MATERIAL AND METHOD

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This study of Lp (a) levels in young patients with myocardial infarction was carried out at SSG Hospital, Baroda during the period of June 2007 to October 2008. The patients included all admissions of young patients with myocardial infarction in ICCU in S.S.G. Hospital. Control group included young individual without ischemic heart disease.

Study population:
Study population consisted of 50 patients having AMI and diagnosed by clinical sign and symptoms, 12-lead ECG and biochemical markers like CK-MB. Control group included 50 young individuals without ischemic heart disease.

Inclusion criteria:-
All patients aged between 20-40 years.

1) Severe chest pain lasting for > 30 minutes and not responding to sublingual nitroglycerin tablets significantly.
2) Presence of pathological Q wave along with ST segment elevation and subsequent T wave inversion appearing in anterior leads, inferior leads or right leads corresponding to anterior wall, inferior wall and right wall myocardial infarction respectively.
3) Significant rise in CK-MB isoenzyme on 1st or 2nd day.

Exclusion criteria:-

1) Patients with uncontrolled Diabetes mellitus.
2) Patients with nephrotic syndrome.
3) Patient's treatment with any of lipid lowering agents.

Methodology:
After admission, a relevant history and clinical examination was carried out and 12-lead ECG was taken in every patients of MI. Patients were treated with thrombolysis, anticoagulation, aspirin, clopidogrel, nitroglycerin, beta blockers, ACE inhibitors, ionotropic support, atropine and laxatives wherever applicable.

Following investigations were carried out in all patients and controls:

1) Hemogram
2) Urine examination
3) FBS & PP_2BS
4) Blood urea and serum creatinine
5) Serum CK-MB
6) Lipid profile
7) Serum Lp(a)

SAMPLE COLLECTION AND PREPARATION

The serum Lipoprotein (a) level and lipid profiles were measured 10-15 days after the attack of myocardial infarction. The serum Lp (a) levels and lipid profile were measured in a 12 hour fasting blood sample in both case and control groups.

5 ml of blood was collected in a plain bulb for estimation of Lp (a) and lipid profile. 2 ml of blood was collected in a fluoride bulb for blood sugar estimation.

10 to 12 hours of fasting prior to sample collection is required. Serum is preferred. Blood should be centrifuged within 30 minutes or kept on ice until centrifugation.

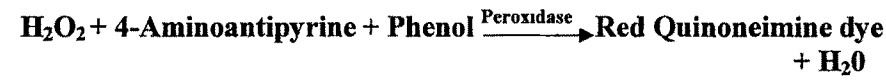
METHODS FOR DIFFERENT PARAMETERS USED IN STUDY:

SERUM CHOLESTEROL:

Method: PAP method

Principle:

Cholesterol esterase hydrolyses esterified cholesterol to free cholesterol. The free cholesterol is oxidized to form hydrogen peroxide which further reacts with phenol and 4-aminoantipyrine by the catalytic action of peroxidase to form a red coloured quinoneimine dye complex. Intensity of the colour formed is directly proportional to the amount of cholesterol present in the sample.



Procedure:

Wavelength	520 (490-540)
Reaction type	End point
Reaction Temperature	37° C
Incubation Time	20 min

Pipette in tubes according to following table

	Reagent Blank (µl)	**Standard (µl)**	**Test (µl)**
Test	--	--	10
Standard	--	10	--
D/W	10	--	--
Reagent	1000	1000	1000

Mix, incubate for 20 min at 37° C. Measure the absorbance of the sample (Abs-T) and standard (Abs-S) against the reagent blank within 60 min.

Calculation:-

Serum cholesterol concentration (mg/dl) = $\frac{\text{Abs-T} \times \text{Conc. Of standard}}{\text{Abs-S}}$

TRIGLYCERIDE:

Method: GPO method

Principle:

The triglycerides present in the serum are catabolised into glycerol and free fatty acids by lipoprotein lipase. Liberated glycerol is converted to glycerol-3-phosphate in presence of glycerol kinase and ATP Glycerol-3 –phosphate is acted upon by glycerol-3-phosphate oxidase to form hydrogen peroxide. This together with phenolic compound and 4-aminoantipyrine in presence of peroxidase gives the purple colour complex. The intensity of the colour is measured at 546 nm (530-570 nm) or green filter and corresponds to the triglycerides concentration.

