MATERIAL & METHODS
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(A) Case material

(B) Methodology

1. Diagnosis of diabetes
2. Hypertension
3. Peripheral vascular disease
4. Diabetic nephropathy
5. Fundus examination for diabetic retinopathy
6. E.C.G. and Master's two step exercise test
7. Skin biopsy for microangiopathy
8. Serum lipoproteins
9. Other biochemical investigations

Case material

The study group will consist of 75 patients attending diabetic clinic and/or patients admitted in M.L.B. Medical College and Hospital, Jhansi. Each case was subjected to a detailed history and clinical examination using the proforma appended. Past history and family history of diabetes, hypertension, anginal pain or myocardial infarction will be enquired particularly.

Detailed clinical examination will be done with particular stress on blood pressure, cardiovascular manifestations and for any evidence of peripheral vascular disease.
Second toe digital pressure (Agarwal et al., 1977) was measured in every case for any evidence of latent peripheral vascular disease.

Routine investigations were done in all cases including urine for sugar (glycosuria) and albumin (for diabetic nephropathy). Glucose tolerance test was done in every case. Serum cholesterol and serum lipoproteins, screening / X-ray chest P.A. view, 12 leads electrocardiogram, Fundus examination shall be done in every case. Stress test (Master et al., 1929) was done in every case until there was a contraindication for it.

Control group

25 normal healthy subjects derived from patients admitted in indoor and which were age and sex matched served as control. In each control case diabetes mellitus was excluded by glucose tolerance test.

1. Criteria for diagnosis of diabetes mellitus

Diabetes will be diagnosed on basis of criteria laid down by W.H.O. expert committee (1965).

(i) A person with diabetic response to glucose tolerance test (venous blood sugar levels more than 130 mg/100 ml, 2 hours after giving the load of 100 g glucose) whose fasting blood sugar levels is below 125 mg/100 ml(venous).

(ii) A person with diabetic response to glucose tolerance test
with fasting blood sugar levels more than 125 mg/100 ml (venous).
The blood sugar levels will be done by Folin Wu method, 1920.

2. Hypertension

Hypertension will be diagnosed when systolic pressure is equal to or greater than 160 mm of Hg and/or diastolic pressure (Kentkoff's phase-5) is equal to or greater than 95 mm of Hg on three consecutive visits. (W.H.O. technical reports series No. 628).

3. Peripheral vascular disease

Peripheral vascular disease will be diagnosed on the basis of following criteria-

1. Second toe pressure less than 55% of systolic blood pressure (Latent peripheral vascular disease).

2. Intermittent claudication.

3. Ischaemic rest pain.

4. Absent arterial pulsation with pallor, coldness or gangrene of feet.

**Digital pressure of second toe** (Agarwal et al., 1977)

The second toe was blanched by squeezing and then the pressure in a proximal cuff (5 cm x 3 cm) was raised near brachial systolic pressure, it was then gradually released and the pressure at which the arterial inflow into the digit distal to cuff was resumed, recorded as digital pressure. The restoration of arterial flow was indicated by appearance
of a pink flush in the skin.

4. Diabetic nephropathy

Nephropathy was diagnosed on the basis of marked albuminuria (Joslin et al., 1971).

5. Fundus examination for diabetic retinopathy

According to Ballantyne (1944), the diabetic retinopathy can be graded as following -

(i) Microaneurysm alone or accompanied by minute haemorrhages or punctate exudate. Retinal veins are frequently dilated.

(ii) Dot and Blot haemorrhages with or without waxy exudate, discrete or confluent, some times circinate but not in the form of macular star.

(iii) Gross vascular changes including irregular expansion of veins and formation of knots, loops, coils and leases, sheathing of veins and intra-retinal dilated plexus simulating neovascularization or venous thrombosis.

(iv) Proliferative changes involving preretinal and vitreous neovascularization with or without obvious connective tissue formation. But rarely with intra-retinal new vessel formation, vitreous haemorrhage and retinal detachment may lead to ophthalmoscopy impossible.

6. E.C.G. and Master's two step test

12 lead E.C.G. was done in all cases. Master's two step test was performed in selective cases and
abnormalities in E.C.G. were diagnosed on the basis of criteria
given by Blackburn et al (1960).

