of Total Free and Ester Cholesterol

Principle: Certain sterols in acetic acid solution give a red colour with ferric chloride and sulphuric acid.

Procedure: Into a conical centrifuge tube graduated to 10 ml was added 0.5 ml of serum and 9.5 ml of acetone alcohol mixture, blowing it in from a pipette in order to disperse the serum to give a fine flocculent protein precipitate. The tube was placed in a water bath at 60-70° C for 10 mins. The tube was then cooled and the volume was adjusted to 10 ml marks with solvent. The tube was capped, with aluminum foil and then centrifuged for 3 minutes.

The concentration of cholesterol in the solution at this stage is $1/20^{th}$ of that in the original serum.

For total cholesterol 2 ml of the extract was transferred to a boiling tube and evaporated to dryness in a water bath at 60-90°C being careful not to overheat and char. Then 6 ml of glacial acetic acid was added and placed for a few minutes in boiling water until the residue is dissolved and cooled.

For estimation of free cholesterol 5 ml of the extract was transferred to a centrifuge tube. Evaporated to a volume of about 1 ml, cooled and added 1 ml of digitonin solution. Mixed by swirling and allowed to stand for 10 minutes. Then centrifuged at 3500 RPM for 10 mins. The supernatant was removed and the tube was left to drain for 5 minutes, 4 ml of acetone was added to disperse and wash the precipitate and drained. The tube was dried by brief immersion in a boiling water bath. 7.5 ml of glacial acetic acid was then added and loosen the precipitate by tapping the tube. The tube was placed in a boiling water bath for a few minutes to dissolve the precipitate mixed and cooled. 6 ml of the solution was transferred to a container or a boiling tube for the colour reaction.

Determination of HDL cholesterol:

Principle: All the lipoproteins chylomicrons, very low density lipoproteins VLDL and low density lipoproteins (LDL) except HDL are precipitated by phosphotungstic acid in the presence of magnesium (Mg++) ions, leaving HDL in the supernatant the cholesterol estimated in the supernatant will be HDL - cholesterol.

Procedure: 0.1 ml serum is taken in a centrifuge tube, 0.1 ml of phosphotungstate reagent is added and mixed on a Vortex shaker for 10 secs then 50 µl of magnesium chloride solution was added and mixed centrifuged for 30 min at 3500 RPM carefully the clear supernatant was removed for analysis avoiding any surface deposit. This clear supernatant was subjected to HDL cholesterol estimation by the method of Wybenga *et al.*

Procedure:

1. To each of three screw capped vials (13 x 100 mm) with Teflon lining labeled “Blank” “Standard” and “unknown” added 5.0 ml cholesterol reagent.
2. Added 50µl cholesterol standard and 50µl serum or plasma (do not use oxlated plasma) to the “standard” and unknown vials respectively and mixed the contents of each vial thoroughly for at least 10 sec.

3. Inserted all vials into a heating block (such as the Down Diagnostest Heating block; Set at 100°C – standard and unknown, vials should be heated for the exact same period.
4. Remove all vials exactly after 2 mins and immerse them in tap water (20°C or cooler) for 5 min. Remove, dry the exterior of the vials, screw on the caps tightly and mix their contents by inversion (if screw capped vials are not available) inversion can be accomplished with regular test tubes, by placing parafilm on the top and holding it on tightly with the thumb.
5. Read AX and AS versus the blank at (510 nm) or with a filter wit nominal wave length in the region calculations: - mg of cholesterol/100ml = Ax/AS x 0.1 x 100/0.05.

= AX/AS x 200

VLDL was determined using the formula – Triblycarides/5 and LDL was estimated by subtracting VLDL and HDL from total cholesterol which was estimated earlier.

Triglycerides Principle:

Glycerol moiety is oxidized to formaldehyde and the later condensed with ammonia and 2,4 pentanedione (acetyle acetone) to produce 3,5 disacetyl 1,4 dhydro toludine which is yellow in colour and has absorption at (405 nm).

Procedure:

0.1 ml of serum/plasma and standard were taken in screw capped tube. The volume was made up to 4 ml with isoproponal. The contents were mixed and 0.4 gm of washed alumina was added to the tubes. These were placed on mechanical stirrer for 15 min and then centrifuged.