Triglycerides $+H_2O \xrightarrow{\text{Lipoprotein lipase}}$ **Glycerol + Fatty acid**

Glycerol + ATP $\xrightarrow{\text{Glycerol kinase}}$ **Glycerol-3-phosphate + ADP**

Glycerol-3-phosphate + $H_2O \xrightarrow{\text{Gly-3-phos. Kinase}}$ **Dihydroxyacetone Phosphate +** H_2O_2

H_2O_2 **+ 4-Aminoantipyrine + Phenol** $\xrightarrow{\text{Peroxidase}}$ **Red Quinoneimine dye +** H_2O

Procedure:

Wavelength	546 (530-570) nm
Reaction type	End point
Reaction Temperature	37° C
Incubation Time	10 min

Pipette in tubes according to following table

	Reagent Blank (µl)	**Standard (µl)**	**Test (µl)**
Test	--	--	10
Standard	--	10	--
D/W	10	--	--
Reagent	1000	1000	1000

Mix, incubate for 10 min at 37° C. Measure the absorbance of the sample (Abs-T) and standard (Abs-S) against the reagent blank within 30 min.

Calculation:-

Serum Triglyceride concentration (mg/dl) = $\frac{\text{Abs-T} \times \text{Conc. Of standard}}{\text{Abs-S}}$

HDL-CHOLESTEROL:

Method: PTA method

Principle:

The chylomicron, VLDL and LDL are precipitated by addition of phosphotungstic acid magnesium chloride. After centrifugation, high density lipoproteins fraction recovered as clear supernatant its cholesterol content is estimated by enzymatic method.

Serum + Phosphotungstic acid ⟶ supernatant (HDL) + ppts (Other fractions)

Procedure:

Wavelength	505 (490-530)
Reaction type	End point
Reaction Temperature	37° C

Pipette in tubes according to following table

Sample	200 µl
Precipitating Reagent	200 µl

Mix well, incubate it for 10 min and then after centrifuge. Separate the clear Supernatant and proceed as below with it.

	Reagent Blank (µl)	Standard (µl)	Supernatant Test (µl)
supernatant	--	--	50
Standard	--	50	--
D/W	50	--	--
Reagent	1000	1000	1000

Mix, incubate for 10 min at 37° C. Measure the absorbance of the sample (Abs-T) and standard (Abs-S) against the reagent blank within 30 min.

Calculation:-

Serum HDL-cholesterol concentration (mg/dl) = $\frac{\text{Abs-T} \times \text{Conc. Of standard}}{\text{Abs-S}}$

VLDL-CHOLESTEROL:

This was calculated by using Friedewald's equation as below.

Serum VLDL-Cholesterol conc. (mg/dl) = $\frac{\text{Triglyceride}}{5}$

LDL-CHOLESTEROL:

This was calculated by using Friedewald's equation as below.

Serum LDL-Cholesterol conc. (mg/dl)
= Total cholesterol – [HDL-Cholesterol+VLDL-Choleterol]

SERUM LIPOPROTEIN (a)

Method: Turbidimetric test

Principle:

Latex particles coated with antibodies anti-Lp (a) are agglutinated when mixed with samples containing Lp (a). The agglutination causes an absorbance change, dependent upon the Lp (a) contents of sample that can be quantified by comparison from a calibrator of known Lp (a) concentration.

Procedure:

Wavelength	578 (540-600)nm
Reaction type	End point
Reaction Temperature	37° C

Pipette into a cuvette:

Diluent	800 µl
Latex	200 µl
Sample	15 µl

Mix and read the absorbance after10 seconds (A1) and after 4 minutes (A2) of the sample addition.

CALCULATIONS:-
Lp (a) concentration in the sample is calculated by interpretation of its (A2-A1).