MATERIAL AND METHODS
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The study was conducted on patients attending the medical out patient department and admitted to the wards of M.L.B. Medical College, Hospital, Jhansi during the period from Feb., 1991 to April, 1992. Those patients who had symptoms of malabsorption syndrome included in this study. Their prior written consent was taken. All these patients remained admitted in the hospital during the whole period of study.

All the patients were subjected to detailed interrogation about their diseases and specific symptoms suggestive of malabsorption. A thorough physical examination was done to look for signs of deficiency of vitamins and minerals and protein energy malnutrition. The following criteria were employed for selection of the subjects for this study.

1. History of diarrhoea for 3 months or longer and loss of weight.

2. One or more of the following features:
   b. Glossitis            e. Oedema of dependent parts of body
   c. Stomatitis.          f. Emaciation or cachexia.

The subjects investigated in this series fulfilling the above criteria were broadly put in
two groups:

1. Patients of chronic diarrhoea with no detectable primary cause.

2. Patients of chronic diarrhoea with a known etiology other than protozoal or helminthic infestation.

Total number of 58 patients were studied. Patients under the age of 12 years were not included in the study because they attended the Pediatric Medicine out patient department of the hospital and complete follow up was not feasible.

Twenty control subjects were studied. They were patients convalescing from relatively minor medical illness. None of the control subject had any known history of gastrointestinal disease.

**INVESTIGATIONAL PROCEDURE**

1. A detail clinical history and physical examination.
2. Naked eye and microscopic examination of stool.
3. Routine haematological examination: Hb\%, TLC, DLC, ESR etc.
4. For anaemic RBC count (Red blood cells count), PCV, (Packed cells volume), MCHC (Mean corpuscular haemoglobin), MCV (Mean corpuscular volume), MCHC (Mean corpuscular haemoglobin concentration), peripheral blood picture, bone marrow examination.
5. Total serum proteins (albumin and globulin).
6. Serum cholesterol.
7. Urine albumin, sugar, and microscopic examination.
8. Stool for ova and cysts, trophozoits.
   The quantitative determination of fat in timed stool collection.

Depending upon the requirement suggested by history and physical examination, selected patients were also be subjected to barium meal follow through examination sigmoidoscopy, Montoux test, chest X-ray, upper gastrointestinal endoscopy and other relevant investigations.

Based on the results of the faecal fat estimation, D-xylose test and other relevant tests the diagnosis of malabsorption syndrome was considered or excluded. Efforts were also made to find out the cause of malabsorption in individual cases.

METHODS

1. Determination of total fat contents of feces.

Indications:

**Patient's Preparation**

Patient was given 75 gms of fat (butter) for 6 days, 72 hours stool for the last 3 days was collected. The fat content of the stool sample was estimated by the method of Van de Kamar (1949) and expressed as grams of fat excreted per 24 hours. Stool fat excretion of more than 5 gm/24 hours signifies fat malabsorption.

**Fallacies**

In accuracy of proper times stool collection is the major fallacy. In patients whose bowel movements are infrequent, it may be necessary to use marker, but we did not enemate or marker used for collection of stool.

**ESTIMATION OF FECAL FAT (VAN DE KAMER et al., 1949).**

This is a convenient and rapid method and is particularly suited for use in connection with fat balance test. Results are expressed in terms of fatty acids.

**Principles**

Feces are saponified with concentrated KOH in ethanol and the fatty acids are liberated with HCl. The fatty acids are extracted with alcohol and petroleum ether and determined by titration of an aliquot of the petroleum ether extract with alkali.
**Reagents**

Ethanol 96% containing 0.4% amyl alcohol. Ethanol 96% neutral to thymol blue, Potassium hydroxide 33%. Hydrochloric acid 25%, specific gravity petroleum ether boil point 40-60° or 60-80° when evaporated to dryness this must leave no residue which can be titrated or saponified with alkali NaOH, 0.1 N thymol blue 2% in 5% ethanol.

**Caution**

The entire procedure should be carried out in a hood smoking and naked flames should not be permitted.