After centrifugation 2 ml of the supernatant fluid was transferred to appropriately marked tubes.

Then added 0.6 ml of saponification reagent to the tubes and incubated at 60-70°C for 15 min. After cooling added 1ml of sodium metaperiodate solution and mixed well and 0.5 ml of acetyl acetone reagent was added and was mixed again. The tubes were incubated at 50°C for 30 min and after cooling the colour was read as 405 nm using spectronic 21.

Malonaledehyde Estimation

Principle:

The malonaldehyde in the serum reacts with thiobarbituric acid in acidic medium and gives rise to a pink colour complex which is measured at 530 nm in spectrophotometer.

Procedure:

This is done by following the medium of thiobarbituric acid adduct as per study done by Mahfouz *et al.,* (1986) with minimal modification (Mahafouz *et al.,* 1986)/ (Wade *et al.,* 1989) (Valipasha *et al.,* 1984). 0.5 ml of serum and 0.5 ml of 0.9% saline and 1 ml of 10% TCA and centrifuge at 3000 RPM for 30 mins.

1 ml of supernatant was taken and 0.25 ml of 0.67% of thiobarbituric acid freshly prepared was added. Boil in boiling water bath for 30 mins cool immediately in ice cold water and take reading at 530 nm.

RESULTS & DISCUSSION

Table 1 & 2 presents the data on mean levels of serum told cholesterol, free and ester cholesterol levels.

In the normal controls the mean levels of serum total cholesterol, free and ester cholesterol are 184.1 ± 20.56; 56.1 ± 5.79 and133.9 ± 16.79 mg/100 mnl respectively. In smokers with CHD the serum total cholesterol was significantly raised the values being 224.4 ± 28.32 mg/100 ml ($P < 0.001$) serum free cholesterol was also significantly raised in them [the levels being 103.7 ± 14.58 ($P < 0.001$)] (Table 1 & 2).

Table 3 presents the data of ester cholesterol level in smokers and non-smokers.

The ester cholesterol in smokers group was 120.7 ± 17.72 mg% and was significantly lower from the control group ($P < 0.002$). Lecithin cholesterol acyl transferase activity in smokers was significantly lowered being 3.7 ± 1.5 as compared to 5.345 ± 0.006 in normal controls ($P < 0.001$) (Table 3).

There was no significant difference in HDL cholesterol in both the normal cases and smokers with coronary heart disease (65.5 ± 18.26 and 60.8 ± 17.18) (Table 4).

However LDL cholesterol levels are significantly raised in smokers with CHD the levels being 133.3 ± 33.31 and 92.25 ± 22.13 mg% in control group ($P < 0.001$) (Table 5).

The mean serum triglyceride and VLDL level in smokers with CHD was 151.15 ± 66.67% mg% and 30.3 ± 13.65 respectively and was significantly higher than normal group [101.05 ± 29.36 ($P < 0.001$) and VLDL cholesterol level 22.75 ± 8.87 ($P < 0.05$) the malonaldehyde levels were significantly raised in smokers with CHD as compared with normal non-smokers (341.45 ± 115, 34, 165.35 ± 36.13 ($P < 0.001$)] (Table 6 & 7).

Table 1: LCAT activity expressed percentage fall in free cholesterol in normal non-smokers

Sl. No.	Before incubation			After incubation			
	Total Chole-Sterol Mg%	Free Chole-Sterol Mg%	Ester Chole-Sterol Mg%	Total Chole-Sterol Mg%	Free Chole-Sterol Mg%	Ester Chole-Sterol Mg%	LCAT Activity Unit
01.	224	67	155	224	58	166	4
02.	153	48	105	153	39	114	4.3
03.	187	47	140	187	37	150	5
04.	184	50	134	184	43	141	4
05.	225	61	164	225	52	173	4
06.	203	58	145	203	47	156	5
07.	168	51	107	168	40	128	6
08.	154	52	102	154	42	112	6
09.	192	62	130	192	53	139	5
10.	205	63	142	205	51	154	5
11.	177	56	121	177	46	131	6
12	169	50	119	169	41	128	5
13.	159	49	110	159	38	121	6.6
14.	163	53	110	163	42	121	6.5
15.	190	60	130	190	41	141	6
16.	193	62	131	193	51	142	6
17.	167	55	112	167	46	121	5
18.	202	64	138	202	52	150	5
19.	177	56	121	177	46	131	7
20.	190	58	132	190	48	142	5.5
Mean ± SD	184.1± 20.56	56.1± 5.79	133.9± 16.79	184.1± 20.56	46.15± 5.66	138.05± 7.22	5.345± 0.866

