Lipid Profile And Incidence Of Abetalipoproteinemia In Chronic Diarrhea Among Children Below Five Years Old
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Objectives:
This study was conducted to estimate the lipid profile in children below five years old with chronic dearrhia who were admitted in AL-ZAHRA’ hospital in Najaf in the period since 1st march to 31st July 2006 and to find out the incidence of ABETALIPOPROTEINEMIA (a rare autosomal recessive disease with potential lethal complications among them) for a better clinical approach to these patients.

Abstract:
This study was conducted to estimate the lipid profile in children below five years old with chronic dearrhia who were admitted in AL-ZAHRA’ hospital in Najaf in the period since 1st march to 31st July 2006 and to find out the incidence of ABETALIPOPROTEINEMIA (a rare autosomal recessive disease with potential lethal complications among them) for a better clinical approach to these patients.
Method And Patients:

20 patients were investigated, male to female ratio was 3:2, and by evaluating their history, comparing their growth parameters (weight, length, and head circumference) with a referral charts, then examination of their deep tendon reflexes, a sample of 5 ml. of venous blood was drown for detecting their lipid profile and searching for acanthocytosis in their blood films in addition to a stool sample send to the lab for general examination.

Results:

80% of the patients were hypolipedemic (below normal values) but non of the patients fulfilled the criteria of Abetalipoproteinemia which include failure to thrive associated with acanthocytosis of the RBC, negative deep tendon reflexes with sever hypolipidemia (a total cholesterol level below 50 percent of their normal values, triglyceride below 20 mg./dl, absence of low density lipoprotein LDL, and undetectable levels of the very low density lipoprotein, VLDL).

Conclusion:

Chronic diarrhea could be associated with hypolipidemia as a negative acute phase reactant to the stress of acute illness while the incidence of Abetalipoproteinemia among collected patients was zero percent.

Introduction:

Transportation of lipids: (Lipids are biologic compounds largely or entirely composed of nonpolar groups. As a consequence of this property, they are readily soluble in nonpolar solvents and relatively insoluble in water. Some lipids function as fuels, others as components of membranes or as precursors of hormones, and still others in a variety of additional specialized roles. The nonpolar nature of lipids is of central importance to many of these functions but poses special problems for their transport throughout the body. Because lipids are nonpolar, they must be transported in the circulatory system by water vehicles. Lipids are carried both by a protein, serum albumen, and by aggregates of lipids and proteins known as lipoprotein.

Free fatty acids are transported by serum albumen, a protein specialized for the transport of several nonpolar substances.

Triglycerides and cholesteryl esters are transported by several types of lipoprotein particles, all of which have a structure like that shown in (Fig 1).
The lipids to be transported are contained within the nonpolar core. Water solubility is conferred on the particle by a surface monolayer of amphipathic lipids (cholesterol and phospholipids) oriented with their polar groups to the outside. A number of proteins, called apolipoproteins, are attached to the surface or integrated into the particle. These proteins mediate:

1. The secretion of the particles from the cells which they are formed.
2. The delivery of the core material to the target tissue.
(3) The catabolism of the remnants that remain when the particles are exhausted of their contents.

There are 4 major classes of lipoprotein particles. Their functions and structures are summarized as:
- **Chylomicrons**: deliver dietary triglycerides to peripheral tissues and dietary cholesterol to liver. Secreted via the intestinal epithelial cells.
- **VLDL**: Deliver hepatic triglycerides to peripheral tissues. Secreted by the liver, majority of remnants converted to LDL.
- **LDL**: Deliver hepatic cholesterol to peripheral tissues formed at the liver from remnants of VLDL.
- **HDL**: mediate centripetal transport of cholesterol. Some derived from the surface components of chylomicrons. Others secreted by the liver.

**Abetalipoproteinemia (ALP)**: a rare autosomal recessive condition that affects both sexes, but predominantly males (70%). Characterized by fat malabsorption, spinocerebellar degeneration and pigmented retinopathy. The biochemical hallmark of this disease is the strikingly abnormal plasma lipid & lipoprotein profile.

Total cholesterol & triglyceride levels are extremely low there are no detectable plasma chylomicrons, VLDL, or LDL & apo B is absent from plasma. This disease is caused by mutation in the gene for the microsomal triglyceride transfer protein which mediates the intracellular transport of membrane associated lipids, in the intestine and liver and is necessary for the normal formation of chylomicrons in the enterocytes and VLDL in the hepatocytes.