Master's two step exercise test involves the
performance of 15 to 25 trips up and down, two steps, each 9
inches high in a period of 1½ minutes. The number of trips
varies, depending on age, weight and sex of patients (Master
and Oppenheimer, 1929). The test is discontinued if pain is
experienced, but is repeated if patient remains symptom free
and E.C.G. remains normal. E.C.G. was recorded immediately
and for up to ten minutes after the conclusion of test. ST
segment depression of 0.5 mm is suggestive of myocardial
ischaemia, provided ST segment is horizontal or with downward
sloping.

7. Skin biopsy

Skin biopsy was taken from leg and haemotoxylin
and P.A.S. staining was done in all cases and grading of micro-
angiopathy was done as described by Moor and Frew (1965). The
salient features of the various grades were as follows -

1. **Mild or grade I** - Slight thickening of basement membrane in
arterioles.

2. **Moderate or grade II** - Arterioles and capillaries showing
moderate thickening of basement membrane and slight endo-
theelial proliferation.

3. **Severe or grade III** - Changes were pronounced in arte-
rioles. The basement membrane was thickened and showed
splitting. Endothelial proliferation was moderate and plump cells bulged into lumen. In capillaries simple thickening of basement membrane with slight endothelial proliferation was present.

4. **Grade IV** – The appearance was similar to grade III but greater degree of splitting in basement membrane of vessels was some times so pronounced that rings of basement material was seen far out in surrounding stroma.

8. **Lipoprotein electrophoresis** (Ivor Smith, 1948)
   (i) Electrophoresis
   (ii) Drying and staining of the strips
   (iii) Densitometry

**Apparatus**

(i) Vertical tank for electrophoresis
(ii) Voltage stabilizer
(iii) Whatman filter paper No. 3
(iv) Micropipette
(v) Densitometer

**Reagents**

(i) Barbitone buffer
(ii) Distilled water
(iii) Sudan black - B
(iv) Ethanol 45% and 55%

**Barbitone Buffer**

The constituents of barbitone buffer are -
(i) Sodium diethylbarbiturate - 41.2 gm.
(ii) Barbitonic acid - 7.32 gm.

The sodium salt is dissolved in 500-600 ml cold water. The barbituric acid is dissolved in 100 to 200 ml of warm water and added to previous solution and whole is made to 1000 ml. This stock solution is diluted to one volume into a total of four volumes for use. pH value was adjusted at 8.6 ± 0.05 with help of N HCl (if necessary).

**Sudan black - B**

A saturated solution of Sudan black-B is prepared by adding an excess of dye to warm ethanol solution. The solution is cooled, allowed to stand a few minutes and filtered.

**Electrophoresis**

This was performed at room temperature in barbitone buffer of ionic strength, 0.5 and pH 8.6. 0.02 ml of plasma was applied with micropipette in a streak on a Whartman paper No. 3 near the negative pole of cell. Electrophoresis was carried out for 16 hours (overnight run) at constant voltage (110 V.) with a current of 0.1 milliamp./cm. width of the strip. Optimal voltage gives a beta lipoprotein migration of at least 1.5 cm. and also causes the best resolution of beta and pre-beta fractions. The polarity of the electrodes was reversed with each successive run to minimise pH changes and buffer crystallisation on the electrode wire.
Staining

After the completion of the run, strips were placed in an oven at 80° to 100° c for 20 minutes. Excessive heating yellows the paper and may slightly inhibit dye uptake by lipoprotein.

The strip is stained with sudan black-B for 30 minutes and washed with a quick rinse in 50% ethanol and thoroughly in 40% ethanol until most of back ground clears. When back ground clears completely, the strips are hanged for drying.

Densitometry

The strips were scanned densitometrically using an Aplab densitometer and coloured filter of 530 μ. The areas under the various peaks were integrated planimetrically and the various lipoprotein fractions were calculated.

Criteria for typing hyperlipoproteinaemia

The cases were classified into one of the 5 types of hyperlipoproteinaemias according to the criteria laid down by Fredrickson and colleagues (1967).

9. ESTIMATION OF CHOLESTEROL (Zak et al., 1953)

Estimation of cholesterol was done by technique of Zak et al (1953) and upper normal limit for serum cholesterol was taken as 250 mg/100 ml (Gupta et al., 1979).