Results of the study indicate that there is significant change in lipid profile of smokers with coronary heart disease as compared with normal non-smokers. There is significant increase in serum total cholesterol with an increase in free fraction and decrease in ester cholesterol. Plasma lecithin cholesterol acyl transferase activity is also significantly lowered in smokers with coronary heart disease.

Result of the present study thus are greatly supporting the surgeon's new message. On cigarette packages "Quitting smoking now greatly reduces serious risk to your "health".

A study of serum lipid profile along with lecithin cholesterol acyl transferase activity and serum malonaldehyde levels has been carried out among smokers with coronary heart disease and among normal non-smoker adult males of similar age group.

Table 2: LCAT activity expressed as % fall in free cholesterol in smokers with CHD

Sl. No.	Before incubation Total Chole-sterol Mg%	Free Chole-sterol Mg%	Ester Chole-sterol Mg%	After incubation Total Chole-sterol Mg%	Free Chole-sterol Mg%	Ester Chole-sterol Mg%	LCAT Activity Unit
01.	221	101	120	221	98	123	2
02.	231	110	121	231	105	126	3
03.	204	103	101	204	98	106	3
04.	234	116	118	234	108	126	4
05.	197	98	99	197	90	107	3
06.	239	107	132	239	102	137	2
07.	211	92	119	211	85	126	3
08.	196	87	109	196	81	115	3
09.	218	107	111	218	94	124	6
10.	181	65	86	158	58	93	5
11.	224	105	119	224	96	128	4
12	273	104	169	273	94	179	4
13.	220	102	118	220	97	123	2
14.	255	125	130	255	113	142	5
15.	207	104	103	207	99	108	2
16.	242	106	136	242	93	149	5
17.	260	114	146	260	109	151	2
18.	224	106	118	224	92	132	6
19.	275	140	135	275	127	148	5
20.	206	82	124	206	71	135	5
Mean ± SD	224.4± 28.32	103.7± 14.83	120.7± 17.72	226.6± 28.41	95.5± 14.82	128.9± 19.83	3.7± 1.5

P < 0.001 P < 0.001 P < 0.0021 P < 0.001

Table 3: Serum cholesterol and LCAT activity in normal non-smokers and smokers with CHD

	Normal group Mean± SD	Smokers with CHD Mean± SD
Serum total Cholesterol mg%	184.1± 20.56	**224.4 ± 28.32
Serum free Cholesterol mg%	56.1± 5.79	**103.7 ± 14.88
Serum ester Cholesterol mg%	133.9± 16.79	**120.7 ± 17.72
LCAT activity Enzyme units	5.345± 0.866	**3.7 ± 1.5

**(P < 0.02)*
*** (P < 0.001)*

Present study indicate that a significant rise in serum cholesterol, specially the free cholesterol with a decrease in ester fraction and a decrease in the activity to lecithin cholesterol acyl transferase while HDL – C was normal, there as significant increase of LDL – C and serum triglyceride levels. All the above findings are suggestive of strong predisposition of smokers to coronary heart disease. An elevation in serum malonaldehyde is also present in smokers. This has been suggested to be due to reactive peroxy

radicals and acetaldehyde in cigarette smoke making the cells vulnerable to peroxidative damage. A rise in serum malonaldehyde has also been suggested to enhance the tendency for atherosclerosis by altering APOB of LDL and thereby facilitating greater uptake of LDL by macrophages which will form foam cells in atherosclerotic plaques, further adding to the risk of smokers to coronary heart disease.