The most prominent and debilitating clinical manifestations of ABL are neurological and usually begin in the 2nd decade. The first sign of the disease usually the loss of deep tendon reflexes, followed by decreased distal lower extremities vibratory and proprioceptive senses and cerebellar signs such as dysmetria, ataxia, and spastic gait.

The clinical outcome is a variable, but the result in untreated patients is often severe ataxia and spasticity by the 3rd or 4th decade. These severe effects on the CNS are the ultimate cause of death in most patients and often appear by the 5th decade or earlier.

Patients of ABL also acquire a progressive pigmented retinopathy.

The 1st ophthalmic symptoms are decreased night and color vision, daytime visual acuity usually deteriorates to virtual blindness by the 4th decade. The majority of clinical symptoms of ABL are the result of defect absorption and transport of fat soluble vitamins, especially vit. E.

The basic defect is lack of synthesis of apoprotein B which appears to be essential for the formation of low density lipoprotein LDL, very low density lipoprotein VLDL, and chylomicrons. Consequently, all these lipoproteins are absent from the serum.

High density lipoprotein HDL is also reduced in concentration. Plasma triglyceride concentrations are so low to be frequently undetectable, and cholesterol concentrations are typically less than 50% of normal.

Related disorder, (homozygous familial hypolipoproteinemia FHLP), mimics the signs and symptoms and the characteristic lipid levels of ABL, but believed to be of different mutation.

The non familial forms of hypobetalipoproteinemia are secondary to a number of clinical states such as occult malignancy, malnutrition, and chronic liver disease.
Frequency: (In the US: ABL and FHBL are rare inborn errors of lipoprotein metabolism. ABL occurs in fewer than 1 in 1 million persons. FHBL occurs in approximately 1 in 500 heterozygotes and in approximately 1 in 1 million homozygotes. Approximately one third of ABL and FHBL cases result from consanguineous marriages. Internationally: Frequency is similar to that reported in the United States.)

(It is important to consider these disorders in children who have any of these previously mentioned symptoms, because prevention of vit. E deficiency appear to prevent the devastating retinal and neurological manifestations.)(8) Symptoms of ABETALIPOPROTEINEMIA.
(Failure to thrive f(grow) in infancy.
Fatty stools that appear pale in color.
Frothy stools.
Abnormally foul – smelling stool.
Protruding abdomen.
Developmental delay.
Poor muscle coordination that usually develops after age 10.
Muscle weakness.
Slurred speech.
Curvature of spine.
Progressive decreased vision.
Balance and coordination difficulties.

Signs and tests: (VLDL concentration, usually below 1.3 mmol./L (50 mg/dl) together failure of chylomicrons formation after a fat meal and triglyceride level below 0.2 mmol./L (15mg/dl), are suggestive of the diagnosis.

Acanthocytosis of RBC and failure of rouleaux formation (the best seen in a fresh red undiluted blood film) are always present, but are not pathognomonic of ABL (Fig 2).

Figure (2): scanning electron micrograph of the red cells in a patient with ABL, most of the RBCs show the deformity of acanthocytes a few show the appearance of normal biconcave discs. and diagnosis must be confirmed by demonstrating absence of LDL by electrophoretic and immunochemical methods.
Intestinal biopsy shows normal villous architecture with swollen epithelial cells laden with fat, similar appearance may be found in primary hypo-B lipoproteinemia. 

Stool collection that shows elevated fat levels.

Low levels of fat-soluble vit. A, D, E, or K.

Ophthalmology exam showing retinal degeneration.

Absent or low apolipoprotein B levels in blood.

Genetic testing may be available for mutations in the APOB or MTP genes.

EMG or verve conduction velocity testing may show demyelination of peripheral nerves.

Lipid measurement: (Lipids can be measured individually as TC, TG, or HDL-C. using these measurements and the Friedewald equation when TG are less than 400 mg/dl, LDL can be calculated (LDL = TC - [ HDL+ TG/5 ]).

TG/5 represents the VLDL cholesterol content of serum or plasma.

Children should be on their regular diet for 4-6 weeks before testing.

Significant stress can lead to transient decreases in lipid levels or transient lipid abnormalities (e.g., hypertriglyceridemia following diabetic ketoacidosis). During acute illness, lipids should not be measured unless hypertriglyceridemia is believed to be the underlying cause of the disease.

(Lipoproteins are negative acute phase reactants, and their concentrations decline within 24 hours of severe acute stress.