**Procedure**

1. Give the patient 75 gm butter fat daily for six days.
2. Collect the stool for the last three days (72 hours) for estimation.
3. Weight about 5 gm of faces in a 150 ml elhenmeyer flask.
4. Add 10 ml of 33% potassium hydroxide and 40 ml of ethanol containing 0.4% amyl alcohol and mix well.
5. Boil the mixture for 20-30 minutes under a reflux condenser.
6. After boiling add 17 ml HCl (25%) and again cool and mixture.
7. Add 50 ml of petroleum ether.
8. Close the flask with a rubber stopper and shake flask vigorously for one minute (20 times making).

9. Allow petroleum ether to separate.

10. Transfer the 25 ml contents into a small elhenmeyer flask and a small pieces of filter paper is added to prevent irregular boiling.

11. Evaporate the petroleum ether to dryness at 90° a stream bath.

12. Add 10 ml of neutral ethanol and mix well.

13. Titrate the fatty acids with sodium hydroxide (0.1 N) using thymol blue as an indicator.

Calculations

An average molecular weight 284 is assessed for fatty acids, hence:

\[
\frac{A \times 284 \times 1.04 \times 2 \times G}{10,000 \times Q} = \frac{5.907 \times A \times G}{100 \times Q}
\]

where, \( A = \) ml of 0.1 N NaOH required for titration.

\( Q = \) feces taken for analysis (in gm).

\( G = \) gm feces per 24 hours.

The factor 1.04 is used because the petroleum ether layer increase 1% in volume when shaken with the HCl reagent and because 3% of the fatty acids remain in the acid alcohol layer.
**D-Xylose Absorption Test**

This test was done to assess the integrity of jejunum to absorb carbohydrates.

**Reagents**

1. **P-Bromoaniline reagent, 2% solution (2 gm/100 ml).**
2. Saturated solution of thiourea in glacial acetic acid prepared fresh and stored the reagent in dark glass bottle (maximum a week).
3. **Zinc sulphate 5%**.
4. **Barium hydroxide (0.3 N)**
5. **Stock standard solution**: Dissolved 200 mg of xylose in 100 ml of saturated benzoic acid.
6. **Working standard** is prepared by dilution the stock solution to 1 to 10 and 1 to 20 with saturated benzoic acid. These contain 0.2 and 0.1 mg xylose per millilitre respectively.

**Patient's preparation**

The patient was fasted over night and empties his urinary bladder before the test. He was preferably resting in bed. The test was done by giving 25 gms of D-xylose in 250 ml of water orally. He was given a glass of water every hour to ensure a good urinary volume. The total urine can be collected in the next 5 hours and can be assessed for D-xylose. Normal 5 hours excretion is more than 4.2 gms. Five hour urinary
excretion less than 4 gms after a 25 gms is indicative of D-xylose malabsorption. The test can be done with 5 gm D-xylose, when 5 hours urinary excretion is less than 1.0 gm is consider abnormal.

We estimated D-xylose in blood. After two hours of giving D-xylose to patient blood sample collected with potassium oxylate(Pinch) then it is estimated by calorimeter.

**Method**

1. The test was done after fasting over night.
2. Patient was allowed to empty the bladder completely.
3. He was given an oral dose of 25 gms D-xylose in 250 ml water.
4. He was not allowed to take any thing during the study.
5. He was given 250 ml water to drink after one hour.
6. Blood sample was collected after 2 hours.
7. Xylose content can be determined in urine. But we determined in blood by colorimetric estimation.

**COLORIMETRIC ESTIMATION OF D-XYLOSE**

**Principle**

Xylose was determined by the formation of furfural and its reaction with parapromoaniline acetate to form a pink colour product at 70°C and in presence of the antioxidant thiourea. The estimation has a high
degree of specificity.

**Procedure for Blood**

1. De-proteinize 1 volume of blood sample after adding of 1 volume of water and 1 volume of zinc sulphate (5%) and BaOH (0.3 N).
2. Filter and proceed as described above using 1 ml filtrate.
3. Used standard containing 0.1 mg xylose per ml.

**Barium Meal Study**

Small intestine was carried out with micropaque the non-flocculable barium sulphate 4 ounces of barium sulphate, suspended in an equivalent quantity of water was given to the over night fasting patient. Roentgenographic observations were made at intervals usually half an hour. A 6 hours film was taken routinely in all the patients. In few patients 7 film at 9 hours were also taken.