College of pharmacy

Clinical laboratory training

Fifth stage

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Lecture: 4

Cholesterol, Triglycerides and Lipoproteins

Cholesterol, Triglycerides and Lipoproteins

Disorders of lipids are very important in medicine owing to their strong relations to many diseases, thus early detection of deranged blood lipid profile is important. The **"lipid profile"** usually includes total cholesterol (TC), triglyceride (TG), and lipoproteins which can be classified into high density lipoprotein (HDL), low density lipoprotein (LDL) and very low density lipoprotein (VLDL).

Selection of patients for investigation

Plasma lipids should be measured in individuals with the following conditions:

1- Coronary heart disease (CHD), cerebrovascular disease and peripheral vascular disease.

2- A family history of premature CHD (occurring at age <60 years).

3- Other major risk factors for CHD (e.g. diabetes mellitus and hypertension).

4- Patients with clinical features of hyperlipidemia.

5- Patients whose plasma is seen to be lipemic.

6- To determine the relative risk of the development of acute pancreatitis attributable to hypertriglyceridemia.

7- To confirm that eruptive xanthoma, lipemia retinalis, and palmar xanthoma are the result of elevation of triglyceride-rich lipoproteins.

8- To determine whether secondary hypertriglyceridemia is produced as a side-effect of certain drugs.

9- As a follow-up measurement to determine the effectiveness of diet, exercise, or lipid lowering drugs.

Important considerations

Age: cholesterol levels increase with age.

Sex: women have lower level LDL and total cholesterol than men except after menopause. Men had higher triglycerides and total cholesterol and lower HDL levels compared to women.

At midcycle, the time of maximum estrogen secretion the plasma cholesterol and TG tend to be highest.

Orally administered estrogen reduces LDL cholesterol levels and increases HDL cholesterol levels in postmenopausal women.

Pregnancy increases total cholesterol, LDL cholesterol, HDL cholesterol and triglycerides levels.

Season: cholesterol levels are slightly higher in cold weather. Higher levels were observed in winter and lower in spring and summer.

Food intake:

- Ingestion of fatty meals may cause elevated TG levels. Ingestion of monounsaturated fat reduces cholesterol.

- Plasma triglyceride concentration is reduced when sucrose intake is reduced. Eating sugar and other carbohydrates raises triglycerides.

- Large protein meals at lunch or in the evening also increase the serum cholesterol for at least 1 hour after a meal. In vegetarian individuals, the LDL- cholesterol & VLDL-cholesterol are reduced by 37 % and 12%.

- Individuals who eat many small meals throughout the day tend to have concentration of LDL and HDL that are lower than when same type and amount of food is eaten in three meals.

Life-style : lipids are higher in sedentary and poor diet habits.

To determine lipid profile, the patients should be on their usual diet for 2 weeks and are neither gaining nor losing weight before the blood is taken. Patient should fast for at least 12 hours before sampling. Blood should be drawn after an overnight fast.

- Chylomicrons are cleared within 6 – 9 hours and their presence after 12 hours fast is abnormal. Chylomicrons, being derived from dietary fat, should normally have been cleared; a pathological disturbance may thus be inferred if they are present.

- In health, in the fasting state, plasma is **clear**. Following a meal, it often becomes **opalescent** owing to the light-scattering properties of chylomicrons and VLDL. At triglyceride concentrations above about 4 mmol/L, the plasma becomes increasingly **turbid**; with severe hypertriglyceridemia, it appears **milky (lipemic)**. If plasma is left undisturbed, **chylomicrons float to the surface**, leaving a clear infranatant layer; **VLDL remain in suspension**. The LDL do not scatter light and, even at high plasma cholesterol concentrations, the plasma remains clear.

Medical conditions: many drugs and diseases like thyroid, liver, and kidney diseases affect lipid profile.

Acute illness: It is recommended that lipoproteins measurement should be made no sooner than 8 weeks after any form of trauma or acute bacterial/viral infection and 3 - 4 months after child birth. When lipid studies are done on a patient who has had a myocardial infarction or stroke, blood should either be taken within 24 hour or after an interval of three months, because the **metabolism of lipoproteins is disturbed** during the convalescent period and analytical results may be misleading.

Positional variations occur, cholesterol levels are lower when sitting, versus standing and lower when recumbent verses sitting. Decreases of as much 10% in concentration of TC, LDL-C, HDL-C and apo-A-I and B, have been observed after 20 minutes recumbence.

Prolonged venous occlusion leads to increase in cholesterol concentration by 10 - 15%. Ideally, the tourniquet should be in place no longer than one minute to prevent hemoconcentration which can cause falsely elevated results.

Mild exercise produces a slight decrease in concentration of cholesterol and TG that may persists for several days. Those who walk for about 4 hours each week have an

average cholesterol concentration 5% lower and HDL-C concentration 3.4% higher than inactive persons.

Smoking: the plasma cholesterol , triglyceride and LDL cholesterol concentration are **higher** by about 3 %, 9.1 % and 1.7 % respectively **in smokers** than in non-smokers. The HDL cholesterol is lower in smokers than in non-smokers.