Laboratory procedure for lipid measuring

Cholesterol testing: Add 0.5 ml. of patient’s serum to one ml. of cholesterol reagent, centrifuge for 5 min. then wait for 5 min. in a water bath, then read in wavelength of (500). Use the no. 6.6 as a multiplying figure to estimate the result of TC.

Normal level of cholesterol is 150 – 200 mg/dl.

Triglyceride: Same procedure using the triglyceride reagent instead of cholesterol reagent, and using the figure 6.6 as a factor to multiply the end result by.

High density lipid: Add 0.5 ml of patients serum to 0.05 ml of precipitant of HDL from the kit, wait 10 min., centrifuge then take one ml. of reagent of cholesterol and add it to 0.05 ml of supernant and wait for 5 min. in water bath, read in 500 n.m (nanometer).

Principle of the procedure: (Chylomicrons, VLDL,and LDL lipoproteins are precipitated by the addition of phosphotungastic acid in the presence of magnesium ions a supernant obtained after centrifugation contains high density lipoproteins HDL. The cholesterol bound to the HDL. is determined using the cholesterol RTU reagent.

Role of vitamin E: (Most of the clinical symptoms of ABL are the result of defects in the absorption and transport of vitamin E. Normally, vitamin E is transported from the intestine to the liver and is then repackaged in the liver and incorporated into the assembling VLDL particle by a specific protein termed the tocopherol-binding protein. In the circulation, VLDL is converted to LDL and vitamin E is transported by LDL to peripheral tissues and delivered to cells via the LDL receptor. Patients with ABL are markedly deficient in vitamin E because of the deficient plasma transport of vitamin E, which requires hepatic secretion of apoB-containing lipoproteins. Most of the major clinical symptoms, especially those of the nervous system and retina, are primarily due to vitamin E deficiency. This hypothesis is supported by the fact that other disorders involving vitamin E deficiency are characterized by similar symptoms and pathologic changes.)
(Low cholesterol levels with steatorrhea are also found in celiac disease, but without acanthocytosis, neurological findings or the typical lipoprotein pattern.(5) A clinical difficulty is that the children with celiac disease often appear to have a truncal ataxia with hypotonia which may be part of celiac toxic encephalopathy.(4)

In cystic fibrosis, there are respiratory symptoms and an elevated sweat chloride.(5)

Treatment: (Consult a nutritionist or other medical professional for dietary instruction. Large doses of vitamin supplements containing the fat-soluble vitamins (vitamin A, vitamin D, vitamin E and vitamin K) are given.

To avoid intestinal symptoms, avoid eating long-chain triglycerides. Thus, the diet should contain no more than 5 ounces of lean meat, fish, or poultry per day. Use skim milk instead of whole milk.

Since a certain amount of fat is needed for normal growth and development in all people, medium chain triglycerides are alternatively used as the major source of fat in the diet. These are absorbed from the gut differently than other fats, and thus avoid the intestinal symptoms. Medium chain triglycerides are taken as a dietary supplement, typically under the supervision of a physician or nutritionist.

Prognosis: The outcome is related to the degree and progression of neurological and visual problems. Severe forms of the disease lead to irreversible neurological disease before age 30.

Complications: Blindness:
- Mental deterioration.
- Loss of function of peripheral nerves, ataxia.

Prevention: High doses of fat soluble vitamins may be able to slow progression of some problems such as degeneration of the retina and decreased vision..(7)website 2

Hyperlipoproteinemas: (symptomatic hyperlipoproteinemia of childhood are divers and include disorders with accompanying sever hyper triglyceridemia, hyper cholesterolemia, or both.

Familial lipoprotein lipase (LPL) deficiency is characterize by sever hyper triglyceridemia, caused by functional absence of endothelial enzyme LPL. this enzyme is responsible for intravascular hydrolysis of dietary triglyceride, which is carried by chylomicrons. Patients who have this condition, have lipemic plasma in the fasting state, and triglyceride concentrations that typically are 1500-25000 mg. dl. cholesterol concentration measured in mg/dl typically are 10% -20% of the triglyceride conc. patients who have this disorder almost universally have recurrent bouts of abdominal pain and may develop pancreatitis. other signs of LPL deficiency, include hepatosplenomegalay, and eruptive xanthomas, which are small acne like papules on the buttocks, shoulders, and extensor extremities. Diagnosis of deficiency require assay of the enzyme activity in the plasma samples after injection of heparin.Both the symptoms and the characteristic lipid abnormalities of this disorder are responsive to a very low -fat diet -.