Table 4: Lipid profile in normal non smokers

Sl. No.	Serum Triclyceride mg%	HDL Chol. mg%	LDL Chol mg%	VLDL Chol. mg%
1.	112	65	137	22
2.	82	58	79	16
3.	98	69	98	20
4.	105	64	69	51
5.	119	59	142	24
6.	126	70	108	25
7.	107	48	99	21
8.	100	69	65	20
9.	88	73	101	18
10.	122	75	108	22
11.	129	61	90	26
12.	82	64	89	16
13.	94	70	70	19
14.	105	79	63	21
15.	129	64	100	26
16.	87	61	115	17
17.	116	65	78	23
18.	96	58	125	19
19.	134	67	83	27
20.	108	71	97	22
Mean ± SD	101.05 ± 29.36	65.5± 18.26	92.25± 22.13	22.75± 8.87

Table 5: Lipid profile in smokers with CHD

Sl. No.	Serum Triclyceride mg%	HDL Chol. mg%	LDL Chol mg%	VLDL Chol. mg%
1.	108	57	142	22
2.	98	68	143	20
3.	112	81	101	22
4.	146	64	141	29
5.	156	55	111	31
6.	90	69	152	18
7.	86	50	144	17
8.	147	77	90	29
9.	164	45	140	33
10.	88	63	70	18
11.	305	64	99	61
12.	88	61	194	18
13.	98	82	118	20
14.	94	80	156	19
15.	141	76	103	28
16.	278	55	131	56
17.	265	55	152	53
18.	147	34	161	29
19.	224	35	195	45
20.	188	45	123	38
Mean ± SD	151.15 ± 66.67 P< 0.01	60.8± 17.18 N.S.	133.3±33.31 P< 0.001	30.3±13.65 P< 0.05

Table 6: Lipid profile in normal non-smokers and smokers with CHD

	Normal Non smokers (Mean ± SD)	Smokers with CHD (Mean ± SD)
Serum Trilycerides mg%	101.05 ± 29.36	**151.15 ± 66.67
Serum HDL Cholesterol mg%	65.5 ± 18.26	60.8 ± 17.18 (N.S)
Serum LDL Cholesterol mg%	92.5 ± 22.13	***133.35 ± 33.31
Serum VLDL Cholesterol mg%	22.75 ± 8.87	*30.3 ± 13.63

****P < 0.001*
***P < 0.01*
**P < 0.05*

Table 7: Serum Malonaldehyde levels is normal non-smokers and smokers with CHD

Sl. No.	Serum Malonaldehyde in normal Non smokers nanomoles/100 ml	Serum Malonaldehyde in smokers with CHD nanomoles/100ml
1.	173	213
2.	186	272
3.	181	245
4.	152	298
5.	122	283
6.	216	362
7.	192	250
8.	208	256
9.	218	413
10.	162	309
11.	128	352
12.	101	229
13.	194	192
14.	144	261
15.	181	608
16.	205	517
17.	137	325
18.	101	453
19.	181	421
20.	125	570
Mean ± SD	165.35 ± 36.13	341.45 ± 115.34

** P < 0.001*

REFERENCES

Alt Schula (1974). Atherosclerosis: *Medical Clinics of North America* 58, 397-395

Astrup F. Kjeldem K. (1973) *Medical Clinics of North America* 58, 323 – 347.

Deming QB, Mosaback EH, Bevans Metal BP (No Date). Cholesterol Content of serum and tissues and atherosclerotic disease. *Clinical Genetics* **9** 558-556.

Elder HA and Gidez LI (1982). The clinical significance of the plasma high density lipoproteins. *Medical Clinics of North America* **66**(2) 431-440.

Gorder T, Castelli WP, H Jortland MC *et al.*, (1977). High density lipoprotein as protective factor against coronary heart disease. *American Journal of Medicine* **62**(5) 125 – 129.

Jihan Talib, Jair Kwan, Aldwin Suryo Rahmanto, Paulk writing and Micheal J Davies (2014). The smoking-associated oxidant hypothiocyanous acid induces endothelial nitric oxide synthase dysfunction. *Biochemical Journal* **457**(1) 89-97.
Tahmeen Jameel (2008). Lecithin Cholesterol Actyle Fransferase activity and Lipid profile in Type 2 Diabetes Mellities. M.D (Thesis), NTR university of Health Sciences.
Timmis GC (1985). *Cardiovascular Review* (Grune and stratton Orlando) 9 -29.