Alcohol ingestion: when moderate amount of alcohol is ingested for 1week, the serum TG concentration is increased by more than 20 mg/dL. Prolonged moderate ingestion of alcohol may increase HDL-C concentration. Alcohol should not have been taken on the evening before blood sampling. **Alcohol is a common cause of hypertriglyceridemia even in patients who have otherwise fasted**.

Either plasma or serum can be used when TC, TG and HDL-C are measured. Serum can be used when it is necessary to store samples for weeks or months. It is generally recommended that plasma be stored in the liquid state when it is to be used for lipid and, particularly, lipoprotein analysis or for lipoprotein electrophoresis studies. **The levels of all lipoproteins may decrease with storage**.

Total Cholesterol

Normal or desirable value: < 200 mg/dL (< 5.18 mmol/L SI units)

Border line high value: 200 - 239 mg/dL (5.18 - 6.19 mmol/L SI units)

High value: > 239 mg/dL (> 6.20 mmol/L SI units)

To convert mmol/L to mg/dL: Multiply mmol/L by the number 39

Specimen

1- Fasting is required, the patient is instructed to fast 12-14 hours before testing. Only water is permitted also no alcohol should betaken 24 hours before test. Total cholesterol, the HDL cholesterol concentrations **change very little** between fasting and non-fasting conditions.

2- Usually serum used in analysis, obtained from venous blood. The serum total cholesterol measurement depends on colorimetric enzymatic methods by

spectrophotometry which have become the most popular methods for its analysis.

Triglycerides

Desirable value: < 150 mg/dL (< 1.7 mmol/L SI units)

Border line high value: 150 - 199 mg/dL (1.7 - 2.25 mmol/L SI units)

High value: 200 – 499 mg/dL (2.26 - 5.64 mmol/L SI units)

To convert mmol/L to mg/dL: Multiply mmol/L by the number 88

Specimen

1- Usually serum used in analysis, obtained from venous blood. The blood should be rapidly centrifuged to minimize the spontaneous hydrolysis of triglycerides to glycerol and fatty acids in the blood. If the blood sample cannot be analyzed for triglycerides within 24 hours, freezing the samples at −20°C, preferably at −40°C to −60°C, or colder (such as −80°C) is recommended, using tubes specifically designed for low temperature storage.

2- Fasting is required for 12-16 hours before testing. Only water is permitted also no alcohol should be taken 24 hours before test. A fasting sample is **essential** for triglyceride analysis, since triglyceride levels increase as soon as 2 hours postprandially and reach a maximum at 4 to 6 hours. Samples drawn from non-fasting patients are not suitable for analysis.

3- Most current methods use chemical or enzymatic procedures to determine the glyceride glycerol concentration, which is then converted to the equivalent mass concentration of an average triglyceride.

Lipoproteins

Normal value of HDL : 40 - 60 mg/dL (1.04 - 1.55 mmol/L SI units)

Values of HDL > 60 mg/dL: Considered a negative risk factor of heart disease

Values of HDL < 40 mg/dL: Considered a major risk factor for heart disease

Optimal value of LDL : < 100 mg/dL (< 2.6 mmol/L SI units)

Near optimal value of LDL: 100- 129 mg/dL (2.6 - 3.35 mmol/L SI units)

Border line high value of LDL: 130 - 159 mg/dL(3.36 - 4.11 mmol/L SI units)

Normal VLDL level is between 2 and 30 mg/dL (0.1 to 1.7 mmol/L). Normal value ranges may vary slightly among different laboratories.

Specimen

- Usually serum used in analysis, obtained from venous blood.

- Fasting is required for 12-14 hours before testing. Although non-fasting conditions do not appear to influence the blood HDL-C levels in most people, the postprandial lipemia has the potential of interfering with many of the analytical methods. To minimize this analytical problem, it is always good laboratory practice to request fasting specimens. Only water is permitted also no alcohol should be taken 24 hours before test.

- Serum or plasma can be used as the sample, but since plasma is usually preferred for lipid analysis, the HDL analysis will most frequently be performed on the same plasma specimen. The sample should be removed from the blood clot within 2 hours and may be stored at 4°C for up to 2 days. If specimens are to be kept for longer than 48 hours, they should be frozen. The HDL is relatively labile, and freeze/thaw cycles have been shown to affect some of the precipitation methods. However, if specimens are frozen, they should be kept at temperatures below −50°C, at which temperature they are stable for up to 2 years. Once thawed, specimens should be gently mixed.

Triglyceride, total cholesterol and HDL cholesterol concentrations can easily be measured in the laboratory. Most clinical laboratories utilize the convenient method of Friedewald to estimate or calculate LDL cholesterol as following:

LDL = total cholesterol − (HDL +VLDL)

LDL mmol/L= total cholesterol – (HDL + TG/2.2)

This formula is invalid if the triglyceride concentration exceeds 4.5 mmol/L.

LDL mg/dL= total cholesterol – (HDL + TG/5)

This formula is invalid if the triglyceride concentration exceeds 400 mg/dL.