Familial type 5 hyperlipidemia in childhood: This is, also associated with sever hypertriglyceridemia, with triglyceride level of 500- 1000 mg/dl and the same set of symptoms and signs, as LPL deficiency. in contrast to patients who have LPL deficiency which usually selectively affects clearance of chylomicrons, patients who have type 5 hyperlipidemia have elevations of both chylomicrons and VLDL. cholesterol level
typically are 20%-50% of the plasma triglyceride level. a consistent enzymatic or molecular defect has not been identified among patients who have this disorder. affected children also respond to very low-fat diet.

**Heterozygous familial hypercholesterolemia:** This affects approximately 1 in 500 children, and is caused by dominantly inherited defect in the cellular receptor for LDL. children who have this condition, have mean plasma total and LDL cholesterol levels of approximately 280 mg/dl and 235 mg/dl respectively. their triglyceride level are normal. although the majority of affected children are asymptomatic, up to 10% may develop tendon xanthomas by the 2nd decade of life. these are appreciated as discrete areas of thickened tendons most frequently, affecting the Achilles and/or extensor tendons of the hand. symptomatic coronary artery disease which presents in up to 50% of affected males by age 50, is uncommon before the 3rd decade of life)\textsuperscript{(8)}

**Patients And Methods:**

This study was carried out in department of pediatrics in Al-Zahra’a hospital in Najaf, 20 patients with chronic diarrhea (diarrhea more than two weeks duration) were investigated, 12 of them 60% were males, and 8, 40% of them were females. with age ranges between two months to five year old.

After obtaining a history from relatives of the babies regarding name, age, duration of diarrhea, character of stool (regarding it’s color, amount, greasy, frothy, and whether there was a history of similar illness in the same family or among relatives and number of affected persons in the same family, also the history inquired whether the parents were relatives or not, and degree of relationship. Then the babies were submitted for checking their growth parameters regarding weight in KG. length in Cm., head circumference in Cm. and comparing each of these parameters with a reference charts obtained from (National Center for Health statistics in collaboration with the National Center for chronic diseases prevention and health promotion 2000).

Then estimation was done for the presence of deep tendon reflexes of both knees, followed by obtaining a sample of 5 ml. of venous blood to estimate the lipid profile and to look for acanthocytosis or burr cells which are the usual finding in Abetalipoproteinemia. Also a stool sample was send to the lab for testing appearance and consistency.

Then analysis of results of lipid profile were compared with normal values of the hospital lab.

Statistical analysis was carried by using a statistical analysis system program on main frame computer.
Results:
Sex distribution of the presented chronic diarrhea patients is illustrated in (fig 3).

Out of 20 patients, there were 60% males and 40% females, with male to female ratio 3:2.
Duration of diarrhea range between 2 – 12 weeks with mean of 7 weeks.
45% of total patients were born to a related parent of 1st degree, 40% of the infants had a history of same illness in the same family, and 65% of them were shown to have positive history among relatives.
Weight distributions among patients in this study ranges as such; 75% of them were below 3rd centile (include all of the females and around 66.6% of the males). the weight of the other patients distributed above 3rd centile (fig 4).
Regarding length parameter, it was shown that 30% of them were below 3rd centile (fig 5).
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Head circumference estimations, it was shown that 15% of the patients had head circumferences below 3rd centile, the other patients fall within upper limits in the growth chart for age and sex. (fig 6).
For the cholesterol estimations, the study showed:
85% of the patients had cholesterol level below 150 mg/dl (the normal level is 150 –
200 mg/dl) among them only 5% had cholesterol level below 50 mg/dl as patients
with ABL have while 15% of total patients had normal cholesterol level.
Also for the Triglyceride level, 15% of them shown to be of low level (below
65mg./dl), but not to the critical level to support the diagnosis of
Abetalipoproteinemia or even sever hypolipoproteinemia which recommend a
level below 20 mg./dl.(fig7).

7 Patients i.e 30% total cases had LDL below normal values of 13-36mg./dl & 4 of
them (20%) of total cases had very low level i.e below 4 mg./dl of Non of the patients was
estimated to have absent level of low density lipid as essentially recommended for the
diagnosis of Abetalipoproteinemia. For the very low density lipid VLDL, there were
15% of patients, who have very low levels (below 10 mg./dl). But not to the
recommended value of being (not detectable) as the study searches for.

Non of the patient had a positive blood film for acanthosytosis as the
Abetalipoproteinemia patients have, while all the patients were anemic with a
hemoglobin levels ranging between 6.5 gm/dl to 9 gm./dl, anemia was of hypo
chromic microcytic type.

All patients showed positive deep tendon reflexes.

General stool examination revealed that patients stool share the character of
being loose, bulky, malodor, greasy, yellowish to greenish in color.

Statistical analysis: For patients who were below five years old admitted to the pediatric
wards in the hospital during the period of research, there was:
.2882 no. of total admission to the hospital for all reasons.
1125 is the no. of patients who were admitted for diarrhea.
130 is the no. of patients admitted for chronic diarrhea.
Nov of the patients proved to have abetalipoproteinemia.

Analysis:
Incidence of diarrhea among whole admitted patients, was 39%.
Incidence of chronic diarrhea among whole admissions was 4.5%.
Incidence of abetalipoproteinemia among whole admissions was "0%".
Incidence of abetalipoproteinemia among patients with chronic diarrhea was "0%".

Discussion:
- Sex distribution among patients with chronic diarrhea showed that male to female ratio was 3/2 which mean a male predominance for chronic diarrhea among age group selected in the research.
- Mean duration for diarrhea was 7 weeks which give a strong indication for proper management for these patients.
- 45% of patient were born to a relative parents and 45% of patients had a history of same illness among relatives which indicate a possible genetic factor for their health problem. (chronic diarrhea).
- The weight of 75% of the patients were distributed below the 3rd centile which should pay a serious medical attention for a proper interference and prevention of further retardation in growth and development.
- Length and head circumference parameters was less affected (30% and 15% for length and head circumferences respectively) which fits the expectation of sequential retardation of growth parameters for wt, length and then the head circumference if malnutrition accompanied the diarrhea.
- Total serum cholesterol was below normal level in 85% of patients (below 150 mg./dl) which indicate an impaired fat absorption in patients with chronic diarrhea which is a reasonable indication to supply these patients with heavy doses of fat soluble vitamins to overcome possible complications for their shortage (.as mentioned in this text that most complications of a ALP is due to vit. E deficiency, also it is an indication to avoid consuming high chain triglyceride and encourage use of skim milk instead of whole milk.)
- The triglyceride of 15% of the patients were of low level which may indicate a mild impairment of chylomicrons levels in intestinal endothelium which accompany the chronic diarrhea we have to mention here that we haven't the facility to check for chylomicrons in blood. the hypolipedemia could be explained gust(as a negative acute phase reactance to a stress of acute illness.)
- Some of results showed low levels of LDL but not to the critical level of confirming diagnosis of ABL by being undetectable. the same conclusion for the VLDL level.
- Deep tendon muscle reflexes were positive in all the patients which unfit the possibility of ABL as a cause for the chronic diarrhea.
- Non of the blood films was positive for acanthositosis (which should be positive since birth)^2 in ABL cases.
• General stool examination revealed a share character of being loose bulky, malodorous, greasy, which fits the stool character of malabsorptive diseases for any cause, and it is not pathognomonic for ABL.

• None of the patients fulfilled all the criteria of a beta lipoproteinemia by having retardation of growth together with a total cholesterol below 50 mg/dl, triglyceride below 20 mg/dl, absence of L.D.L, undetectable V.L.D.L with acanthocytosis & a negative deep tendon reflexes.

Conclusion: As this study showed & for this sample of patients, during this limited period of time, and in this local hospital that chronic diarrhea could be associated with hypolipidemia which necessitate avoidance of fatty diet in case of diarrhea and that THE INCIDENCE OF A BETALIPOPROTEINEMIA WAS ZERO PERCENT.

Hypolipoproteinemia in some of the patients could be explained as a negative acute phase reactant to the stress of acute illness, or secondary to malabsorption.

Recommendations: To overcome cases of hypolipoproteinemia which could be due to a negative phase reactant, it would be of value to follow patients with hypolipoproteinemia for re-estimation of lipid profile after 4-6 weeks after control of diarrhea.

A comparative study for incidence of hypolipoproteinemia among wider age group may give a helpful clue for a higher incidence of the disease by demonstrating well progressed features of neurological and visual manifestations.

In addition to the above, finding more incidence among older age group would mean that Abetalipoproteinemia among younger age group could be subtle and encourage providing a prophylactic doses of vit. E to alleviate a possible future harmful neurological and visual manifestations.

References:
