Gut Barrier and Absorptive Indices in Constipated Children and its psychological effects

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List of Abbreviations

APCs	Antigen-presenting cells
BMI	Body mass index
CNS	Central nervous system
CO_2	Carbon dioxide
[^{51}Cr]EDTA	[^{51}Cr] ethylene diamine tetra-acetic acid
EGF	Epidermal growth factor
GLUT2	Glucose uptake and transporter
H_2	Hydrogen
HPLC	High-performance liquid chromatographyt
HRP	Horseradish peroxidase
IgG	Immunoglobulin G
IgG	Immunoglobulin G
K	Potassium
M cells	Microfold cells
MHC	Major histocompatibility complex
MW	Molecular weight
Na^+	Sodium ion
NGF	Nerve growth factor
PEGs	Polyethylene glycols
S-IgA	Secretory immunoglobulin A
T_4	Serum thyroxin
TGF-α	Transforming growth factor-α
TSH	Thyroid-stimulating hormone

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ABSTRACT

Constipation in children can be defined by a stool frequency of less than three per week, or painful bowel movements often accompanied by severe discomfort, screeming, and stool withholding maneuvers in young children, or stool retention with or without encopresis, even when the stool frequency is three or more per week ***(Loening-Baucke, 1996)***.

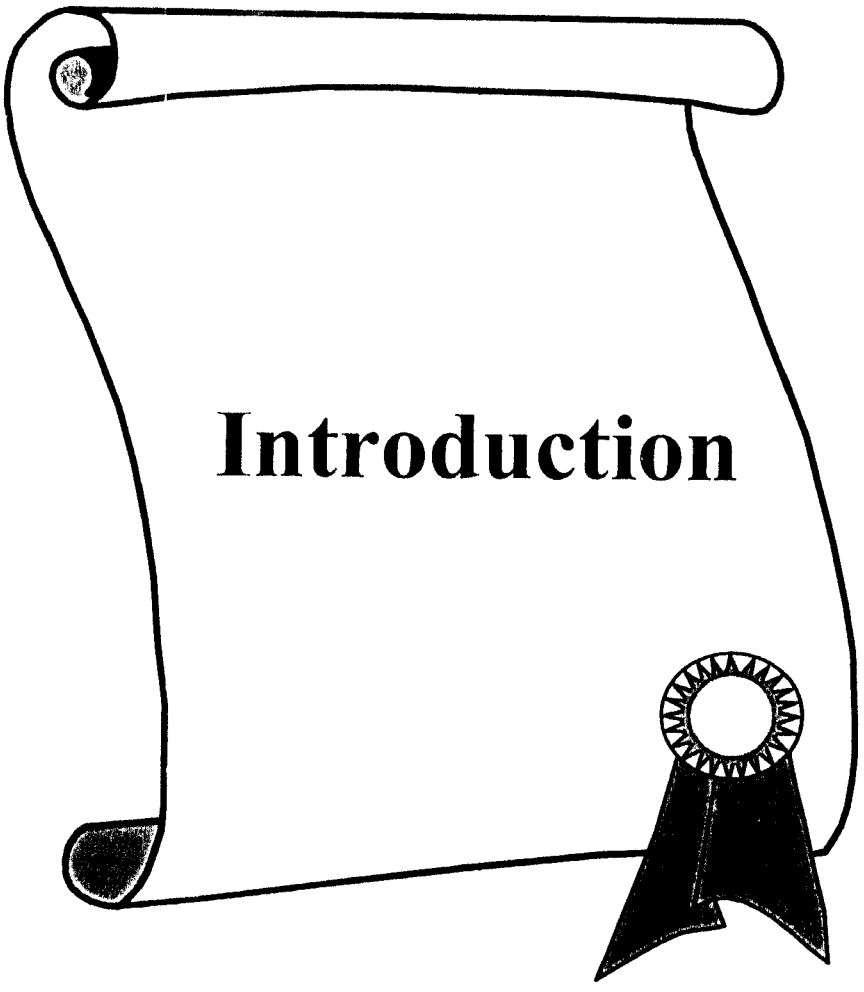
We hypothesized that constipation is associated with hypomotility which can lead to small intestinal bacterial overgrowth which can lead to malabsorption.

The study was carried out on 56 children with chronic constipation. They were recruited in the period between June 1999 and December 2000. They were 34 males and 22 females. Their ages ranged between 4 years and 9 years with a mean age of 5.90 ± 1.18 years. A cohort of 56 healthy children were chosen as a control group.

All children were subjected to assessment of fecal α_1-antitrypsin as a measure of protein malabsorption, fecal fat by semiquantitative steatocrite method, breath hydrogen test to diagnose carbohydrate malabsorption and assessment of mannitol, lactulose and lactulose / mannitol ratio as a measure of gut mucosal permeability.

The results of the study revealed that fecal α_1-antitrypsin as a measure of protein-loosing enteropathy was significantly higher among patients (3.14 ± 3.83) compared to controls (0.26 ± 0.08), fecal fat as a measure of fat malabsorption was significantly higher among patients (6.76 ± 5.68) than controls (2.08 ± 0.84). The different readings of lactose breath hydrogen testing were not different between cases and controls and lactose malabsorption is significantly more frequent in patients (23.2%) compared to controls (7.1%).

We can come to conclusion that constipation is not only a disease of the colon but the rest of the gut may be involved with malabsorption and gut barrier distribution.


Introduction

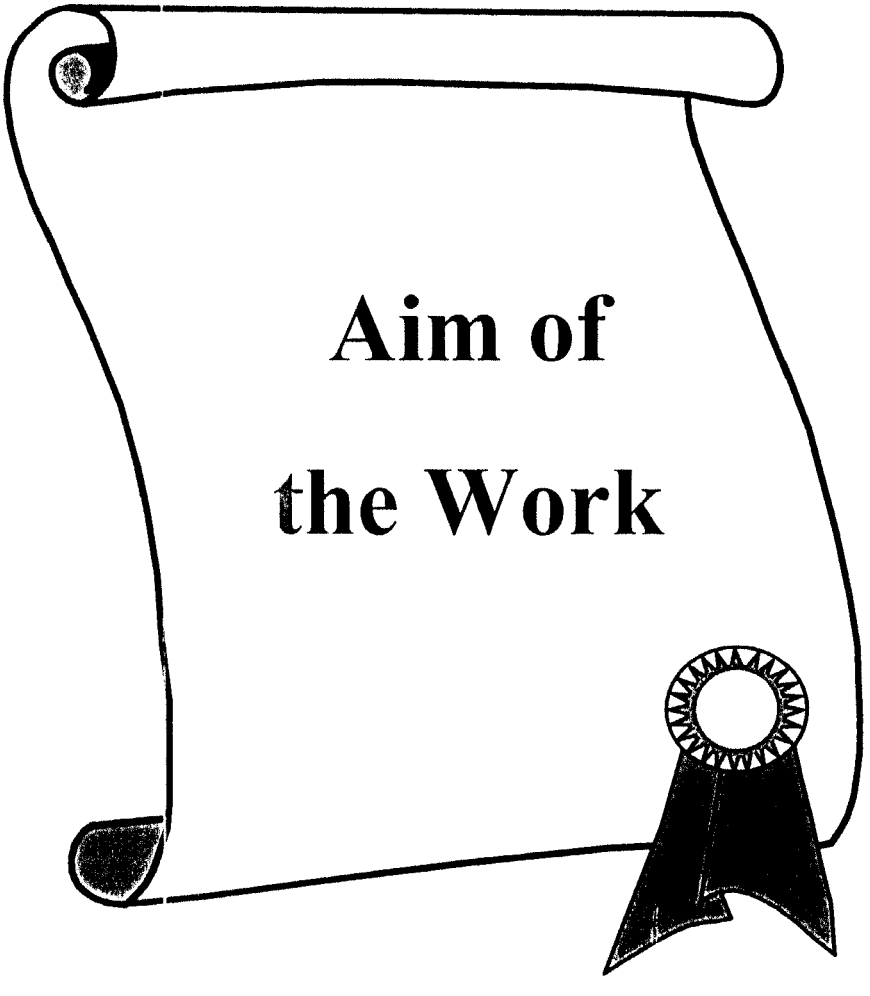
INTRODUCTION

Constipation is a frequent problem among infants and children. It occurs in 16 – 37% of children below 12 years ***(Issenman et al., 1987 and Koch, 1995)***.

Constipation may be a part of generalized hypomotility of the gut in whole or in part ***(Koch 1995)***.

Cow milk allergy was reported recently to present as chronic constipation rather than diarrhea in some children ***(Iacono et al., 1998)***.

Gut contamination is a usual complication of decreased gut motility. This may involve the small intestine. As a consequence the absorptive capabilities may be altered in such a situation ***(Rintala et al., 1994)***.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AIM OF THE WORK

1) Assessment of possibility of malabsorption in cases with constipation.

2) Assessment of gut barrier in constipated children to clarify the relation to allergy.


Review of
Literature

CONSTIPATION

Linguistic:

The word constipation comes from the Latin word "constipare" meaning to crowd together.

Definition:

Constipation can be defined as the passage of small hard feces infrequently and with difficulty ***(Marie Fallon and Bill O'Neill, 1997).***

It can refer to the passage of hard stools, infrequent defecation or difficulty in passing stools ***(Donatelle, 1990).***

In children, constipation generally refers to a stool frequency of less than three times per week ***(Alexander et al., 1996).***

According to ***Loening-Baucke, (1996)*** constipation is usually defined in terms of alterations in the frequency, size, consistency, or the ease in passage of stools.

Constipation in children can be defined by a stool frequency of less than three times per week, or painful bowel movements often accompanied by severe discomfort, screaming, and stool withholding maneuvers in young children, or stool retention with or without encopresis, even when the stool frequency is three or more per week ***(Loening-Baucke, 1996).***

The definition of constipation remains elusive. What may appear to be constipation to parent seems normal to another ***(Fitzgerald, 1987).***

Infrequent, hard, and painful bowel movements signals a mild-to-moderate problem in bowel function ***(Leonard et al., 1986).***

All children were considered to be constipated because they had either less than 3 bowel movements per week or painful bowel movements, or a rectal impaction, or an abdominal fecal mass on physical examination, or all four ***(Loening-Baucke, 1993).***

Incidence:

Constipation is a common and frequently underestimated problem in children, which may cause abdominal pain or contribute towards urinary tract disorders ***(O'Regan et al., 1985).***

Iacono et al. (1998) defined chronic constipation as having one bowel movement every 3 to 15 days and, in many cases, having abdominal symptoms as well.

Although the exact incidence of constipation is not known, it is estimated to occur in 5 to 10 percent of children ***(Di Lorenzo et al., 1992).***

Constipation is slightly more common in boys ***(Lloyd-Still, 1987).***

Constipation accounts for approximately 3 percent of visits to pediatric ambulatory clinics and 20 to 25 percent of visits to pediatric gastroenterology clinics ***(Loening-Bauke, 1994 and Susan et al., 1999).***

In U.S study, 16 percent of parents of 22-month old reported that their children had constipation ***(Issenman et al., 1987).***

In United Kingdom, 34 percent of 4-to-7 year old children were said to be constipated. The frequency was 37 percent among 1005 Brazilian children who ranged in age from birth to 12 years ***(Zaslavsky et al., 1988).***

Complaints of constipation and soiling comprise up to 3 percent of large clinic outpatient pediatric health visits ***(Levine, 1975).***

Chronic constipation with encopresis occurs in 7-year old school children at a rate of 2.3% in boys and 0.7% in girls ***(Bellman, 1966).***

Constipation is one of the most common abdominal symptoms of childhood with an estimated incidence varying from 0.3% to as high as 8% ***(Loening-Baucke, 1993).***

Physiology of Normal Defecation

Although the various phases of gastrointestinal motility play an important role in normal defecation other non-motility factors may play a critical role ***(Weaver, 1988).***

Very special control mechanisms are developed in the body to prevent loss of wind, stool and urine. Unconscious regulation of bowel movements is a normal phenomenon after birth. Conscious regulation of bowel movements is achieved at an average age of 28 months ***(Brazelton, 1962).***

Fecal continence is the body's ability to recognize when the rectal ampulla fills; to demonstrate whether the content is formed stool, liquid stool, or gas, and to retain the content until emptying is convenient. The major structures responsible for continence and defecation are the external anal sphincter, puborectalis muscle, internal anal sphincter, and rectum. The factors that are responsible for maintaining fecal continence and that also facilitate defecation are the high pressure zone in the anal canal, the anal and rectal sensory and reflex mechanisms, the visco-elastic properties of the rectum, and the stool volume and consistency ***(Loening-Bauck, 1996).***

Normal defecation involves synchronized autonomic and voluntary function ***(Scharli, 1970).*** A descending fecal bolus

distends an empty rectum, stimulating sensory receptors in the bowel wall and pelvic floor. Ascending sensory fibers allow conscious awareness of rectal distention. There is transient contraction of voluntary striated muscle external anal sphincter, and the puborectalis sling, the so-called inflation reflex. Transmission of the nerve impulse distally by the myenteric plexus in the lower rectal wall produces reflex inhibition of the smooth muscle internal anal sphincter, the rectosphincteric relaxation reflex ***(Denny-Brown and Robertson, 1935).***

Internal anal sphincter relaxation occurs proportionate to the volume and rate of rectal distention. This is followed by inhibition of the external anal sphincter ***(Parks et al., 1962)*** involving reflex and facilitory cortical pathways ***(Freckner, 1975).***

Puborectalis relaxation allows widening of the anorectal angle (normal 60 to 105° increases to 140°), producing an unobstructed anal pathway, increased abdominal pressure associated with peristalsis in the rectal wall allows expulsion of feces with emptying of the entire rectum ***(Terry and Hatch, 1988).***

The anorectum of the developing infant undergoes both structural and function maturation. The rectum increases in length and is accompanied by the appearance of rectal valves

and forward angulation of the anorectal junction ***(Terry and Hatch, 1988).***

The frequency of bowel movements changes during the first year or two of life but not much thereafter. The actual frequency of bowel movements depends a lot on whether the infant is breast-fed or bottle-fed. By about 4 or 5 months of age, most children are having between two and three bowel movements per day. After 1 year of age, the frequency is reduced to an average of one and one-half to two bowel movements per day. By 3 or 4 years of age, there is an average of one and one-half bowel movements per day, which remains constant until later adult years, when the frequency of bowel movements in the elderly population tends to decrease significantly ***(Weaver, 1988).***

Table (1): Frequency of bowel movements per day with advancing age

Percentile	1 wk	2 mo	4 yr	50 y
90^{th}	6.9	4.1	2.3	1.9
50^{th}	3.8	2.4	1.2	0.9
10^{th}	1.4	1.0	0.8	0.6

Adopted from ***Weaver, (1988)***

Etiology

Symptomatic constipation has a large impact on health expenditure ***(Tedesco and Di Piro, 1985).***

Constipation may be the result of different pathogenetic mechanisms. Several studies have categorized constipated patients by their symptoms ***(Lennard Jones, 1984)*** or by motility patterns ***(Meunier et al., 1979).***

A wide variety of clinical conditions are associated with or cause chronic constipation. Systematic classification has been proposed based on the presence or absence of fecal retention, presumed pathophysiology, or the presumed anatomic site of dysfunction. A differential diagnosis is presented in the next table relating to defined disease within or outside of the colon ***(Lennard Jones, 1985).***

I) Unassociated with structural abnormality of the anus, rectum, or colon or defined physical abnormality:

1) Faulty diet or habit (excessive cow's milk, inadequate bulk)
2) Chronic underhydration
3) Undernutrition
4) Idiopathic slow transit constipation (onset adolescent females)
5) Symptoms of "irritable bowel syndrome"
6) Chronic retention constipation

II) **Structural disease of the anus, rectum or colon:**

1) Anal stenosis
2) Painful disorders of the anus (fissures, dermatitis)
3) Anorectal malformation (imperforate anus, anterior ectopic anus, anteriorly located anus)
4) Colonic stricture (inflammatory bowel disease, necrotizing enterocolitis)
5) Aganglionosis and/or abnormal myenteric plexus
    a. Congenital - Hirschsprung's disease
    b. Acquired – Chagas disease
    c. Pseudo – obstruction
6) Idiopathic megarectum and megacolon
7) Redundant sigmoid colon

III) Secondary to abnormality outside the colon

1) Endocrinology and metabolic:

a) Pregnancy b) Hypothyroidism
c) Hypercalcemia d) Diabetes insipidus
e) Infantile renal acidosis f) Hypokalemia
g) Uremia

2) Neurologic :

a) Damage to sacral outflow or spinal cord
b) CNS disorder (included cerebral palsy)
c) Infectious polyneuritis d) Amyotonia congenita

3) Connective tissue disorders

4) Psychologic

a) Depression b) Anorexia nervosa

c) Denial of bowel action d) Withholding

5) Drug side effect:

a) Anesthetics b) Anticholinergics

c) Anticonvulsants d) Antidepressants

e) Barium f) Bismuth

g) Hematinics (iron) h) Metallic intoxication (lead)

i) Opiates

(Adopted from Lennard-Jones, 1985)

Altered bowel habits may be related to an abnormal colonic transit pattern ***(Lanfranchi et al., 1984).*** However, colonic motility and transit may be similar to healthy subjects in some constipated patients ***(Shouler and Keighley, 1986).*** As different mechanisms for the altered bowel habit are identified, new medical and surgical approaches to the treatment of severe constipation can be developed ***(Kreek et al., 1983).***

Chronic constipation is a frequent and distressing complaint in childhood, and a specific cause can not usually be determined ***(Clayden, 1976).*** Multiple pathophysiologic mechanisms may be responsible for the symptom ***(Meunier et al., 1984).***

Most chronic constipation in infants and young children is thought to have a psychogenic cause ***(Abrahamian and Lioyd-Still, 1984),*** or to be related to an intestinal motility disorder ***(Staiano et al., 1991).***

Chronic constipation in the first 2 to 3 years after birth may be a manifestation of cow milk allergy ***(Iacono et al., 1995).***

Recent studies in adults have demonstrated abnormalities of gastrointestinal hormones that affect motility in functional bowel disorders ***(Preston et al., 1985).*** Gastrointestinal hormones are reported to play a role in colonic motility and in the cause of constipation ***(Wrenn, 1989).***

There is evidence to suggest that, in childhood constipation, chronic distention of the rectosigmoid with large amounts of fecal material, actually decreases the ability to sense this distention with diminished sensation, the child is unable to feel an urge to go to the bathroom, which further compounds the problem with constipation and encopresis ***(McClung et al., 1993).***

Impaired rectal sensation, higher maximal rectal compliance, or both, were found in 97% of constipated children ***(Meunier et al., 1979).*** Higher rectal compliance, impaired

rectal contractility, and/or impaired rectal sensation can impede defecation.

Difficulties with defecation are common in infants and children. Most often the problem is short lived and of little consequence, but chronic constipation most often follows an inadequately managed acute problem ***(Fitzgerald, 1987).***

Childhood constipation most often is related not to a disorder of colon function but to learned behaviour aimed at avoiding painful defecation. Such retentive posturing results in the accumulation of a large fecal mass in the rectum ***(Fleisher, 1976)***. Retentive constipation, known also as functional fecal retention. Constipation develops gradually in some children as a result of a progressive decrease in the frequency of bowel movements and a progressive increase in the difficulties in passing an excessively firm stool. In others, an acute episode of constipation may follow a change in diet or environment. A febrile illness, a period of dehydration, or bed rest ***(Loening-Baucke, 1993).***

A primary cause for the constipation may be identified in a small proportion of patients. Examples of these include Hirschsprung's disease, local organic pathology, such as hypothyroidism and extrinsic neurological disorders, such as spinal dysraphism or cerebral palsy ***(Kiely et al., 1979).*** In the

vast majority of cases however, no primary cause can be, and the condition is labeled as "idiopathic constipation".

The cause of chronic idiopathic constipation in children under the age of six years are still debated ***(Loeníng-Baucke and Cruikshank, 1986).*** The two main hypotheses about its causation are that its is psychogenic ***(Olatawura, 1973)*** and that it results from disturbances in intestinal motility ***(Loening-Baucke, 1990).***

However, numerous studies have shown that psychological problems are more often the consequence than the cause of constipation ***(Keuzenkamp-Jansen et al., 1996).*** The role of alterations in motility still need to be clarified ***(Younoszai and Tolaymat, 1989).***

The overwhelming majority of constipated children suffer from idiopathic functional constipation. This term refers to the absence of organic disease, although several studies have found neuromuscular anomalies in the colon and rectum of these children ***(Loening-Baucke, 1993) (Corazziari et al., 1985).***

The cause of functional constipation is poorly understood, but clearly multifactorial ***(Loening-Baucke, 1995).*** A vicious cycle of painful bowel movements and fecal retention, as well as

dietary, psychological, and social factors play a very significant role in this condition ***(Dan Poenaru et al., 1997).***

Intolerance of cow's-milk protein occurs in 0.3 to 7.5 percent of otherwise normal infants ***(Schrander et al, 1993).*** More recently, gastroesophageal reflux and chronic constipation caused by anal erythema, and fissures and fistulas and proctitis have been attributed to intolerance of cow's milk protein ***(Cavataio et al., 1996; Iacono et al., 1995).***

Iacono et al. (1998) provide further evidence that intolerance of cow's milk protein is a cause of chronic constipation in children.

Functional constipation:

Stool retention results when stool expulsion has not occurred for several days. When stool retention persists, then formed, soft, or semiliquid stools leak to the outside around the accumulated firm stool mass. When stool retention remains untreated for a prolonged period of time, the rectal wall becomes stretched and a megarectum develops. The interval between bowel movements become increasingly longer, and the rectum becomes so large that the stored stool can be felt as an abdominal mass that sometimes reaches up to the umbilicus (megarectum). In some cases, stool distends the whole rectum, a megacolon ***(Loening-Baucke, 1996).*** The etiology of chronic functional

constipation is still unknown. Some children demonstrate unconscious contractions of the external anal sphincter during defecation ***(Loening-Baucke, 1990).*** This paradoxical reaction is probably based on fear of a painful defecation and may contribute to the pathogenesis of chronic functional constipation because it limits colonic evacuation. Familial and constitutional factors seem to play a part in the etiology of chronic functional constipation ***(Lowery et al., 1985).*** No single mechanism is responsible for chronic functional constipation. Constitutional and inherited factors, such as intrinsic slow motility and psychological factors contribute to chronic constipation ***(Swarwick, 1991).*** If defecation is painful, the child avoids the pain-producing activity by withholding stool. The rectum adjusts to the contents, and the urge to defecate gradually passes. As the cycle is repeated, successively greater amounts of stool are built up in the rectum with longer exposure to its drying action, and a vicious cycle is started ***(Partin et al., 1992).***

The Pathophysiology

Normally, distension of the rectum produces conscious awareness and initiates activity in the anal sphincter. A transient pressure spike occurs in the external anal sphincter followed by relaxation of this striated muscle. Immediately, the internal anal sphincter undergoes relaxation (recto-sphincteric relaxation reflex), resulting in a sustained lowering of anal sphincter pressure.

Resting anal sphincter pressure:

In constipation, baseline anal pressure has been found to be increased ***(Holschneider, 1983).*** That increased anal pressure might function to produce an outlet obstruction of the rectum ***(Martelli et al., 1978).***

Rectosphincteric relaxation reflex:

Reflex internal anal sphincter relaxation may be altered in severe constipation, the depth of relaxation may be diminished ***(Leoning-Baucke, 1984).*** The minimal volume of rectal distension (critical volume) required to elicit the relaxation reflex is often increased ***(Cacchiatra et al., 1984).*** Diminished relaxation of the internal anal sphincter during defecation could result in anal outlet obstruction due to a persistently high pressure in the distal anal canal.

Rectal sensitivity-conscious awareness:

Threshold volume is the volume of rectal distension required to produce conscious awareness. It is related to the critical volume (the volume required to initiate internal anal sphincter relaxation). The threshold volume is consistently increased in constipation ***(Meunier et al, 1984).*** Critical volume and threshold volume increased in parallel in constipation without encopresis ***(Sondheimer, 1985),*** while in those with encopresis, internal anal sphincter relaxation often occurs at volumes that do not stimulate conscious awareness.

Rectal compliance:

It is the volume of the ease with which the rectum may be distended. It is increased in constipated patients with soiling, while it is more normal in those with constipation alone ***(Suzuki et al., 1980).*** Abnormal compliance and motility is the result of constipation and not its cause, but might contribute to severity.

Transit time:

Constipation may be defined by delay in gastrointestinal transit velocity for the entire intestinal tract ***(Hinton et al., 1969).*** This delay is mainly in the colon, because delayed small intestinal transit is rare ***(Cacchiatra et al., 1984).***

External anal sphincter:

There is unconscious active external anal sphincter contraction during defecation in some constipated patients ***(Wald et al., 1986).*** Probably both external anal sphincter and puborectalis are involved. This response is seen more often in children, usually males, with constipation and encopresis ***(Loening-Baucke and Cruik-Shank, 1986).***

Expulsion failure:

Expulsion failure is independent of rectal size and most other measures of anorectal motility in constipation ***(Loening-Baucke and Cruikshank, 1986).***

It does correlate with paradoxical external anal sphincters contraction and thus with more persistent and severe constipation, encopresis, treatment failure, and a form of constipation persisting into adulthood.

Stool form:

The ability to defecate stool of altered form or hardness is further impaired in constipation. More effort and time is required to expel objects from the rectum if they are small and hard than if large and soft ***(Bannister et al., 1987).***

Clinical Picture

Males predominate throughout childhood. The age of onset is frequently under 1 year (25 percent) and peaks at ages 2 through 4. Definable events such as diet changes, rectal fissures, intercurrent illness, moving to new home, changing schools, birth of a sibling, travel, or family problems are present at onset in a minority of cases ***(Abrahamian and Lioyd-Still, 1984).*** Problematic or failed toilet indicates the early onset of a stooling disorder. Frequently, problems are noted before there is any attempt at toiling training. The most common symptoms associated with constipation are infrequent defecation, passage of voluminous and hard stools, and difficulty in passing stools. Some children with chronic constipation present with recurrent abdominal pain or discomfort. The pain is usually intermittent and localized to the periumbilical area. Children under two years

of age may engage in a screaming episode in anticipation of or during defecation ***(Loening-Baucke, 1994).*** Loss of appetite and abdominal distention may also accompany the condition. Urinary problems (enuresis and urinary tract infection) are seen with overt constipation, while children presenting with urinary complaints have an even higher rate of associated overt or occult constipation. Only 20 percent of children having significant psychologic problem are often secondary to constipation ***(Abrahamion Lioyl-Still, 1984).*** Rectal prolapse is uncommon. Abdominal pain in association with abdominal mass, fecal impaction, and soiling, suggesting increased severity and/or chronicity of retention ***(Monar et al., 1983).*** Other clinical manifestations depend on the underlying disorder.

Complications:

Encopresis, or fecal soiling, is a complication of long standing constipation ***(Corazziari et al., 1985).*** If neglected, constipation may result in impaction and, in severe cases, large bowel obstruction. Rectal prolapse may be the result of severe chronic constipation ***(Leung, 1986).*** Hirschsprung's disease may be complicated by toxic enterocolitis. Gastrointestinal bleeding is a danger sign for eneterocolitis of Hirschsprung's disease.

Recurrent urinary tract infections may also be associated with constipation ***(Zempsky and Rosentein, 1988).*** Treatment of constipation results in cessation of recurrent infections.

Constipation is also associated with enuresis, vesicoureteral reflux and upper urinary tract dilatation ***(Dohil et al., 1994).***

Assessment of constipation:

I) HISTORY

An accurate history is essential for effective management. Inquiry should be made about the frequency and consistency of stools, nausea, vomiting, abdominal pain, distention and discomfort, mobility, diet and any other symptoms. In patients with a history of diarrhea, care should be taken to distinguish true diarrhea from overflow due to fecal impaction ***(Marie Fallon and Bill O'Neill, 1997).***

Careful questioning about access to a toilet or commode is important. Limited mobility may mean that using the toilet or commode is avoided. Other issues, such as lack of privacy or the need for nurses or carers to help with toileting, can exacerbate constipation.

Dietary history:

The relationship of diet to the onset of constipation is important. The introduction of solid foods, inadequate fluid intake, change from breast to bottle feeding, change from formula to cow's milk, or excessive intake of cow's milk may result in constipation.

Psychosocial history:

A detailed psychosocial history should be taken, and any emotional stress should be noted. In particular, family stress should be considered.

II) EXAMINATION:

A complete physical examination is essential, for example, bradycardia, dry skin and myxedema suggest hypothyroidism. Anemia and bluish line on the gum suggest lead poisoning.

The weight, height and head circumference should be plotted on standard growth charts. A constipated patient may have malodorous breath, or the smell of fecal leakage may be obvious. Bacterial degradation of hard stools can results in leakage, of which the patient has no warning. Particular attention should be paid to the general appearance of the patient, the state of hydration and evidence of weight loss. General observation may reveal that a patient is in pain, confused or disoriented, or unable to reach the toilet. Failure to thrive suggests Hirschsprung's disease or hypothyroidism. Abdominal distention, visible peristalsis, and borborygmi can suggest obstruction. Abdomen should be examined for evidence of distention, unusual tenderness or organomegaly. Palpation may reveal an easily palpable colon with indentabel and mobile (and rarely tender) fecal masses. In contrast, tumour masses are

usually hard, not indentable, fixed, and often tender. In chronic constipation, regardless of the cause, fecal masses are often palpable in the left lower quadrant of the abdomen and in the suprapubic area.

The spinal and anal areas should be carefully inspected for spinal anomalies such as meningomyelocele or tumor, abnormal location of the anal orifice, anal fissure, and skin tag (sentinel pile), fecal soiling, perineal disease or deformity, and rectal prolapse. In constipation, complicated by obstruction, auscultation of the abdomen may reveal high pitched tinkling bowel sounds, although the abdomen can also be silent.

Digital examination of the rectum or stoma is crucial if constipation is suspected. This will immediately reveal hard stools, tumor masses, or concomitant disease such as hemorrhoids, an anal fissure, or perianal ulceration. The rectum or stoma can be empty in constipation- hard or even impacted stools can lie higher in the bowel. Rectal examination in patients with Hirschsprung's disease, revealed that the rectum is usually empty and tight and, on withdrawal of the examining finger, feces or gas is often explosively discharged. If rectal examination can be performed without causing the child much discomfort and without significant anal spasm, a local cause for pain can be excluded.

III) INVESTIGATION:

Occasionally, despite an accurate history and examination, the diagnosis of constipation is still not clear. Laboratory tests should only be ordered when indicated by the history and physical examination.

Blood studies:

If hypothyroidism is suspected, serum thyroxin (T_4) and thyroid-stimulating hormone (TSH) levels should be determined. Determination of serum calcium or K level should be considered if hypercalcemia or hypokalemia is suspected. Measuring the serum lead level is helpful if lead poisoning is suspected.

Plain abdominal film:

A simple, quick and easily available method of assessing constipation in children is required both for initial assessment and for monitoring response to treatment. Plain abdominal radiography is, potentially, an ideal candidate for this ***(Clayden, 1992).***

Anorectal manometry:

Anorectal manometry measures pressure events in the rectum and anal sphincter areas. Anorectal manometric assessment of children with mild constipation is not necessary. The main role of anorectal manometry is in the evaluation of children with severe constipation in whom the diagnosis of

Hirschsprung's disease needs to be excluded and in patients with fecal incontinence in whom anal pressure and rectal sensation should be assessed. In patients with Hirschsprung's disease, the internal anal sphincter does not relax during rectal balloon distention and the rectosphincter reflex is absent ***(Loening-Baucke, 1983).*** Numerous manometric studies have been performed in children with functional constipation and encopresis ***(Loening-Baucke, 1989).*** The abnormalities found in these children include increased threshold to rectal contractility as compared with controls ***(Loening-Baucke and Gruikshank, 1986).*** In follow up, after 3 years of therapy, most children show continuous abnormalities of anorectal function, leaving them at risk for recurrent problems ***(Loening-Baucke, 1984).*** Another abnormality is the failure of the external anal sphincter and pelvic floor to relax during defecation attempts ***(Emery et al., 1988).*** This abnormality is found in many constipated children who respond poorly to treatment ***(Loening-Baucke, 1989).***

When history or physical examination suggests Hirschsprung's disease or when constipation is persistent despite adherence to the treatment program, then anorectal manometry should be performed.

Barium enema:

It is unnecessary in uncomplicated cases of constipation. If the rectosphincter reflex is absent or atypical, the barium

enema and rectal biopsy need to be performed to evaluate for Hirschsprung's disease and other neuronal disorders. Barium enema is helpful in the assessment of Hirschsprung's disease in which a transition zone between aganglionic bowel and ganglionic bowel may be observed, in other neuronal disorders in which extensive bowel dilatation may be observed, and in the evaluation of the postsurgical patient operated on for anal atresia or Hirschsprung's disease ***(Loening-Baucke, 1996).***

Rectal biopsy:

Histologic and histochemical information is obtained through rectal biopsy. The presence or absence of ganglion cells can be evaluated from superficial suction rectal biopsy ***(Meier-Ruge, 1992).*** False-negative results are possible. Absence of ganglion cells with increased staining of nerve trunks with acetylcholinesterase stain is diagnostic for Hisrschsprung's disease.

Full thickness biopsies are necessary for the evaluation of other abnormalities of both the myenteric and submucosal plexuses, such as in hypoganglionosis ***(Ariel et al., 1985).***

MANAGEMENT

Treatment should be directed at the underlying cause whenever possible. For example, an anal fissure can be treated with topical application of an anesthetic ointment and attempts to soften the stool. Children with Hirschsprung's disease may require surgical correction. In recent years, a one-stage pull-through for surgical treatment of Hirschsprung's disease has been performed in the first months of life, with good medium-term results ***(Teitelbaum et al., 1997).*** A variety of treatment programs, including pharmacologic ***(Perkin, 1977)*** behavioral and medical ***(Wright and Walker, 1978).*** Dietary ***(Olness and Tobin, 1982),*** psychologic and surgical ***(Clayden and Lawson, 1976)***, as well as combinations of these, have been employed in the treatment of chronic constipation and encopresis.

The treatment of constipation with or without fecal soiling is comprehensive and has four phases, including education, disimpaction, prevention of reaccumulation of stools, and reconditioning of normal bowel habits ***(Navarro et al., 1990; Loening-Baucke, 1991).***

Phase (I): Education

The stooling problem is not caused by a disturbance in the psychological behaviour of the child and is not the parent's fault. Soiling occurs involuntarily and usually without the knowledge of the child. In most cases, a detailed plane eliminates parent's and children's frustration and improves compliance for the prolonged treatment that is necessary. Some parents do not possess the skills necessary to effectively manage their child's behaviour, specifically in relation to following a demanding regimen. These parents need to be identified so that educational efforts can be optimized. In addition, a coring relationship is established. Therapy should be appropriate for the severity of the constipation and the age of the child ***(Loening-Baucke, 1996).***

Phase (II): Disimpaction

For disimpaction, hypertonic phosphate enema can be used, with 30 ml/5kg body weight in young infants. An adult-sized enema (135 ml) can be used for children weighing more than 20 kg. In most children, one to two enemas result in good bowel clean-out. Hypernatremia, hyperphosphatemia, hypocalcemia, hypokalemia and dehydration have occurred in few children after hypertonic phosphate enemas ***(Forman et al., 1979).*** Normal (i.e. isotonic) saline enemas may be used but are often not effective. Cleansing soap-subs enemas should be avoided because they can result in bowel necrosis, perforation,

or death ***(Brandt, 1984).*** Tap-water enemas should be avoided because they can cause water intoxication, hypervolemia, dilution of serum electrolytes, seizures, or death ***(Ziskind and Gelliss, 1958).*** Children with megarectum or megacolon who do not respond to phosphate enemas can be disimpacted with a hyperosmolar milk-of-molasses enema (1:1 milk and molasses) with the infusion stopped when the child indicates discomfort (200 – 600 ml). The milk-of-molasses enema may need to be repeated. Lavage solutions given orally or by nasogastric tube until clear fluid is excreted through the anus can be used for disimpaction. An average of 12 L given over 23 hours at a rate of 14 ml to 40 ml/kg/hr is required for disimpaction ***(Ingebo and Heyman, 1988).*** Other physicians have used 750 ml to 1000 ml/hr of lavage solution for children more than 12 years of age until fecal effluent consisted of clean fluid. It is recommended to give metoclopramide 5mg to 10mg orally 15 minutes prior to the lavage solution to reduce nausea and vomiting ***(Koletzko et al., 1989).***

An efficient, comfortable and safe way for disimpaction using pulsed irrigation-enhanced evacuation is reported ***(Gilger et al., 1994).*** Five gallons of warm water were given more than 25 to 30 minutes as automated small pulses (25 ml) lasting a few seconds, alternating with gravity drainage of feces and water into a sealed bag. Although disimpaction was not complete after the procedure, all patients continued to empty their colon

afterwards. All patients were effectively disimpacted 12 hours later. For a child who vehemently fears enemas, the fecal mass can be softened and liquefied with large quantities of mineral oil or osmotic agents with the administration continued until the fecal mass is passed. Soiling increases dramatically, and abdominal pain and cramping may increase during oral disimpaction ***(Loening-Baucke, 1996).***

Phase (III): Prevention of reacumulation of stools

Adequate intake of fluid and fiber is essential in the prevention and treatment of constipation. During infancy, this intake can be assured by adding excess carbohydrates (corn syrup or brown sugar) and prune juice to the diet. In older child, large quantities of fluid, prune juice, bran crab and fruits and vegetables high in bulk are helpful ***(Alexander et al., 1996).*** Dietary fibers increase water retention and provide substrate for bacterial growth. It is beneficial for children with fecal incontinence who also have loose stool consistency.

Switching from cow's-milk formula to a formula containing whey, protein alleviates constipation in most infants. In most constipated patients, daily defecation is maintained by the daily administration of laxatives beginning on the evening of the clinic visit. Laxatives should be used according to age, body weight and severity of the constipation. Suggested dosages of commonly used laxatives are given in the next table.

Table (2): Dosage of commonly used laxatives

Laxative	Age	Dose
Malt soup extract	Infant	Breast-fed: 5 – 10 ml in 2-4 oz of water or fruit juice twice daily. Bottle-fed: 7.5-30 ml in day's total formula or 5-10 ml in every second feeding
Karo syrup	Infant	Dose is the same as that of malt soup extract
Milk of magnesia	> 6 months	1-3 ml/kg body weight/day divided in 1-2 doses
Mineral oil	> 6 months	Dose the same as that of milk of magnesia
Lactulose or sorbitol	> 6 months	Dose the same as that of milk of magnesia
Senna syrup	1-5 years	5 ml (with breakfast, maximum 5 ml twice daily)
	5 – 15 years	10 ml (with breakfast, maximum 10 ml twice daily)

(Adopted from Loening-Baucke, 1993)

There is no set dosage for any laxative. There is only a starting dosage for each child that must be adjusted to induce one to two bowel movements per day that are loose enough to ensure complete daily emptying of the lower bowel and prevent soiling and abdominal pain. Once an adequate dosage is established, it is continued for approximately 3 months to help the distended bowel to regain some of its function. Usually,

regular bowel habits are established by that time. Then, the dosage may be reduced in small decrements while maintaining a daily bowel movement without soiling. Laxatives need to be continued for several months and sometimes years at the right dosage to induce daily soft stools. For young infants, melt-soup extract or an osmotic laxative, such as karo syrup, can be added to the milk. The mechanism of action of milk of magnesia is the relative non absorption of magnesium and the resultant increase in the luminal osmolality. In severe constipation with rock-hard stools, the starting dosage of milk of magnesia is 2 to 3 ml/kg/day given with the evening meal. In children who have fecal retention of mostly soft-formed stools, usually 1 ml/kg daily is adequate.

Mineral oil: is converted into hydroxy fatty acids, which induce fluid and electrolyte accumulation. Dosage are similar to those for milk of magnesia ***(Wald et al., 1987).*** Mineral oil should never be force-fed or given to patients with dysphagia or vomiting because of the danger of aspiration pneumonia. Anal seepage of the mineral oil is an undesirable side effect, especially in children going to school. Long-term use of mineral oil has been reported to be safe ***(Clark et al., 1985).***

Lactulose: a non-absorbable carbohydrate, is hydrolyzed to acids by the colonic flora, causing increased water contents by the osmotic effects of lactulose and its metabolities. Sorbitol

(70%) is another non-absorbable carbohydrate that is inexpensive and easily taken by children when mixed in soft drinks.

Senna: has an effect on intestinal motility and on fluid and electrolyte transport and stimulates defecation. Senna is used when liquid stools produced by osmotic laxatives are retained; when the child refuses milk of magnesia, mineral oil and sorbitol and in children with fecal incontinence secondary to organic or anatomic causes ***(Clayden, 1992).***

Treatment options are medical therapy (e.g. stools softeners, laxatives, suppositories, phosphate enemas, or large-volume saline enemas), frequent manual removal of feces, avoidance of constipating diet or medication, behaviour modification, biofeedback training, or a combination of these ***(Loening-Baucke, 1996).***

Phase (IV): Reconditioning the child to normal bowel habits by regular toilet use

Toddler:

Toilet training attempts are discouraged in children fewer than 2.5 years of age who are resistant to potty sitting. These children can be put back into diapers or pull-ups. First, normal bowel patterns are accomplished and then toilet training can be started or restarted.

Older child:

Defecation trials are very important and are necessary in any treatment program. The child is encouraged to sit on the toilet for 3-5 minutes, three to four times a day following meals. The gastrocolic reflux, which goes into effect shortly after a meal, should be used to his or her advantage. The children and their parents need to be instructed to keep a daily record of bowel movements, fecal soiling, and medication use. This helps to monitor compliance and to make appropriate adjustments in the treatment program by parents or physicians. If necessary, positive reinforcement is given using star charts, little presents, television viewing, or computer game as rewards ***(Loening-Baucke, 1996).***

Psychological treatment:

Adherence to the treatment program improves constipation, encopresis or fecal incontinence in all children. The presence of coexisting behavioral problems often is associated with poor treatment outcome ***(Levine and Bakow, 1976)***. If the coexisting behaviour problem is secondary to chronic constipation, encopresis, or fecal incontinence, then it improves with treatment. Children who do not improve should be referred for further evaluation, because continued problems can be caused by non-compliance or control issues by children or parents. Psychological intervention, family counseling and occasional hospitalization of children for 2 to 4 weeks to get

treatment program started have helped some of these unfortunate children.

Biofeedback treatment:

Biofeedback can be defined as the use of monitoring instruments to detect and amplify selected physiologic processes in order to make previously unavailable physiologic information accessible to the subjects consciousness ***(Hauri, 1975).*** Biofeedback techniques enable individuals to learn voluntary control over autonomically regulated body function, although such control can also develop without the use of machines ***(Brown, 1974).***

Several anorectal abnormalities, including impaired rectal and sigmoid sensation, have been detected in children with chronic constipation and encopresis ***(Loening-Baucke, 1987).***

Impaired rectal sensation, higher maximal rectal compliance, or both were found in 97% of constipated children and were independent of rectal size ***(Meunier et al., 1984).***

An abnormality occurring in 25% to 53% of constipated patients is abnormal defecation dynamics, an abnormal contraction of the external anal sphincter and pelvic floor muscles during defecation ***(Wald et al., 1986).*** An abnormality observed in many children with fecal incontinence is weak anal sphincters. The external anal sphincter and pelvic floor consist

of striated muscles that are under voluntary control. Recently, biofeedback training has been used in the treatment of children with severe chronic constipation ***(Benninga et al., 1993).*** Biofeedback training teaches children to increase the intraabdominal pressure and to relax the external anal sphincter during defecation. These muscles are amenable to biofeedback treatment and can be trained to relax during defecation and to squeeze for prevention of incontinence ***(Loening-Baucke, 1991).***

Studies have reported positive of biofeedback training in constipated children, with success rates varying from 55% to 100% ***(Loening-Baucke, 1995).***

Follow-up visits:

Because the management of chronic constipation, encopresis and fecal incontinence requires considerable patience and effort on the part of the child and parents, it is important to provide necessary support and encouragement through frequent office visits. Parents are encouraged to call. Progress should be assessed by reviewing the stool records and repeating the abdominal and rectal examination to be sure that the problem is adequately managed. If necessary, dosage adjustment is made, and the child and parents are encouraged to continue with the regimen. After regular bowel habits are established, the medication dosage is gradually decreased to 2 dosage that maintains one bowel movement daily and prevent soiling. After

6 months, a further reduction or discontinuation of the medication is attempted ***(Loening-Baucke, 1996).***

Outcome evaluation:

Successful intervention may involve a cure or permanent cessation of constipation and soiling, a marked decline in the frequency of problems or satisfactory improvement requiring continuous or intermittent intervention (that is medicine, behaviour therapy). Excellent results have been reported in 45 to 100 percent of cases followed for periods ranging from 2 months to 3 years. Moderate improvement is reported in 20 – 30 percent, while failure is reported in 25 – 35 percent. Few long-term studies have been reported and it is difficult to track "cures" for long intervals or to understand the long-term outcomes of treatment factors ***(Terry and Hatch, 1988).***

Long-term follow up studies (6.9 ± 2.7 years) in 90 young constipated patients who were initially evaluated and treated before the age of 4 years revealed that 63% had recovered ***(Loening-Baucke, 1994).*** Twelve-month follow up studies in patients with constipation and encopresis who were more than 5 years of age treated at the encopresis clinic at the university of Iowa showed that approximately 50% of patients have discontinued laxatives and have at least three bowel movements per weak without soiling ***(Loening-Baucke, 1987).*** An additional 20% may be weaned off the laxative within 2 years.

The remainder requires laxatives for daily bowel movements or continues with soiling for many years and, in some cases, into adulthood. Fifty-one percent of patients in an Australian study had recovered 1 year after receiving laxatives and behaviour modification ***(Navarro et al., 1990).*** Clayden evaluated the duration of laxative treatment in more than 300 patients in Britain and showed that 22% of patients required regular laxative use for fewer than 6 months, 44% for fewer than 12 months, and 56% for more than 12 months. Stopping the laxatives too soon is the most common cause for relapse ***(Clayden, 1992).***

Prevention

Perhaps the best form of treatment is prevention. A unique opportunity is presented to the pediatrician in this regard. Serving as a co-ordinator of health and wellness supervision and allowed frequent access to the child and family from birth, the pediatrician can provide anticipatory counseling concerning optimal feeding practices, the interpretation of normal bowel function and the early detection of problems.

Education-before the fact:

To stop bowel problems before they occur, it is essential to understand their evolution and treatment. In particular, as part of general anticipatory guidance. Education of parents about the "transition points" in development can facilitate establishment

of normal bowel function. The pediatrician or primary care provider should be alert to possible misperceptions and discourage inappropriate interventions before they occur. Parents then can be protected from viewing their children as "abnormal" and launching an unneeded intervention that is likely to be counterproductive. For example, in the newborn period it is helpful to alert parents to an infant's reaction to normal bowel movements. Such a warning provides a framework for tolerating what appears to be an unusually discomforting experience in their infant. As with much counsel given by a physician, it is far more effective if provided before the occurrence than if offered after the event. Thus anticipating the important transition points in bowel function, just as anticipating developmental stages that can be particularly accident prone, can smooth the transitions and increase parental confidence and competence ***(Leonard et al., 1986)***.

Treating the cause-not the symptom:

The symptoms of constipation and encopresis should be interpreted as a final common pathway of multiple physical, emotional and interactional problems. If lasting benefit is to be obtained from treatment of the symptom, it will be necessary to understand the underlying problems and intervene appropriately, otherwise, symptoms improve only temporarily and the child and family confront therapeutic failure, which in turn increases anxiety and diminishes confidence in themselves and in the

medical system. For example, if a child's constipation results from a pain-retention-pain-cycle, short-term therapy directed only at increasing the number of bowel movements for a brief period will ensure almost certain failure. Instead, it is necessary to modify the experience of having bowel movements by rendering them painless long enough for the child to reverse previously acquired avoidance behaviors ***(Sondheimer and Gervaise, 1982).***

Altering the life-style:

Many children who have difficulties with constipation spend too little time in the bathroom, and even when therapy is instituted, the most challenging aspect involves inducing the child to preserve on a toilet. Optimal management involves establishing a consistent time for using the bathroom in the same way that children are encouraged to take time daily to brush their teeth. Reading or listening to a radio while trying to defecate may promote this.

Encouraging diets that fasten normal bowel habits:

Fiber is an important part of healthful eating, yet fiber is conspicuously lacking in most children's diet. These substances appear to be natural laxatives in that they increase the bulk of stools, shorten transition time and absorb water so as to soften stools ***(Wyman et al., 1976).***

Good sources include unprocessed wheat germ and bran. Fresh fruits and vegetables, dried fruits and whole-grained cereal and breads. Popcorn is a good source that has probably been underused in children's diet. For a family to use diet to change a child's bowel habits or to protect against bowel dysfunction, it is necessary for the entire family to alter its eating habits. An effective procedure would be to increase the amount of fiber in the family's diet gradually with palatable and testy foods. Clearly, children with abnormal bowel function would benefit from increasing their dietary fiber as an adjunct to any other therapy ***(Olness and Tobin, 1982).***

CARBOHYDRATE MALABSORPTION

Infants begin to ingest a disaccharide predominant diet from initiation of breast feeding which is replaced gradually by complex carbohydrate predominant diet in the latter half of the first year.

Physiology:

Carbohydrate digestion and absorption:

Digestion and absorption of carbohydrate occur as a series of steps in the lumen of alimentary canal, on the surface of its lining and within the apical membrane of absorptive cells ***(Heitlinger and Lebenthal, 1988).***

Digestion:

Starch is attacked by ptyalin, the α-amylase in the saliva. In the small intestine, the potent pancreatic α-amylase also act on the ingested polysaccharides. Both the salivary and the pancreatic α-amylase hydrolyze 1:4 α-linkages but spare 1:6 α-linkages, terminal 1:4 α-linkages and the 1:4 α-linkages next to branching points.

Lactase is found only at tip of the villus and is therefore exquisitely sensitive to mucosal injury ***(Alpers, 1987).***

Absorption:

The monosaccharides, are transported into the intestinal absorptive cells by two distinct mechanisms; active transport and facilitated diffusion.

Glucose and galactose are transported by sodium-dependent carrier-mediated active transport ***(Ckane, 1960).*** Once they enter the enterocyte, exist occurs via 2 sodium-independent facilitated diffusion mechanism.

Fructose enters the enterocyte via sodium-independent facilitated diffusion.

A high concentration of Na+ on the mucosal surface of the cells facilitates the transport of some sugars and a low concentration inhibits sugar influx into the epithelial cells. This is because glucose and Na+ share the same contransporter or symport. Since intracellular Na+ is low in intestinal and renal cells, as it is in other cells, Na+ moves into the cell along its concentrating gradient, glucose moves with the Na+ and is released in the cell ***(Ganong, 1993).***

The Na+ is transported into the lateral intercellular spaces, and the glucose is transported by glucose uptake and transporter (GLUT2) into the interstitium and then to the capillaries. The energy for glucose transport is provided indirectly, by the active transport of Na+ out of the cell ***(Ganong, 1993).***

Clinical features of carbohydrate malabsorption:

Malabsorbed carbohydrate acts as an osmotic laxative, water and electrolytes are drawn into the intestinal lumen. The carbohydrate is fermented by the microbial flora to short-chain fatty acids and hydrogen ***(Heitlinger and Lebenthal, 1988).***

The volatile fatty acids act as a cathartic, and the increased fluid and gas act to distend the lumen. The classic presentation is watery, acidic, explosive, fermentative diarrhea. Associated features may include: vomiting, abdominal distention, borborygmi, crampy abdominal pain, flatulence and failure to thrive. In infancy, an excoriated diaper area and signs of dehydration are common. Some children may have the associated features without diarrhea ***(Heitlinger and Lebenthal, 1988).***

Fate of unabsorbed carbohydrate:

The eventual products of carbohydrate fermentation by the colonic flora vary depending on the organisms involved. They include short-chain fatty acids and gases (CO_2, CH_4 and H_2) ***(Miller and Wolin, 1979).***

The formed short-chain fatty acids are efficiently absorbed from the colon accounting from more than 70% of energy of total carbohydrate ingested. This colonic function is called colonic salvage ***(Cummings, 1983).***

H_2 is a metabolic product of bacterial carbohydrate fermentation in the colon ***(Kotter et al., 1982).*** More than 99% of H_2 production occurs in the colon. H_2 diffuses into the portal circulation and is excreted in breath ***(Levitt and Bond, 1981).*** H_2 excretion in breath correlates significantly with total H_2 production ***(Levitt, 1969).*** Sleep produces a marked increase in breath H_2 concentration ***(Solomons and Viteri, 1976).***

Assessment of carbohydrate digestion and absorption:

Stool examination:

The simplest and first examination to be performed is stool pH. An acidic pH, that is < 5.5, is indicative of colonic fermentation of malabsorbed carbohydrate beyond the colonic capacity to conserve short-chain fatty acids ***(Heitlinger and Lebenthal, 1988).*** Estimation of stool carbohydrate content is somewhat more sensitive than stool pH.

Tolerance tests:

As a test of carbohydrate digestion and absorption, tolerance tests are performed to evaluate the rate of increase in serum glucose after a carbohydrate meal.

Breath H_2 test is an attractive technique for the assessment of carbohydrate malabsorption ***(Perman, 1991).*** The amount of H_2 in expired air is proportional to its intraluminal production,

and that precise quantitative of H_2 excretion permits an estimation of the amount of carbohydrate not absorbed in the small intestine ***(Bond and Levitt, 1976).*** This test has repeatedly been demonstrated to be the most accurate indirect indicator of lactose deficiency and carbohydrate malabsorption ***(Perman, 1991).***

Pulmonary H_2 excretion was used to quantitate the small bowel transit time in men. Excessively rapid small bowel transit is commonly considered to be a cause of malabsorption and diarrhea ***(Bond and Levitt, 1975).***

Duodenal intubation:

The most invasive tests. It should be performed in subjects who meet the following criteria:

* Persistent symptoms despite removal of the offending carbohydrate.
* Suspicion of congenital deficit of digestion or absorption or celiac disease or another enteropathy not expected to resolve by removal of the malabsorbed carbohydrate.
* Basal or secretin stimulated fluid that is obtained should be assayed for pH, bicarbonate, pancreatic enzymes, fluid should be stained for giardia and cryptosporidia.
* Intestinal biopsies should be obtained for histologic examination and disaccharidase assay ***(Heitlinger and Lebenthal, 1988).***

Table (3): Assessment of carbohydrate digestion and absorption

Stool examinations:

- pH
- Reducing substances

Tolerance tests:

- Serum glucose
- Breath H_2

Duodenal intubation:

- Pancreatic amylase
- Mucosal morphology
- Disaccharidases determination

(Heitlinger and Lebenthal, 1988)

LIPID MALABSORPTION

Lipid absorption disorders can be categorized into the following classification; (1) defective lipolysis including cystic fibrosis and other pancreatic disorders, (2) possible defective lipolysis due to colipase deficiency and enterokinase deficiency, (3) impaired stabilization as in bile salt disorders, (4) reduced functioning surface area of the small intestine, (5) impaired exit of chylomicrons from the enterocytes, and (6) impaired lymphatic transport ***(Muller, 1982).***

The fat malabsorption was first accurately measured by chemical assessment of fecal fat excretion.

Microscopic assessment of the stools by Sudan stain was negative in 85% of normal persons. The results in cases of steatorrhea were highly positive with more than 100 fat droplets per high power field with the droplet diameter varying from 6 to 75 μm ***(Drummey et al., 1961).***

Later study showed the capacity of Sudan stain to specify the triglycerides and fatty acids in the feces. The cholesterol and phospholipids although they are measured during quantitative fecal fat determinations by Van de Karmer method, yet they are not measured by the Sudan stain. This indicates that Sudan stain is more superior to quantitative fecal assessment, in determining the exact source of the fatty acids ***(Khouri, 1989).***

The application of stable isotopes in pediatric nutrition and gastroenterology was studied. Compounds labeled with ^{13}C enabled quantitative measurements of nutrient malabsorption. The use of labeled trioctanion or other triglycerides would be of particular advantage in the pediatric population because of the simplicity of tests based on $^{13}CO_2$ measurements of respiratory CO_2 ***(Klein and Klein, 1985).***

It was found that ^{13}C labeled triolein is superior to palmitic and trioctanion in the detection of fat malabsorption with sensitivity of 100% and 85% specificity. Pancreatic insufficiency could be differentiated from mucosal disease or bile salt deficiency by an abnormal triolein or trioctanion breath test or both but a normal palmitic acid breath test ***(Watkins et al., 1982).***

The determination of serum triglycerides before and 120 minutes after a test meal, containing 0.5 g/kg body weight butter and 0.5 g/kg body weight sunflower oil is useful for the diagnosis of intestinal malabsorption in children. The serum turbidity test proved less accurate than serum triglycerides test in identifying fat malabsorption and was found to be of poor clinical significance ***(Caprini et al., 1982).***

A more sensitive assessment than two point serum triglycerides test is the fatty meal test. A fatty meal containing

25 gms of margarine and 25 gms of butter fat is given orally, the rise of serum triglycerides and chylomicrons was measured hourly for 5 hours. Serum triglycerides rise of less than 100 mg/dL or than 100% above the basal values and the appearance of less than 7% of chylomicrons were considered pathological ***(Goldstein et al., 1983).***

In one study, 3 tests were compared, namely; serum optical density, serum triglycerides concentration and $^{14}CO_2$ breath test after the administration of a 60 gm fat meal containing 10 μCi glycerol tri[1-^{14}C] oleate. The results of these tests were compared to quantitative fecal fat excretion. It was found that optical density and triglycerides tests were not greatly helpful. In contrast, eight-hour cumulative $^{14}CO_2$ breath excretion provided good diagnostic aid with 7% false positive and no false negative results. This raises the possibility of breath tests as alternatives to quantitative fecal fat excretion ***(West et al., 1981).***

However, ***King et al. (1982)*** documented that ^{14}C triolein test is both inadequately sensitive and specific for routine clinical use.

The fecal fat concentration was used to differentiate between steatorrhea of pancreatic origin and that of non-pancreatic origin. The pancreatic cause is diagnosed if fecal fat

concentration was found > 9.5% provided fecal daily fat was found > 21 g/day ***(Feurle, 1985).***

It was shown that the use of very simple method based on microcentrifugation is helpful to predict the fat content and caloric value of milk ***(Lucas et al., 1978).***

This method was first tried to detect the stool fat content in newborn and hence called steatocrit. The steatocrit volumetrically relates the stool fat column to the stool solid column, which are of very different densities and are thus separated in the capillary centrifuge tubes ***(Phuapradit et al., 1981).***

Later the steatocrit test was used for monitoring fat malabsorption in patients with cystic fibrosis. It was found both of great diagnostic and prognostic help ***(Colombo et al., 1987).*** It was retested again in assessment of fat malabsorption in cystic fibrosis by ***Montalto et al. (1988)*** and ***Iacono et al. (1988).***

In a more recent study, the normal range of steatocrit values during the first 3 months of life were established. These were also correlated with fecal fat content measured quantitatively. The influence of different alimentary regimens on fecal fat excretion was studied as well. They recommended the test as being satisfactory for screening for malabsorption and for frequent monitoring of steatorrhea ***(Iacono et al., 1990).***

PROTEIN MALABSORPTION

Before dietary protein or endogenous protein of the alimentary tract can be assimilated, it must be hydrolyzed by intraluminal digestive processes into small peptides and free amino acids. In normal subjects, 60 – 80% of 1 gm of radioiodinated human serum albumin are absorbed in the proximal jejunum ***(Nixon and Mawer, 1970).***

In general, over 50% of each amino acid is present in peptide form in the intestinal aspirates. Almost all acidic amino acids and proline are present as peptides ***(Chung et al., 1979).***

The peptide transport in the intestine is claimed to be Na+ independent, carrier-mediated ***(Ganapathy and Leibach, 1982).*** The carrier-mediated uptake process consists mainly or exclusively of one transport system of broad specificity for dipeptides and tripeptides. Tetrapeptides, with exceptions, are apparently not transported intact ***(Silk, 1981).***

The soluble peptidases responsible for the intracellular hydrolysis of transported peptides are a complex mixture of various enzymes. They include a dipeptidase ("Master dipeptidase) which is able to hydrolyse many dipeptides with the exception of those with the C-terminal proline and some with N-terminal proline or basic amino acids. This enzyme has neither

tripeptidase nor aminoacyl-B-naphthylamide hydrolase activities ***(Noren et al., 1977).***

A second peptidase is the aminoacyl-proline hydrolase (prolidase) which can hydrolyse dipeptides with C-terminal proline or hydroxyproline ***(Sjostrom et al., 1973).***

A third peptidase is the amino tripeptidase. It can hydrolyse tripeptides only except tripeptides with either charged N-terminal residue (e.g., lysine or glutamic acid) or a proline residue in the second position ***(Doumeng and Maroux, 1979).*** Other cystosol enzymes have been identified in the small intestine ***(Auricchio, 1981).***

The second mechanism for the hydrolysis of dipeptides and tripeptides is brush border hydrolysis followed by the absorption of the released amino acids or dipeptides. The tetra- and higher peptides are hydrolyzed in vitro in the brush border membrane. The brush border enzymes include amino peptidase, dipeptidyl-aminopeptidase; carboxypeptidase and endpeptidase. These can hydrolyse resistant bonds to pancreatic and cytosol peptides ***(Auricchio et al., 1981).***

Assessment of fecal protein loss in diarrhea:

Early studies utilize radioactive chromium chloride in the diagnosis of protein losing enteropathy and malabsorption ***(Walker-Smith et al., 1967).***

Then, ***Crossly and Elliot (1977)*** introduced the demonstration of α-1 antitrypsin in the stool as simple method for diagnosis of protein losing enteropathies. This test was considered as a reliable test for enteric protein loss in children. It was concluded that fecal α-1 antitrypsin can provide a valid estimate of enteric protein loss ***(Hill et al., 1981).***

Later this protein was tested in random fecal samples and was found statistically correlating with the degree of protein loss in the stool ***(Dinari et al., 1984).***

The effect of infant feeding on the fecal α-1 antitrypsin was studied, and it was found that the variability in the results is not easily explained by the type of feeding alone because of significant overlap between the different groups' results and between the normal person and the results of cases with protein losing enteropathy in this study ***(Woodruf et al., 1985).***

In a recent study, it was found that significant quantities of intact human milk α-1 antitrypsin were excreted in the stools of breast milk fed babies. The amount excreted was higher in earlier weeks of age and decreased with infant age till 3-4

months. It was concluded that α-1 antitrypsin could not be used as a marker of intestinal protein loss in the breast fed babies in early infancy ***(Davidson and Lonnerdal, 1990).***

The α-1 antitrypsin was used as a measure of enteric protein loss in patients with necrotizing enterocolitis ***(Shulman et al., 1989).***

Fecal α-1 antitrypsin was measured in cases of chronic inflammatory bowel diseases and it was found to be increased compared to normal content. Yet there was wide range of overlap between results in cases with active and inactive groups. Fecal α-1 antitrypsin was compared with fecal excretion of ^{111}Indium labeled granulocytes in 27 patients with chronic inflammatory bowel disease but there was no correlation. This indicates that protein loss from the intestine is not necessarily inflammatory in origin ***(Fischbach et al., 1987).***

Fecal α-1 antitrypsin was found to be markedly increased in cases of persistent diarrhea associated with enteric pathogens ***(Bhan et al., 1989).***

Measurement of α-1 antitrypsin on non dried stools was found reliable and simple method of assessment of protein malabsorption ***(Catassi et al., 1986).***

THE GUT BARRIER

Introduction:

The intestine is routinely exposed to a limitless variety of macromolecules derived from many sources, including resident bacteria, ingested food, invading viruses, etc. Because of their size, macromolecules can act as antigens- some of which are harmful to the host while others pose no threat. The intestine must deal with this diversity. To do this, a unique immune system exists at mucosal surfaces, which is distinct from the systemic immune system. An important mechanism by which the intestine surveys antigens in the intestinal lumen is by allowing small quantities to cross the epithelium and interact with the mucosal and systemic immune systems. However, excessive or inappropriate exposure of antigens to the intestinal immune system may lead to gastrointestinal disease ***(Sanderson and Walker, 1993).***

Two important intestinal diseases of childhood underscore the notion that it is the molecular structure of macromolecules that is critical to the pathogenesis of some immunologically mediated gastrointestinal diseases. In celiac disease, the appearance of an enteropathy sensitive to gluten is independent on the integrity of the grain protein. Partial digestion by papain of any of the gluten-related family of macromolecules will abrogate its activity. The second disease entity, cow's milk sensitive

enteropathy, responds to a diet in which casein or whey milk protein has been hydrolyzed commercially. Thus, the pathogenic nature of these macromolecules resides within their antigenic structure ***(Ian et al., 1995).***

It is possible that a limited exposure to antigens (which constitutes a normal mechanism of surveying the contents of the intestinal lumen) may at times lead to damage of the barriers to transport, allowing chronic immune and inflammatory responses to develop, as may occur in inflammatory bowel disease and allergic gastroenteropathy. Thus we must consider how uptake is limited so that immune reactions do not work adversely in the host. Furthermore, oral tolerance (the phenomenon whereby prior exposure to an antigen by the enteric route induces a specific immunological unresponsiveness on subsequent systemic exposure to the same antigen) may depend, in part, on the pathway(s) of antigen uptake and the manner in which luminal antigens are handled by the gut. For example, if a luminal antigen is taken up inappropriately, the result may provide the basis for autoimmune states ***(Targan et al., 1987).***

Some mechanisms of macromolecular uptake are specific and constitute a physiologic process by which the molecules can perform their beneficial function. Other mechanisms are nonspecific and may constitute a potentially damaging result that could cause disease.

Physiologic transport:

While the majority of macromolecules necessary for normal body functions are synthesized *de novo*, there are some essential macromolecules that are taken up by the intestinal epithelium from the lumen of the bowel. Physiologic transfer of macromolecules is particularly important during infancy and childhood when organ development is incomplete. Specific transepithelial mechanisms have evolved to facilitate the uptake of a number of proteins, including growth factors and immunoglobulins. Growth factors are present in breast milk including nerve growth factor (NGF), epidermal growth factor (EGF), and transforming growth factor-α (TGF-α) ***(Koldovsky et al., 1991).***

Some factors are important in the growth and differentiation of the intestine and must therefore interact with enterocytes directly, whereas other factors are involved in the development of organ systems outside the intestine, in which case they must not only be taken up by the intestine but must also be transported into the circulation. In the former, the intestine is the target organ, whereas in the latter it acts as a conduit. This is achieved in many cases by binding of luminal factors to specific receptors that can shuttle them across the intestine without intracellular hydrolysis. Receptors are also involved in the transport of immunoglobulins across the

intestine. Since endogenous immunoglobulin G (IgG) concentrations are below protective levels in young infants, protection depends on passive transfer of maternal antibodies. In many animals, this is achieved by receptor-mediated transport across the intestine ***(Simister and Rees, 1985).***

Surveillance of antigens in the gastrointestinal tract involves their uptake by the intestinal mucosa. Lymphoid elements juxtaposed to the intestinal epithelial surface and in specialized aggregates or follicles (Peyer's patches) constitute the mucosal immune system. A normal immune response depends on the B cell recognition of antigen; thus intraluminal and enterocyte destruction of antigenic structure will reduce its antigenicity. The immune response also depends on T cell recognition of peptides that are processed from antigen. Both types of lymphocytes are located adjacent to the intestinal epithelium. The immune responses of the normal mucosa are varied. The mucosal immune system is capable of producing secretory IgA (S-IgA) specific to antigens in the lumen. It is also able to inhibit responsiveness by the systemic immune system (tolerance) to orally ingested antigens. Oral tolerance has been shown in a range of animal species ***(Peng et al., 1990; Strobel, 1990).***

It is possible that this phenomenon plays a role in preventing food allergy and autoimmune states ***(Higgins and Weiner, 1988).***

Many diseases are related to the ingestion of food antigens. Some are quick reactions usually mediated by an IgE response such as urticaria, vomiting, and, most severely, systemic anaphylaxis. Food-sensitive enteropathies tend to be slow to develop and are related to cell-mediated immune response ***(Walker-Smith, 1988).***

Such responses are normally prevented by means of oral tolerance, although the mechanism of this response is still incompletely understood. It is clear, however, that antigen must cross the epithelium in order for the mucosal immune system to function normally in limiting infectious disease and preventing food allergy. Intact antigen is capable of crossing the epithelium through specialized epithelial cells [microfold cells (M cells); follicular epithelial cells], which have characteristics that make them effective in transporting macromolecules. Whether this is the only physiologic pathway of macromolecular entry that does not involve specific membrane receptors is not yet clear. It is possible, however, that some antigens are absorbed by enterocytes not associated with lymphoid follicles and presented to T cells, leading to alterations in the immune response of the intestine. Although there is no *in vivo* evidence yet that

enterocytes can present antigen to T cells, experiments with epithelial cells isolated from the intestine indicate that T cells do recognize antigen that has been taken up and processed by eneterocytes ***(Kaiserlian et al., 1989).***

Receptor-mediated uptake of growth factors:

The growth and differentiation of the small intestine depend on exposure to an array of growth factors that have complementary actions on intestinal epithelial cells. Some factors are synthesized by enterocytes themselves (autocrine), some are delivered from the circulation, and some enter the epithelium directly from the gastrointestinal tract ***(Carpenter and Wahl, 1991).***

Epidermal growth factor found in human milk and saliva, can cross the intestinal epithelium. EGF is a peptide consisting of 53 amino acids that has trophic effects on both adult and neonatal intestine.

Macromolecules are transferred by a mechanism that is altogether different from those that transport nutrients such as glucose and amino acids. Nutrient molecules enter the intestinal cell cytoplasm at the apical membrane and exit via the basolateral membrane. Growth factor macromolecules, on the other hand, transverse the cell in membrane-bound compartments that invaginate from the apical membrane

(endocytosis). The first step in this process is attachment to receptors on the apical surface of enterocytes. Studies of EGF binding to microvillous membranes and isolated enterocytes show that intestinal cells have receptors that are specific for EGF. Other growth factors may use the EGF receptor (i.e., TGF-α) or use their own receptor (i.e., IGF-1).

The mechanisms involved in the transit of membrane-bound ligands from the apical to the basolateral surface of the enterocyte are still poorly understood. In electron microscopic studies, the apical membrane of absorptive cells can be seen invaginating to form endosomes. Further transit into the cell may occur by the movement of separated vesicles ***(Simons and Wandinger-Ness, 1990)..***

Antibody uptake:

The newborn makes very little immunoglobulin and most circulating antibody is IgG-derived passively from the mother. For the most part, in humans IgG is transferred by the placenta during late gestation, whereas in many animals the transfer occurs from maternal milk through the proximal small intestine. The transfer of IgG across the gut is mediated by receptors that bind to the Fc portion of the immunoglobulin molecule ***(Simister and Mostov, 1989).***

Fc receptors are able to cross the epithelial cells. This transcytosis occurs in both directions. Receptors are carried in membranes that traffic from lumen to serosa and return by other membrane transport mechanisms. Some membrane proteins contain specific amino acid sequences that direct the protein within epithelial cells, that is, the polymeric IgA receptor that transports the IgA from the basolateral membrane to the apical membrane. However, the amino acid sequences that determine the movement of the apical IgG Fc receptor, have not yet been elucidated. Transfer of maternal IgG in the neonatal rodent falls markedly at 21 days of age, that is, at weaning (a phenomenon known as closure). This phenomenon is now known to be due to the decrease in the expression of the Fc receptor gene, and it is likely that factors in breast milk may affect Fc receptor gene expression ***(Casanova et al., 1991)***.

Non-receptor Transport:

Cells specialized for macromolecular transport, membranous epithelial cells (M cells)

The generation of secretory immune responses by the intestinal mucosa depends on transfer of antigens across the epithelium. Any loss of the molecular structure of the antibody recognition sites, the epitopes, on antigens during transport would render them unrecognizable by B cells. The passage of intact macromolecules across the gut is at variance with the role of the gut as a macromolecular barrier. In order for

macromolecules to cross the gut in a controlled manner, specialized epithelial cells have evolved that overlay lymphoid follicles. These M cells have few microvilli on their surface and correspondingly little of the glycocalyx that typifies enterocytes. There is also less mucus covering the cell surface. In addition, lysosomal enzymic activity within the cell is reduced. Thus, these components of normal barrier function are less well developed in M cells than in other epithelial cells. Furthermore, there is a deep invagination of their basal membrane into which cells of the immune system can intrude. This invagination is separated by only a narrow band of cytoplasm from the apical membrane. Thus lymphocytes and macrophages can position themselves close to the intestinal lumen ***(Amerongen et al., 1992).***

Amerongen et al. (1992) have reviewed the microorganisms and other macromolecules that are known to be transported by M cells. M cells also transport luminal antigen from the gut and therefore represent the primary physiologic route for nonreceptor transport of macromolecules. This has been shown by electron microscopy using antigens including ferritin and horseradish peroxidase. Soluble macromolecules are incorporated into membrane-bound compartments, transferred across the cell, and extruded from the serosal surface into the interstitium containing lymphoid cells.

An important but as yet unanswered question is whether specific receptors exist on the surface of M cells, which aid the transport of macromolecules across the epithelium. Some infectious agents, including reovirus ***(Wolf et al., 1981)*** and Escherichia coli (strain RDEC-1) ***(Inman and Cantey, 1983),*** bind selectively to M cells. The question of receptors is made more complex by the fact that different agents are taken up in different ways by M cells. For example, poliovirus ***(Sicinski et al., 1990)*** is taken into clathrin-coated pits by endocytosis, whereas reovirus is taken up in vesicles that do not contain clathrin ***(Wolf et al., 1981).***

Enterocytes as antigen-presenting cells

Intact antigens or antigen fragments traverse the M cell and may encounter immunoglobulin in solution or on B cell surfaces as part of an immune response. Effective immune responses to antigenic proteins also require the help of T lymphocytes. Stimulation of T cells in turn depends on exogenous antigen being presented by antigen-presenting cells (APCs). The APCs must internalize, digest, and link a small fragment of the antigen to a surface gylcoprotein- the major histocompatibility complex (MHC) class II or HLA-D in humans – that interacts with a T cell receptor. Various cells of the immune system can act as APCs, including B cells, macrophages, and dendritic cells. The ability of these cells to

present exogenous antigen depends on the expression of MHC class on their surface ***(Unanue, 1984).***

Pathologic transport:

Controlled uptake of macromolecules from the intestine is important in delivering growth factors and immunoglobulins to the circulation. It also enables the mucosal immune system to sample antigen in the lumen. Thus, physiologic transport is dependent on specific mechanisms that control macromolecular entry. However, if macromolecules are taken up non specifically, these regulatory mechanisms could be circumvented. In this case, antigens could cross the epithelium in excessive amounts. Such transport may well set up immune reactions that are not limited to the local immune response. These reactions, in the face of unrestricted antigen entry, may become widespread and thus ultimately cause disease in the gastrointestinal tract or other organ systems.

Non specific transfer can occur by two pathways. First, vesicular traffic moving across the cell will transport molecules that have adhered to receptors on the surface membrane. Second, junctions between cells, which normally act as a barrier, could loosen and become leaky. These non specific pathways become more permeable when the intestine receives an insult or during its developmental stages, thus making chronic gastrointestinal disease more likely at these times.

Mucosal barrier to antigens:

Antigens only gain access to the surface of the intestine after passing a number of mechanisms that act as a barrier. This barrier consists of some components that are under immunological control and others that are non specific. Breakdown in any of these components could result in an increase in the non specific passage of antigen into the intestine. Thus the integrity of these mechanisms is necessary to prevent disease caused by excessive uptake of antigens. Antigen absorption is limited by a number of non immunological factors that operate in the gastrointestinal tract. These include gastric acidity, proteolytic digestion, mucus secretion, and peristalsis. These mechanisms have been reviewed extensively and will not be described in detail ***(Sanderson and Walker, 1993).***

Intracellular transport:

Direct evidence of non specific macromolecular transport through vesicular compartments of enterocytes has been demonstrated in ultrastructural studies of horseradish peroxidase (HRP) in mature intestine ***(Cornell et al., 1971).***

Macromolecules can be taken up by the enterocytes without the involvement of specific receptors. This can occur in two ways. Molecules can bind to the apical membrane in a non specific manner and then be taken up by endocytosis; conversely, molecules in solution close the invaginating

membrane will be engulfed by the developing vesicle. Macromolecules are more adherent to the surface of immature cells than to mature cells and may adhere preferentially because of their structure or charge. This means that when the contents of the lumen have easy access to immature enterocytes, macromolecules will adhere readily. Immature cells are found on the surface of the intestine in the young and when the life span of the enterocyte is reduced, as happens in many enteropathies, including viral gastroenteritis in which villous enterocytes are preferentially destroyed and replaced by increased numbers of immature crypt enterocytes ***(Pang et al., 1983)***.

Various macromolecules, including bovine serum albumin and HRP, are transported more readily in young animals. The passage of these macromolecules falls markedly with age and this is considered another form of "closure". Closure therefore encompasses more than one mechanism. It was originally applied to the cessation of passage of immunoglobulins across the intestine, which is now known to be due to reduction in expression of the Fc receptor gene, but the term, closure also includes cessation of enhanced non specific transfer. A similar, but more subtle, decrease in the transport of antigens is seen in the human newborn ***(Roberton et al., 1982).*** Formula-fed preterm neonates have higher serum concentrations of β-lactoglobulin than term neonates ***(Axelsson et al., 1989).***

Table (4): Enteropathies where the ratio of immature to mature cells increases on the intestinal surface

Celiac disease	Autoimmune enteropathy
Post-enteritis enteropathy	Radiation enteritis
Allergic enteropathy	

(Pang et al., 1983)

Paracellular transport :

Transepithelial transport can occur by the paracellular route as well as through cells. There has been a significant change in our perception of the importance of this route in recent years. It has been appreciated that water, sodium, potassium, and chloride can pass between cells, but there is now evidence that large solutes can, under certain circumstances, penetrate at this site. The rate-limiting barrier to diffusion is the tight junction, which in healthy intestine prevents passage of large macromolecules such as HRP. The structure of the tight junction (***Madara, 1990)*** in freeze-fracture preparations consists of strands that pass between cells. The composition of these strands is not known, but they are likely to be proteins of high tensile strength. It is the number of strands that determines the ionic resistance of an epithelial monolayer. ***Pappinheimer and Reiss (1987)*** have calculated that the rate of uptake from the lumen of molecules smaller than 5500 daltons was proportional to the rate of fluid absorption – a concept known as solvent drag. This gives an effective pore size of 5 nm at the tight junction

(Madara, 1990). Sodium-dependent solute, such as glucose and amino acids, induces expansion of intercellular spaces associated with condensation of microfilaments of the actinomycin ring associated with the tight junction. While these observations have enormous importance on the physiology of absorption of nutrients, their impact on our understanding of macromolecular transport has yet to be fully assessed. The calculated pore radius of the open tight junction (5 nm) is similar to that of small macromolecules; glucose-sodium transport will in fact allow the passage of polypeptides 11 amino acids long (MP-1) ***(Atisook and Madare, 1991),*** but larger immunogenic proteins may not pass through this route under physiologic conditions. HRP, for example, does not pass the tight junctions ***(Atisook and Madara, 1991)*** even when they have been rendered permeable to MP-1.

On the other hand, pathologic insults to the intestine may open these pores sufficiently to allow passage of antigens. The permeability of the gut to macromolecules in disease models needs to be reexamined using Pappenheimer's methodology. Macromolecular markers of different sizes, charge and hydrophilicity have all been used independently *in vivo* in both animals and humans, but the physical characteristics of these molecules have not been used to predict pore size in disease. There is no doubt that uptake of antigens is increased in a number of diseases of the small intestine.

Table (5): Insults that increase macromolecular permeability of the intestine

Intestinal disorders	*Systemic insults*	*Drugs*
Gastrointestinal food allergy	Excessive radiation	Non steroidal
Celiac disease	Extensive burns	anti-inflammatory
Acute gastroenteritis	Septicemic shock	drugs
Chronic intestinal infections	Hypovolemic shock	
Inflammatory bowel disease	Malnutrition	
Surgery		

(Adopted from Atisook and Madara (1991)

EVALUATION OF GUT BARRIER

Measurement of intestinal permeability:

Measuring intestinal permeability in humans requires a probe molecule that should be inert, water soluble, non toxic, non degradable by intestinal bacteria, and non metabolized after absorption. Its transport through the intestinal mucosa should decrease with increased molecular size and follow first-order kinetics. The probe should be measurable with sensitivity, accuracy, and ease in biological fluids such as urine ***(Lifshitz, 1985).***

The different probe molecules used to measure intestinal permeability are xylose, lactulose, erythritol, mannitol, inulin, raffinose, cellobiose, polyethylene glycols (PEGs) of different molecular weights, urea, uric acid, creatinine, and [^{51}Cr] ethylene diamine tetra acetic acid ([^{51}Cr]EDTA) ***(Lifshitz, 1985).***

Inert sugars:

Intestinal permeability tests based on inert sugars usually combine a monosaccharide and an oligosaccharide, which have different permeation characteristics. Their use in combination is becoming more widespread since it allows the principle of differential absorption to be exploited ***(Ford and Walker Smith, 1986).***

Non absorbable monosaccharides are rhamnose [molecular weight (MW) 164] and mannitol (MW182). The first disaccharide suggested as a permeability marker was lactulose (MW342), and with cellobiose (MW342) this is now the most frequently used marked ***(Wheeler et al., 1978; Menzies, 1974).***

The urine collection is usually done over a 5-hour period, although a steady-state method with a single urine collection has been developed, as well as shorter collection periods ***(Nathavitharana et al., 1988).*** Urinary sugar measurements have been made by both quantitative and gas chromatography, recently improved by avoiding the use of ion exchange resins ***(Hamilton et al., 1987)*** and by high-performance liquid chromatography (HPLC) ***(Nathavitharana et al., 1988).***

Polyethylene glycol (PEG)

Since the first description by ***Chadwick et al. (1977),*** the measurement of intestinal permeability with PEG has been based on the use of fractions of different MW (400, 1000, 4000 daltons) to take advantage of the differential absorption principle. Its use in allergic children was described by ***Falth-Magnusson et al. (1984).***

[^{51}Cr]EDTA

[^{51}CR]EDTA (MW357) was introduced more recently as another test substance for detecting alterations in intestinal

permeability ***(Bjarnason et al., 1983)*** and has been used to demonstrate intestinal permeability alterations in gastroenteritis ***(Forget et al., 1985),*** celiac disease, Crohn's disease and cystic fibrosis. Among the several drawbacks of this technique, however, one can underline (a) the use of only probe molecule, which eliminates the advantage of the differential absorption principle; (b) the need to ingest a radioisotope, making the test unsuitable for routine use in children, although the irradiation provided to the body by one test does not exceed that encountered with natural exposure during 1 year ***(Bjarnason et al., 1983),*** and (c) the fact that the [^{51}Cr]EDTA test requires an accurate 24-hour collection, which is not practical for routine use with children.

Principle of differential absorption:

Differential absorption involves the simultaneous use of two or more probe molecules, assuming that they behave similarly in all respects except in their permeation across the mucosa ***(Ford et al., 1985).*** Therefore expressing the urinary excretion of the markers as a ratio may overcome the effects of the many non relevant variables that may influence the individual markers, such as the adequacy of the oral load ingested, the gastric emptying time, the intestinal transit time, dilution of the marker by intestinal secretions, renal clearance, and the completeness of the urine collection.

Lactulose-Mannitol test:

On the morning an overnight fast of at least 6 hours and after voiding and discarding overnight urine, subjects are given a 1.001 mOsm/liter aqueous solution of mannitol and lactulose at a dosage of 0.1 g/kg of body weight of each sugar. During the following 5 hours, all urine including that passed at the end of the 5 hours is collected and the lactulose and mannitol contents are analyzed by gas chromatography. Intestinal permeability tests are done twice. During the fasting intestinal permeability test, the solution of markers is given alone, and subjects are allowed only glucose water during the third, fourth and fifth hours of urine collection. On the following day, a provocation intestinal permeability test is carried out under similar conditions, the solution of markers being given mixed with 100 ml of breast milk or of the formula to be tested. The ratio of the urinary concentrations of oral lactulose and mannitol (L/M ratio, %) is determined.

Epithelial structure and intestinal permeability:

Sugars

The monosaccharides rhamnose and mannitol are thought to pass through aqueous pores in the enterocyte membrane, with a 0.4 nm radius for mannitol ***(Fromter and Diamnod, 1972).*** In contrast, the larger sugars lactulose and cellobiose permeate across the intestinal mucosa either through larger pores, with a 0.52 nm radius, or more predominantly via the intercellular

spaces and at extrusion zones at the villous tips. In patients with villous atrophy related to untreated celiac disease, reduction in mannitol recovery should result from a reduced surface area and number of pores available for diffusion, whereas the increased recovery of larger molecules might be via epithelial discontinuities such as altered tight junctions and cell extrusion zones ***(Strobel et al., 1984).*** The increase in passage of mannitol through paracellular pathways might remain quantitatively limited in comparison with the decrease related to the modifications of surface area ***(Dawson et al., 1988)*** so that during villous atrophy the overall result is a decrease of the urinary recovery of mannitol.

The relation between morphometry of small intestinal biopsy samples and permeability for sugars has been explored by ***Ford et al. (1985),*** using a computer-based image analysis of tracings of the histological sections. There was a strong correlation between crypt depth and the lactulose / rhamnose urinary ratios as well as a negative correlation between the villous height and the same ratio. A positive correlation was also demonstrated between the lactulose/ mannitol ratio and the villous height / mucosal thickness ratio ***(Ukabam and Cooper, 1985).***

The passage of luminal mannitol, however, is still a controversial issue. ***Pappenheimer (1990)*** assumes, on an

experimental basis, that in the presence of glucose, mannitol (together with glucose, amino acids, and other small nutrients) is absorbed passively by solvent drag between absorptive cells. However, the rates of absorption of nutrients by this mechanism are simply proportional to their concentration or to the rate of fluid absorption, which contradicts the recent demonstration of a different effect of the antidiarrheal drug diosmectite on the passage of lactulose and mannitol.

On the other hand, for ***Ferraris et al. (1990),*** the role of solvent drag becomes significant only in extreme conditions and carrier-mediated uptake rates are adequate to explain absorption of glucose and therefore of similar sized solutes. In the latter hypothesis, the passage of mannitol most probably reflects diffusion through epithelial cells, thereby reflecting the absorptive capabilities of the epithelial surface.

Diffusion through the unstirred water layer lining the epithelial border also appears as a rate-limiting step in the uptake of all rapidly absorbed compounds. Thus unstirred water layer thickness, in addition to epithelial transport, would be a major variable influencing the absorption rate of solutes like mannitol. Although speculative, it seems possible that the recent demonstration of improvement of mannitol absorption with the mucus-stabilizing agent diosmectite might result from a reduction of the unstirred water layer thickness ***(Dupont et al., 1992).***

Important modifications of intestinal permeability may be observed even in the absence of frank morphologic abnormalities of the intestinal mucosa. In the study by ***Strobel et al. (1984),*** these abnormalities of intestinal permeability without morphologic alterations of the intestinal mucosa were shown to be associated with an increased intraepithelial lymphocyte count, a finding further documented by ***Hodges et al. (1989).***

Juby et al. (1987) compared the results of a cellobiose/mannitol test and those of a computer-assisted measurement of intestinal mucosal perimeter and lamina propria surface area. A first group of patients showed villous atrophy and abnormal intestinal permeability, a second group had normal permeability and morphology, and a third group had normal mucosae together with abnormal permeability. In the latter group, the perimeter/lamina propria index was significantly reduced compared to the second group. In addition, considering all patients, a significant correlation was shown between the perimeter/lamina propria surface index and the cellobiose/mannitol ratio. This study was important since it showed that the intestinal permeability test was able to detect subtle modifications of intestinal mucosa, which jejunal biopsy with conventional histology cannot reveal.

Polyethylene Glycol

PEG has considerable lipid solubility, so that the absorption characteristics of PEG 400 are similar to those of monosaccharides, for example, rhamnose (MW164), rather than to those of oligosaccharides of similar MW, such as lactulose (MW342) ***(Maxton et al., 1986).***

This is probably the reason why during villous atrophy the permeation of PEG 400 decreases ***(Lifshitz et al., 1986)***, as does that of mannitol or rhamnose, while that of lactulose and cellobiose is increased ***(Cobden et al., 1978).***

It is most likely that PEG polymers cross the intestinal mucosa by two different mechanisms. PEGs with a small MW could pass through aqueous channels in the cell membrane, whereas larger molecules could cross through the paracellular pathways. One of the weaknesses of the PEG test is that a decrease in the transcellular passage could be masked by an increase in the paracellular passage. Expressing the results therefore requires sophisticated mathematical models, which impairs both the reliability of the test and its ease of use in clinical practice.

Non digestive factors modifying intestinal permeability:

A correlation of the lactulose / mannitol (L/M) ratio with body surface area has been shown ***(Nathavitharana et al.,***

1988), almost all studies are done using a standard sugar dose for all body sizes and the authors suggest that the body surface area related dose they used had the opposite effect to that intended- that is, to eliminate any body size effect. They also concluded that the ratio of sugars ingested and the gut permeability to the sugars are not the only factors influencing the excreted sugar ratio: the absorption kinetics of the two sugars might change differently with dose quantity or volume or with the size of the subject.

A urine collection period of 3 hours was shown to produce a better discrimination between patients with normal mucosa and those with severely damaged mucosa than the more conventional 5-hour collection ***(Nathavitharana et al., 1988).*** Such an abbreviated test period and consequent reduction in the period of fasting could be particularly advantageous in children.

Markers may be given in isotonic or hypertonic solution. Hypertonicity is not physiologic but increases the absorption of cellobiose and of lactulose to a greater degree across an inflamed mucosa than across a normal one ***(Wheeler et al., 1978).*** Hypertonicity has no effect on mannitol absorption, whereas when given in an isotonic solution, mannitol reduces the absorption of disaccharides. The reason for using a hypertonic solution is to increase discrimination between patients with mucosal alterations and normal subjects ***(Hamilton***

et al., 1987): the sensitivity of a lactulose / mannitol test was 33% during villous atrophy in a study performed with an isotonic solution, 274 mOsm/liter, whereas a cellbiose test using a hyperosmolar solution (1500 mOsm/liter) had 100% sensitivity for the same kind of mucosal lesions. Raising the osmolarity to 1000 mOsm/liter in our experience ***(Dupont et al., 1989)*** or to 1500 mOsm/liter in others ***(Hamilton et al., 1987, Hodges et al., 1989)*** never induced any diarrheal side effects.

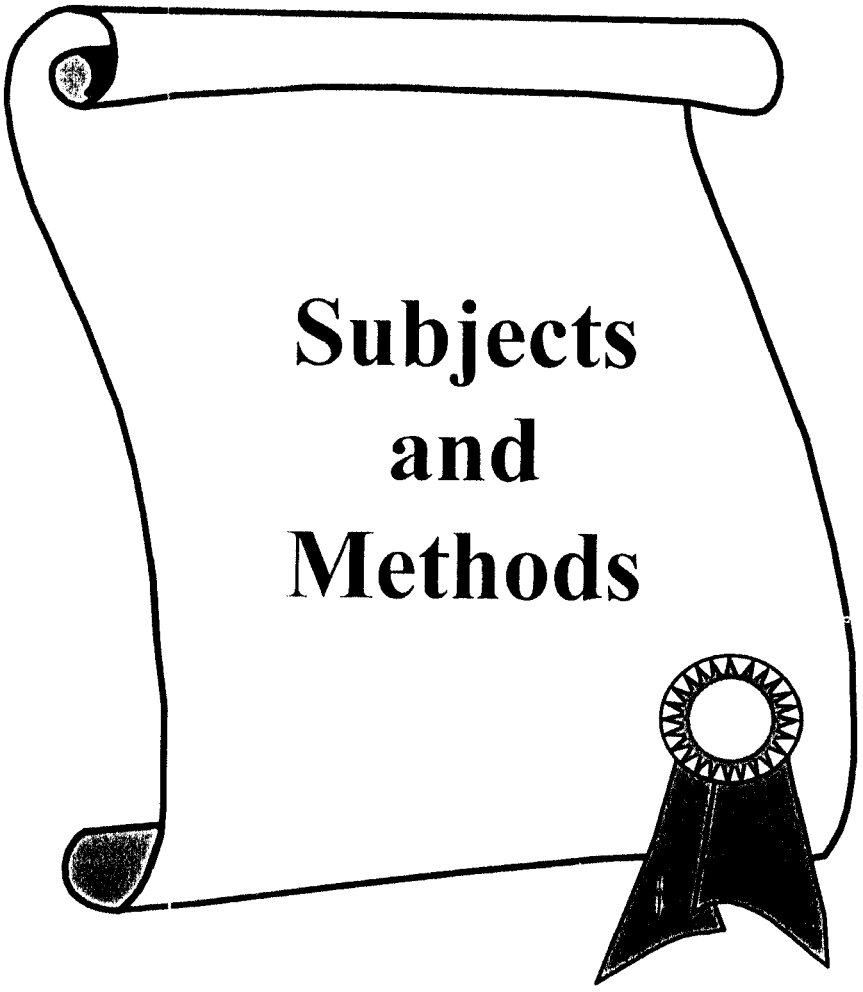
A physiologic increase in the passage of lactulose may occur during the first 2 weeks of life. A non specific modification of intestinal permeability may also occur in response to iron deficiency ***(Hodges et al., 1989),*** nonsteroidal anti-inflammatory agents, or methotrexate treatment.

Effects of probiotics on gut mucosal barrier

Although many clinical benefits have been ascribed to consumption of candidate probiotic strains in gastrointestinal disorders, few studies are available on their effects on the gut defense mechanisms.

To determine the effect of probiotics on gut immune response and antigen transfer, a suckling rat model was used ***(Isolauri et al., 1993).*** Before weaning, the rats were daily gavaged with cow's milk, Lactobacillus GG with milk, or water. The transport rates of horseradish peroxidase (HRP), a macromolecular tracer with a structure corresponding to that of

dietary antigens, across patch-free jejunal segments and segments containing Peyer's patches were studied at the time when gut closure normally occurs. High intact HRP absorption was detected in the group gavaged with milk in comparison with the group gavaged with milk and probiotic strain or with controls. There was a trend to increased proportional transport of HRP across Peyer's patches in the group gavaged with milk and the probiotic strain, and the specific antibody-secreting cell response against dietary β-lactoglobulin was significantly increased, compared to the group given milk or the control group gavaged with water.


Subjects
and
Methods

SUBJECTS AND METHODS

This study was carried out on 56 children with idiopathic chronic constipation, selected among those attending the Pediatric Gastroenterology Clinic, Children's Hospital, Ain Shams University. They were recruited in the period between June 1999 and December 2000. They were 34 males and 22 females. Their ages ranged between 4 years and 9 years with a mean of 5.90 ± 1.18 years.

Enrollment criteria:

1) Constipation more than 3 months duration.
2) Motions less than 3 times per week.

Exclusion criteria:

1) Cases with defined underlying surgical or medical cause of constipation.
2) Cases that recovered within the last 2 weeks.

A cohort of 56 healthy children were chosen as a control group.

All subjects were subjected to the following:

1) Medical history taking with special emphasis on different gastrointestinal symptoms and characters of constipation.

2) Clinical examination including general, abdominal and anthropometric assessment, including measurement of weight, height and their centiles.
3) ***Laboratory investigations including:***
   a) Stool analysis.
   b) Fecal α_1 antitrypsin as a measure of protein malabsorption.
   c) Fecal fat by semiquantitative steatocrite method.
   d) Lactose absorption status by measurement of breath hydrogen after a lactose load using the Quintron apparatus.
   e) Assessment of mannitol, lactulose and L/M ratio by capillary gas chromatography as a measure of gut mucosal permeability.

The techniques of the laboratory procedure:

1) Fecal α_1 antitrypsin:

Samples:

Random samples were taken and stored at –20°C till time of analysis.

Sample preparation:

Aliquot of 1 gm stool was diluted 1 : 10 with 0.9% saline solution. The sample was then homogenized by centrifugation at 2300 rpm for 15 minutes and the supernatant was separated.

Idea:

Measurements by low concentration immuno-diffusion plates.

Method:

20 µl samples of supernatant were placed into the wells of the plates. The precipitation rings were measured after 3 days. The results were assessed using a reference curve constructed through a standard serum –1 AT per gram wet weight of stools.

2) Fecal fat by steatocrite test:

I. Add ½ gm fresh stools + 2½ ml distilled water + 0.06 gms fine sand → homogenisation.
II. Hematocrit tube is filled and sealed with wax at one end.
III. Centrifuge for 15 minutes at 13,000 rpm in hematocrit centrifuge.
IV. Then, allow to stand vertically.
V. 2 layers are always formed ± 3rd layer:
    a. Basal solid (S).
    b. Intermediate liquid ±
    c. Upper layer fatty in nature (F).

$$\text{Steatocrit value} = \frac{F}{F + S} \times 100$$

It is positive if > 2%.

3) Lactose Breath hydrogen test:

a) 0.2 gm of lactose was given diluted in water to the studied cases.
b) Breath samples were collected using the standard syringe and mask method 4 hours after fasting then every ¼ hour after the lactose load till 4 hours.

c) Readings were considered positive if it was more than 20 p.p.m.

Precautions followed before and during the test included absence of antibiotics use two days before the test as well as avoidance of exposure to smoking 12 hours before and during the test.

4) Urinary mannitol and lactulose determination by capillary gas chromatography:

The test was done at least 24 hours after finishing the lactose breath hydrogen test.

Sampling:

5 gms of lactulose and 1 gm of mannitol were given to all patients and controls after 2 hours fasting and fasting was prolonged 2 hours after marker intake. Urine was collected for 5 hours. Thymol was used as preservative and urine was kept frozen (-20°C) until analysis.

Sample preparation:

The pH of the urine was measured using pH meter and adjusted to pH of 5 – 7 with 1 mol/L HCl, if necessary. Then urine was centrifuged for 10 minutes at room temperature.

Technique:

Portions (2 µl) were automatically injected into a model 5880 gas chromatograph with flame ionization detection, equipped with a model 7672A autosampler (both from Hewlett Packard Co., Amstelveen, the Netherlands) and interfaced with a Nelson analytical 3000 data system (Capertine, USA).

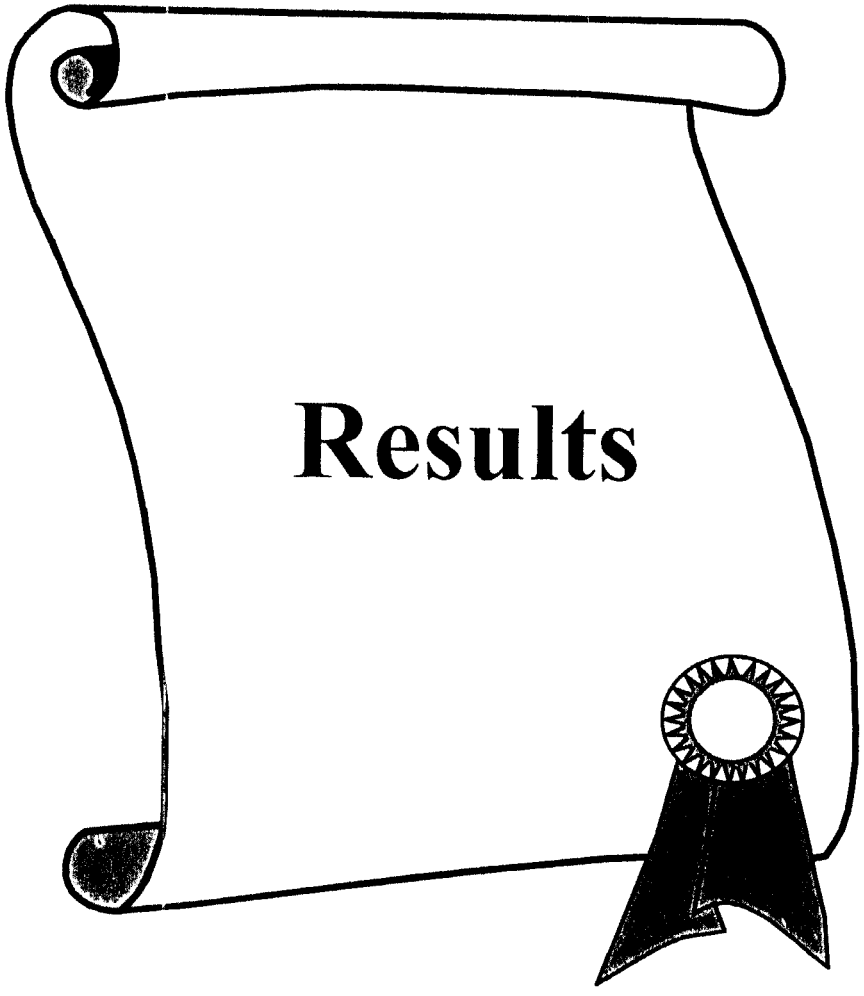
The column was a 25 m x 0.2 mm capillary fused silica coated with a cross-linked methyl silicon (film thickness 0.11 µm). Gas flow rate was 0.6 ml/min., split ratio 1:15, detector temperature 300 °C and injector temperature 280°C. The oven temperature program was: start at 120°C, increase by 3°C/minute to 265°C, then increase with 10 °C/minute to 295 °C and hold for 5 minutes at 295°C.

Principles of prepurification:

Stability of trimethylsilyl ethers towards hydrolysis is much greater than that of trimethyl esters or trimethylsilylated amines. This implies that, provided no inhibitory effect of inorganic salts are experienced, trimethylsilylation of dried biological samples, followed by selective hydrolysis of trimethylesters and trimethylsilylated amines with water and dilute hydrochloric acid, should leave the trimethylsilyl ether derivatives of sugars and polyols intact. These apolar derivatives can subsequently be extracted into hexane. One drop of bis (trimethyl silyl) trifluoroacetamide was added to the hexane layer

after extraction to refurnish a potentially lost trimethylsilyl group to the anomeric hydroxyl group. Using this prepurification, the isolation of neutral sugars and polyols became simple and fast.

The levels were measured as mannitol or lactulose mmol/mol creatinine to avoid fallacious effects of variation of renal functions.


Results

RESULTS

Table (6): Basic statistics of quantitative parameters among controls

Parameter		Mean	Standard deviation	Minimum	Maximum
Age (years)		5.94	1.18	4.00	9.00
Fecal fat (%)		2.08	0.84	1.20	3.40
Fecal α_1 antitrypsin (mg/dl wet stool)		0.26	0.08	0.12	0.35
Lactose Breath Hydrogen (PPM)	Fasting	7.61	2.55	2.00	14.00
	15\'	11.68	3.34	5.00	18.00
	30\'	8.68	3.18	4.00	12.00
	45\'	11.88	3.84	4.00	18.00
	60\'	12.52	10.31	4.00	61.00
	120\'	11.70	3.43	3.00	18.00
	180\'	9.43	3.12	4.00	12.00
	240\'	11.30	3.39	3.00	18.00
Mannitol (mmol/L)		24.96	2.02	21.9	27.8
Lactulose (mmol/L)		0.25	0.014	0.22	0.28
Lactulose / mannitol		0.01	0.001	0.008	0.012

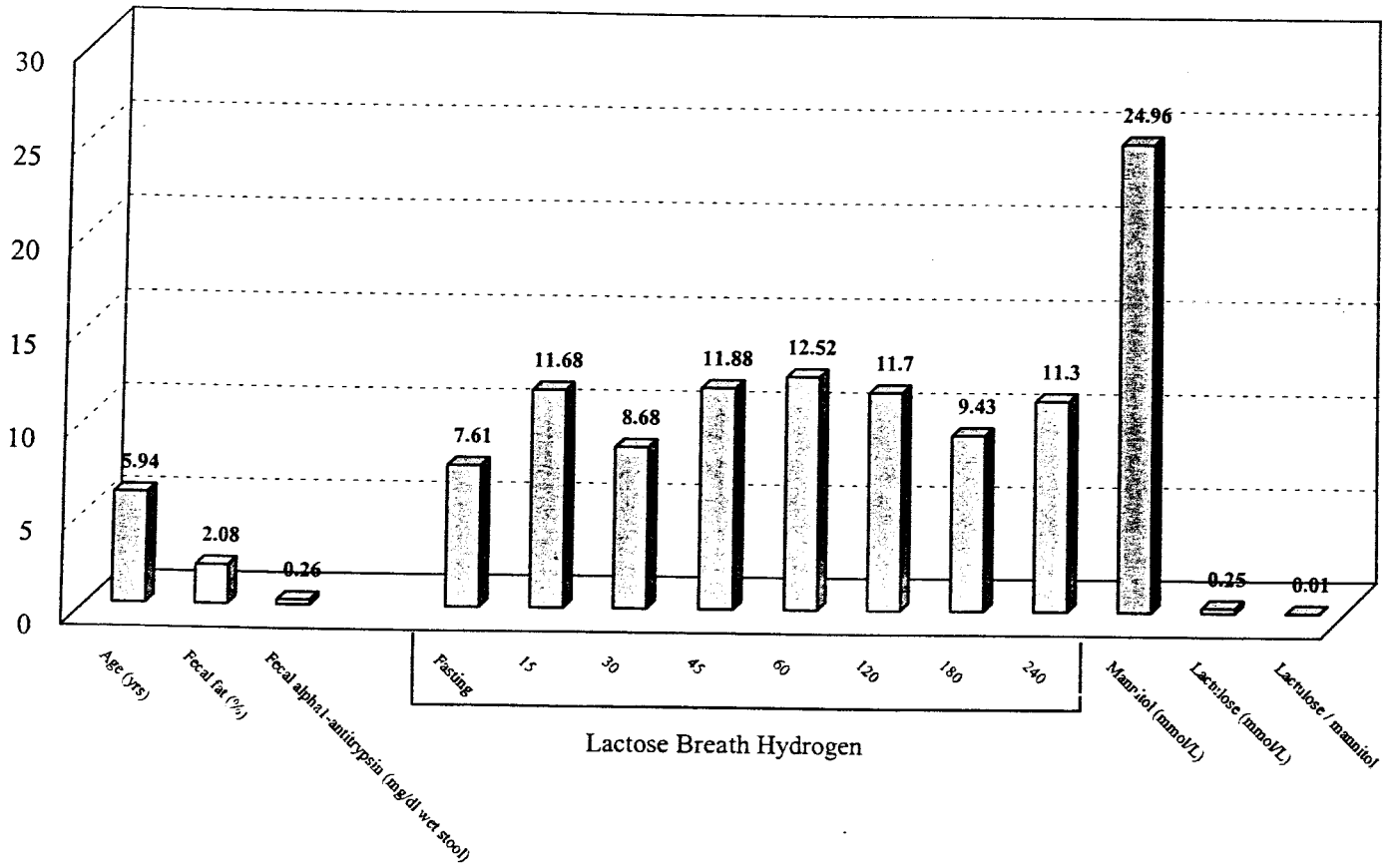


Fig. (1): Basic statistics of quantitative parameters among controls

Table (7): Basic statistics of quantitative parameters among patients

Parameter		Mean	Standard deviation	Minimum	Maximum
Age (years)		5.47	1.58	4.00	9.00
Age of onset (years)		2.09	0.35	1.00	3.00
Duration of constipation (years)		3.36	1.37	2.00	6.00
Frequency of motions (motions/w)		2.20	0.98	1.00	3.00
Treatment-duration (months)		11.36	6.23	6.00	24.00
Weight (kg)		18.77	4.37	13.00	26.00
Height (cm)		106.27	11.18	95.00	133.0
Body mass index (BMI)		16.3	1.8	11.3	19.1
Fecal fat (%)		6.76	5.68	1.20	24.00
Fecal α_1 antitrypsin (mg/dl. wet stool)		3.14	3.83	0.11	11.20
Lactose Breath Hydrogen (PPM)	Fasting	6.93	1.77	4.00	11.00
	15'	11.64	3.35	5.00	18.00
	30'	8.80	3.32	4.00	12.00
	45'	12.52	5.19	4.00	38.00
	60'	16.43	14.40	4.00	61.00
	120'	11.86	3.22	8.00	18.00
	180'	9.59	3.08	4.00	12.00
	240'	11.77	4.28	4.00	18.00
Mannitol (mmol/L)		18.19	0.33	17.8	18.6
Lactulose (mmol/L)		0.354	0.03	0.31	0.38
Lactulose / mannitol		0.02	0.002	0.016	0.021

BMI: Body mass index

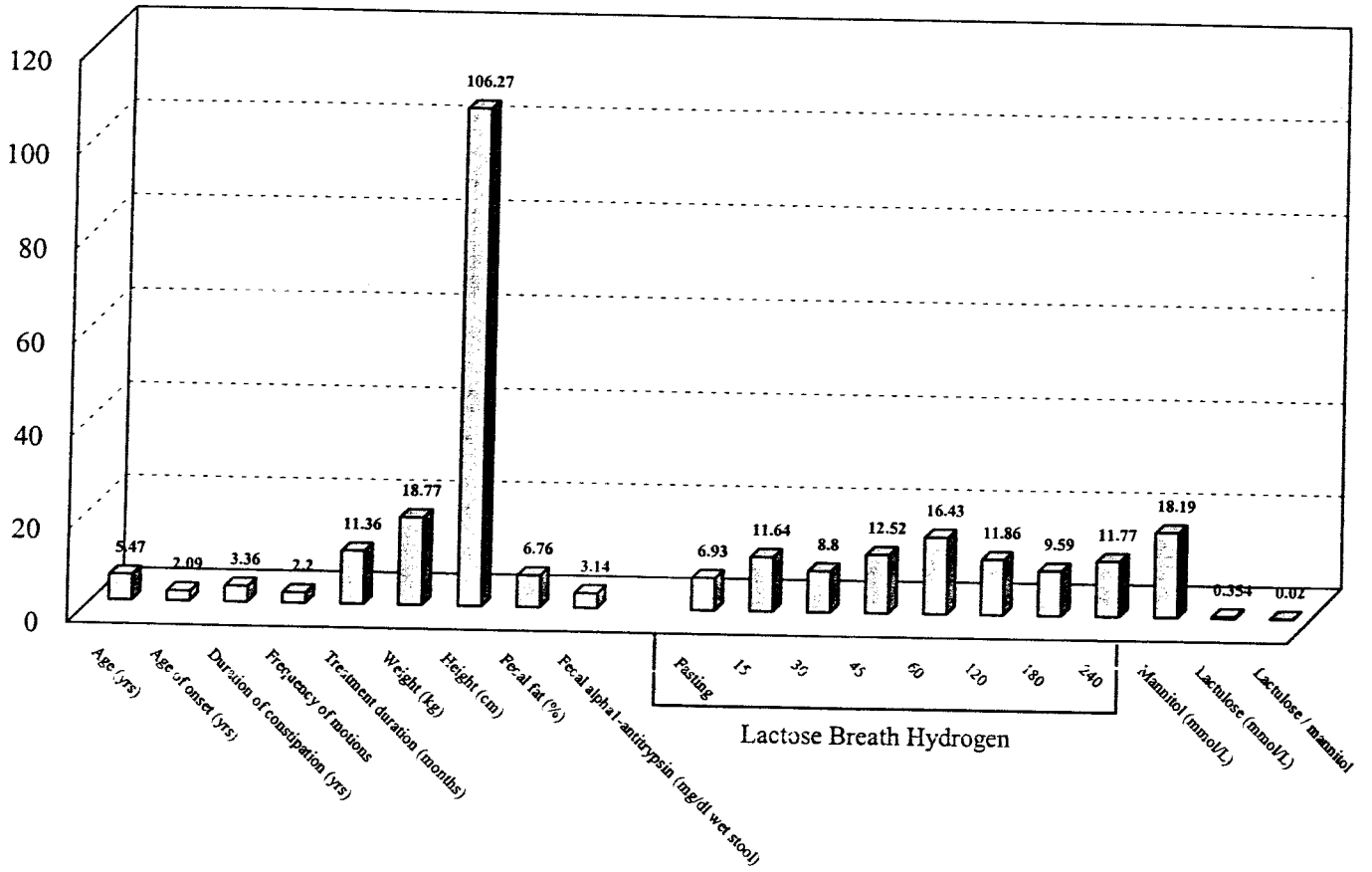


Fig. (2): Basic statistics of quantitative parameters among patients

Table (8): Basic qualitative parameters among controls

Parameter	Frequency	Percentage (%)
Male	31	55.36
Female	25	44.64
Lactose malabsorption	4	7.14

Table (9): Basic qualitative parameters of patients

Parameter	Frequency	Percentage (%)
Males	34	60.71
Females	22	39.29
Socio-economic state		
Low	33	58.93
Average	1	01.79
High	22	39.29
Straining	47	83.93
Pain during defecation	43	76.79
Blood in stool	23	41.07
Fecal soiling	23	41.07
Obsessive toilet training	33	58.93
Social or psychological problems in the family	9	16.07
Treatment		
Lactulose	53	94.64
Sodium picosulfate	3	05.36
Wheezy chest	3	5.36
Anorexia	33	58.93
Vomiting	23	41.07
Urinary tract infection	20	35.71
Dysuria	3	5.36
Anal inflammation	10	17.86
Anal fissure	21	37.50
Giardia lamblia in stool	43	76.79
Entameba histolytica in stool	9	16.07
Lactose malabsorption	13	23.21

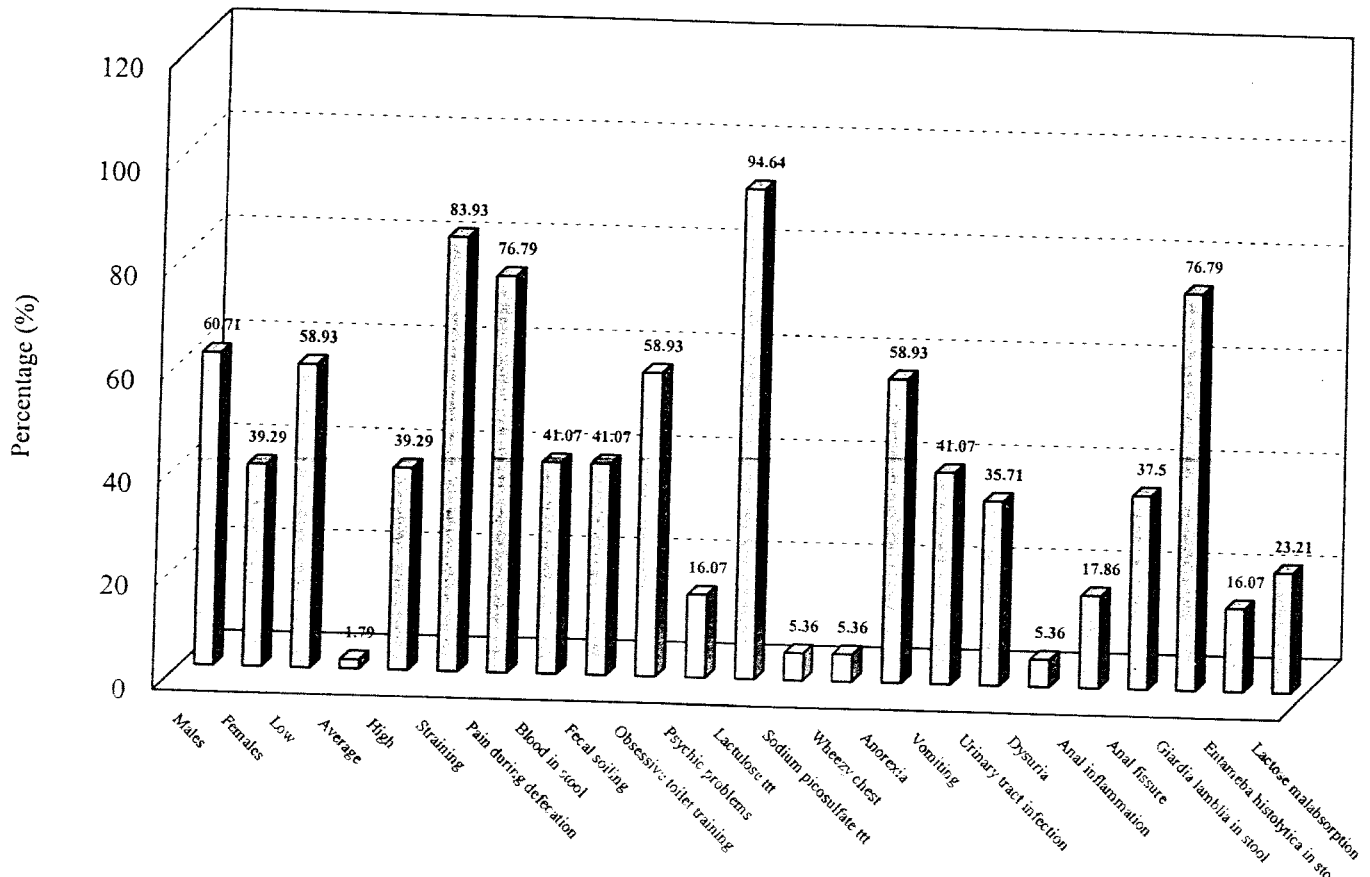


Fig. (3): Basic qualitative parameters of patients

- Table (9) and fig. (3) show that males are predominant (60.71%) in the studied cases.
- Straining and painful defecation are commonly encountered (83.93% and 76.79 respectively).
- Bleeding per rectum, encopresis (soiling) and anal fissure (41.07%, 41.07% and 37.3% respectively) are not uncommon among our series of constipated children.
- Anorexia and vomiting are commonly found among our patients (58.93 % and 41.07 % respectively).
- Obsessive toilet training was common among our cases (58.93%). However, psychological problems are uncommon (16.07%).
- The table also shows that urinary tract infection is not uncommon (35.71%) but recurrent attacks of wheezy chest are rare finding (5.36%).
- Stool analysis demonstrates a common prevalence of giardia lamblia (76.79%) but entameba histolytica is rarely found (5.36% and 16.07% respectively).
- Lastly, the table demonstrates that 23.21% of the patients are lactose malabsorpers .

Table (10): Comparison between mean values of different studied parameters between patients and controls

Parameter		Control	Patient	t	p
Age (years)		5.94 ±1.18	5.47 ± 1.58	1.03	>0.05
Fecel fat (%)		2.08 ± 84	6.67 ± 5.68	6.10	<0.001
Fecal α_1 antitrypsin (mg/dl. wet stool)		0.26 ± 0.08	3.14 ± 3.83	5.63	<0.001
Lactose Breath Hydrogen (PPM)	Fasting	7.61 ± 2.55	6.93 ± 1.77	1.64	>0.05
	15\	11.68 ± 3.34	11.64 ± 3.35	0.06	>0.05
	30\	8.68 ± 3.18	8.80 ± 3.32	0.20	>0.05
	45\	11.88 ± 3.84	12.52 ± 5.19	0.74	>0.05
	60\	12.52 ± 10.31	16.43 ± 14.40	1.65	>0.05
	120\	11.70 ± 3.43	11.86 ± 3.22	0.26	>0.05
	180\	9.43 ± 3.12	9.59 ± 3.08	0.27	>0.05
	240\	11.30 ± 4.39	11.77 ± 4.28	0.57	>0.05
Mannitol (mmol/L)		24.96 ± 2.02	18.19 ± 0.33	24.09	< 0.001
Lactulose (mmol/L)		0.25 ± 0.014	0.35 ± 0.027	30.17	<0.001
Lactulose / mannitol		0.01 ± 0.001	0.02 ± 0.002	40.29	< 0.001

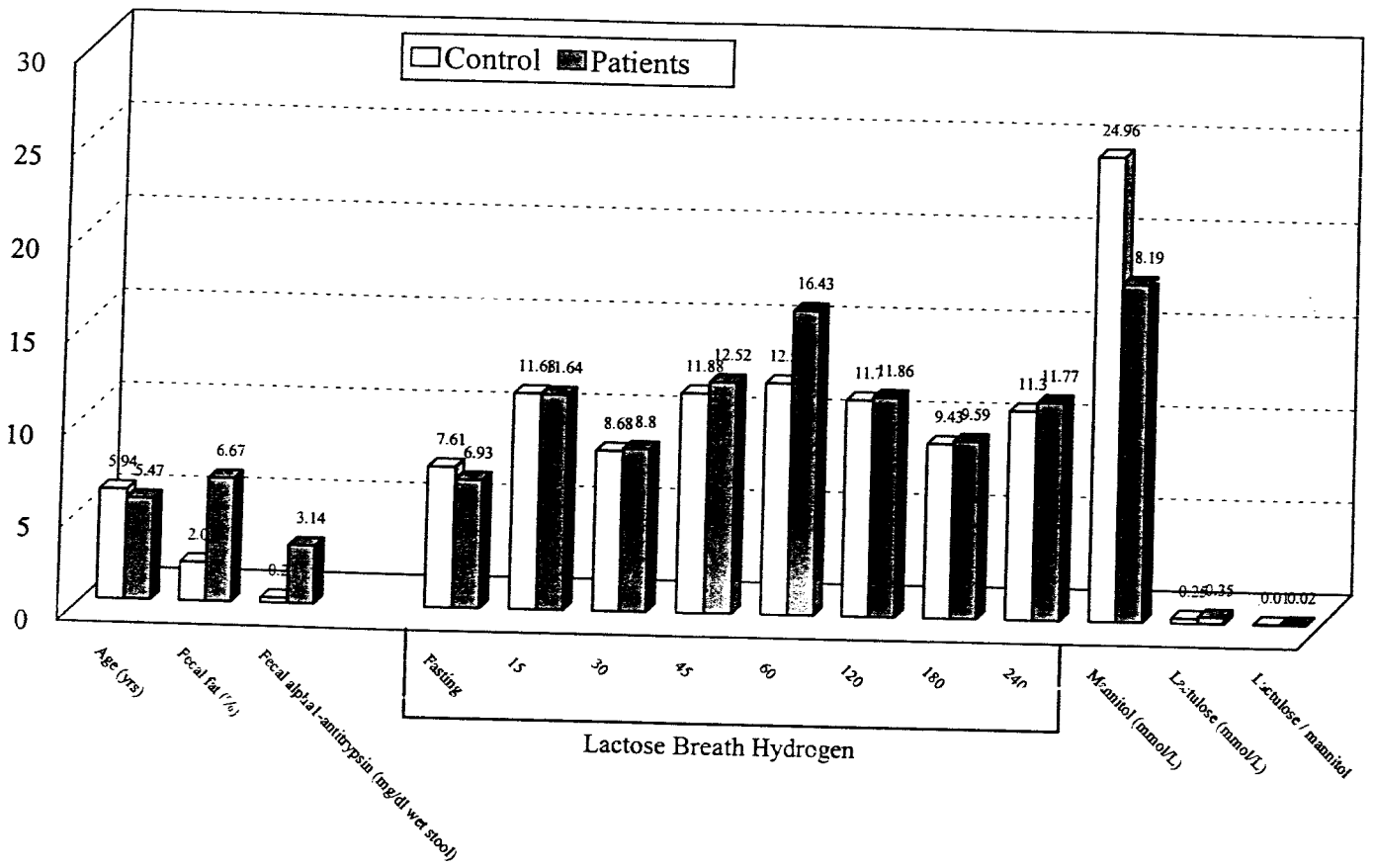


Fig. (4a): Comparisonof the mean values of different studied parameters between patients and controls

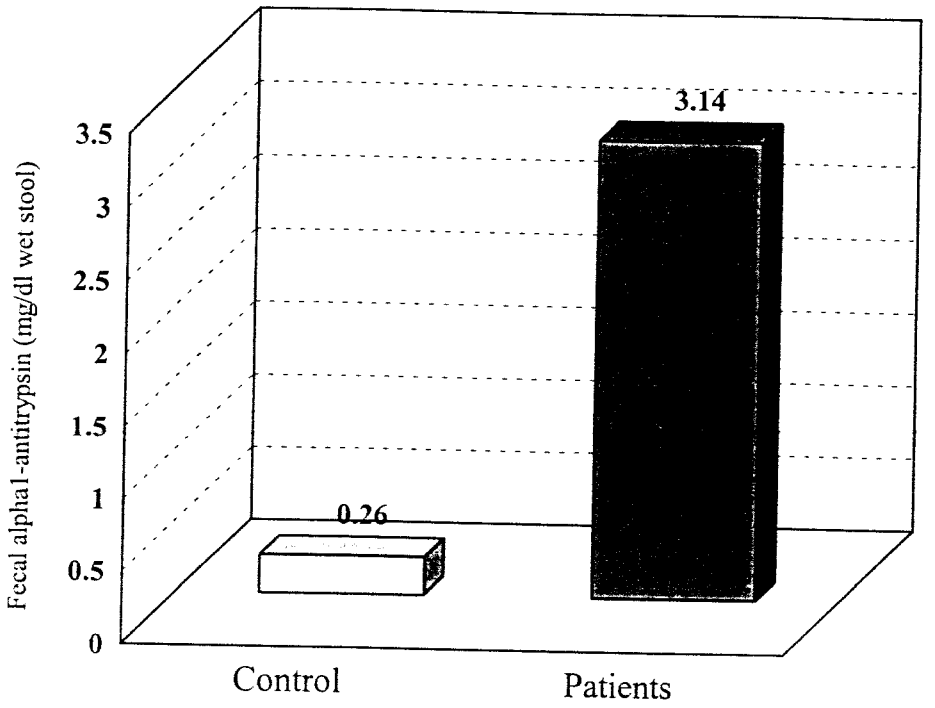


Fig. (4b): Fecal α_1-antitrypsin difference between patients and controls

- Table (10) and fig. (4a&b) show that age was not different between patients (5.47 ± 1.58) and controls (5.94 ±1.18).
- Fecal fat by steatocrite method as a measure of fat malabsorption was significantly higher among patients (6.76 ± 5.68) than controls (2.08 ± 0.84).
- Similarly, fecal α_1-antitrypsin as a measure of protein-loosing enteropathy was significantly higher among patients (3.14 ± 3.83) compared to controls (0.26 ± 0.08).
- Urinary excretion of lactulose and mannitol after oral dose of each shows that urinary lactulose is significantly higher in patients (0.35 ± 0.027) than controls (0.25 ± 0.014). However, urinary mannitol is lower in patients (18.19 ± 0.33) compared to the controls (24.96 ± 2.02). Moreover, the lactulose / mannitol ratio as a parameter of enhanced intestinal permeability is significantly higher in patients (0.02 ± 0.002) compared to controls (0.01 ± 0.001).
- However, the different readings of lactose breath hydrogen testing were not different between cases and controls.

Table (11): Comparison of gender difference and lactose malabosorption between patients and controls

Parameter	Control	Patient	z
Male	0.554	0.607	0.574
Female	0.446	0.393	(> 0.05)
Lactose malabsorption	7.1%	23.2%	2.370 < 0.01

Table (11) and fig. (5a&b) show that there is no gender difference between patients and controls.

However the table shows that lactose malabosrption is significantly more frequent in patients (23.2%) compared to controls (7.1%).

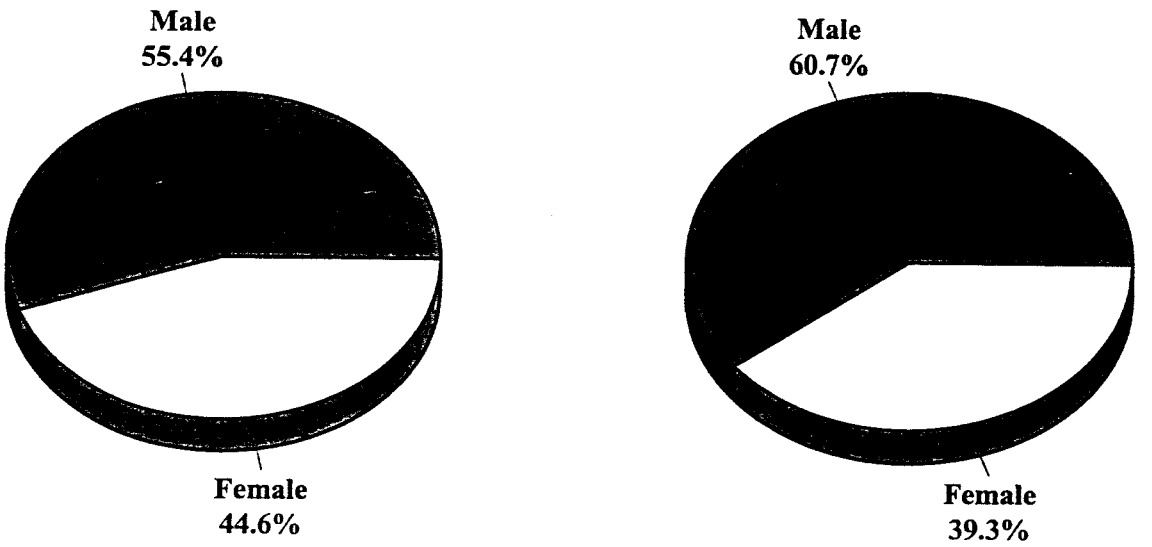


Fig. (5a): Comparison of gender difference between patients and controls

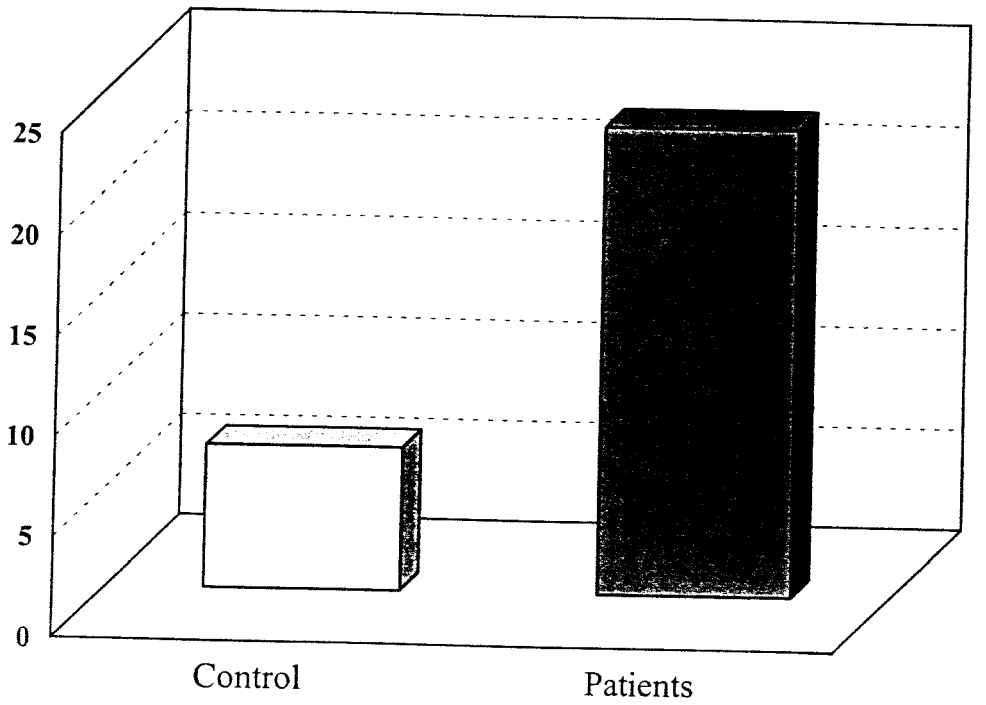


Fig. (5b): Comparison of lactose malabsorption between patients and controls

Table (12): Comparison between mean value of different parameters among male and female controls

Parameter		Male	Female	T	p
Age (years)		4.84 ± 1.57	5.06 ± 0.22	0.77	>0.05
Fecal fat %		1.94 ± 0.81	2.25 ± 0.87	1.38	>0.05
Fecal α_1 antitrypsin (mg/dl. wet stool)		0.28 ± 0.17	0.24 ± 0.10	1.63	>0.05
Lactose Breath Hydrogen (PPM)	Fasting	7.90 ± 2.68	7.24 ± 2.39	0.98	>0.05
	15\	12.74 ± 3.88	10.36 ± 4.85	1.02	>0.05
	30\	8.48 ± 3.39	8.92 ± 2.96	0.51	>0.05
	45\	11.29 ± 3.73	12.60 ± 3.94	1.27	>0.05
	60\	11.94 ± 9.19	13.24 ± 11.71	0.46	>0.05
	120\	11.68 ± 3.13	11.72 ± 3.84	0.04	>0.05
	180\	9.61 ± 3.02	9.20 ± 3.28	0.49	>0.05
	240\	11.87 ± 4.43	10.60 ± 4.33	1.08	>0.05
Lactulose (mmol/L)		22.03 ± 0.25	22.02 ± 0.25	0.09	>0.05
Mannitol (mmol/L)		0.34 ± 0.04	0.34 ± 0.04	0.08	>0.05
Lactulose / mannitol		0.02 ± 0.00	0.02 ± 0.00	0.00	>0.05

Table (12) and fig. (6) show that age, fecal fat (%), fecal α_1- antitrypsin, breath hydrogen readings, mannitol, lactulose and lactulose / mannitol ratio were not significantly different between male and female and controls.

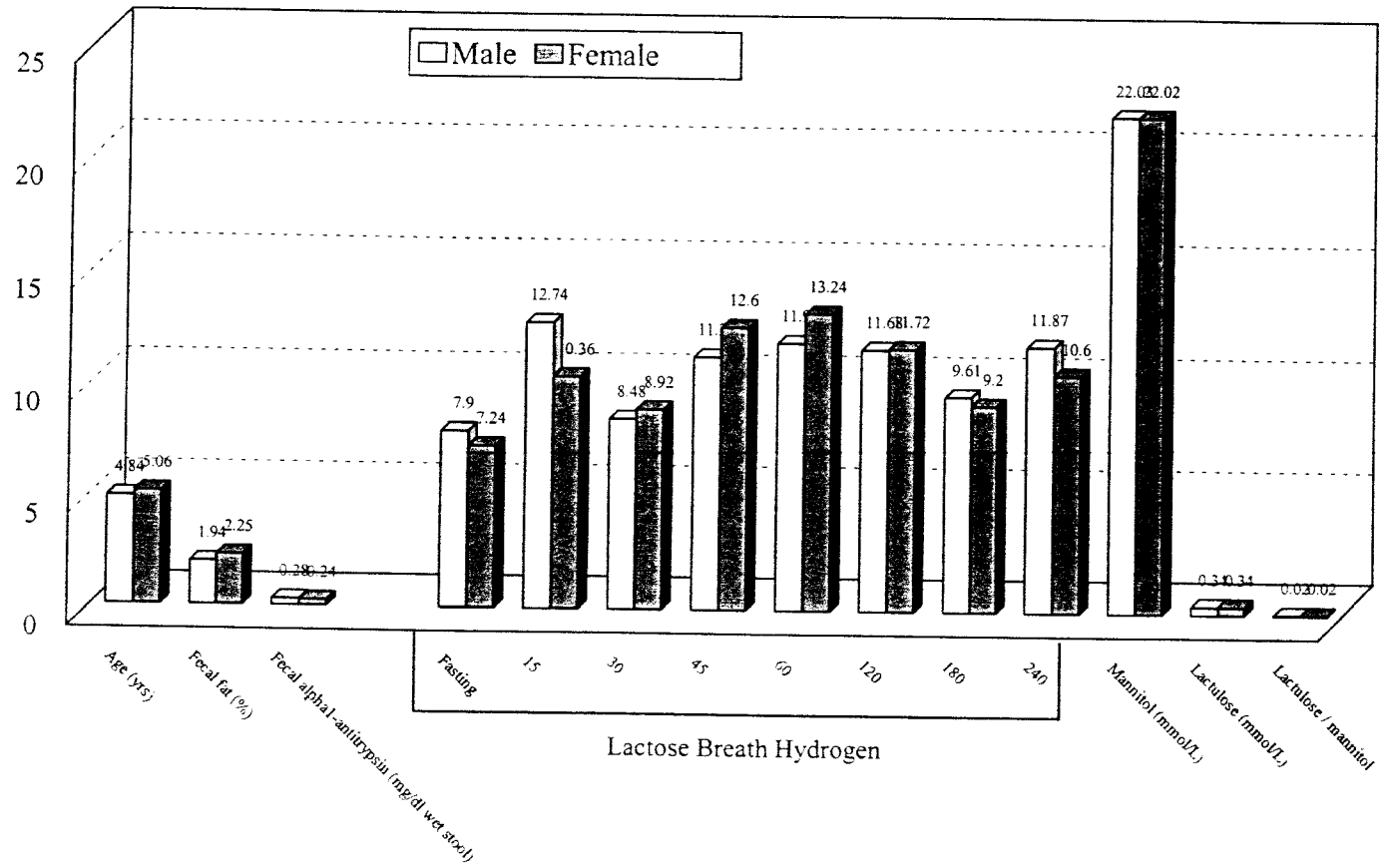


Fig. (6): Comparison between mean values of different parameters among male and female controls

Table (13): Comparison between mean value of different parameters among male and female patients

Parameter		Male	Female	t	p
Age (years)		5.56 ± 1.97	5.22 ± 0.33	1.15	> 0.05
Age of onset (years)		2.15 ± 0.44	2.00 ± 0.00	1.97	< 0.05
Duration of constipation (years)		3.49 ± 1.71	3.00 ± 0.31	1.96	< 0.05
Frequency of motions (motions/w)		2.97 ± 0.17	1.00 ± 0.00	67.00	< 0.001
Treatment-duration (months)		10.94 ± 8.02	12.00 ± 0.00	0.77	> 0.05
Weight (kg)		17.88 ± 5.45	20.14 ± 0.35	2.40	< 0.01
Height (cm)		106.53±14.42	105.86 ± 0.35	0.27	> 0.05
Fecal fat (%)		6.75 ± 6.10	6.23 ± 4.69	1.55	> 0.05
Fecal α_1 antitrypsin (mg/dl. wet stool)		3.72 ± 4.08	2.26 ± 3.30	1.47	> 0.05
Lactose Breath Hydrogen (PPM)	Fasting	7.29 ± 1.34	6.36 ± 2.19	1.69	> 0.05
	15\'	11.35 ± 3.31	12.09 ± 3.45	0.79	> 0.05
	30\'	8.21 ± 3.46	9.73 ± 2.91	1.67	> 0.05
	45\'	11.68 ± 5.85	13.82 ± 3.72	1.67	> 0.05
	60\'	17.32 ± 14.79	15.05 ± 13.99	0.58	> 0.05
	120\'	12.03 ± 3.49	11.59 ± 2.82	0.52	> 0.05
	180\'	10.21 ± 2.78	8.64 ± 3.33	1.63	> 0.05
	240\'	11.35 ± 3.95	12.41 ± 4.77	0.86	> 0.05
Mannitol (mmol/L)		17.40 ± 0.4	18.3 ± 0.33	1.22	> 0.05
Lactulose (mmol/L)		0.36 ± 0.02	0.34 ± 0.12	1.10	> 0.05
Lactulose / mannitol		0.018 ± 0.001	0.021 ± 0.00	0.80	> 0.05

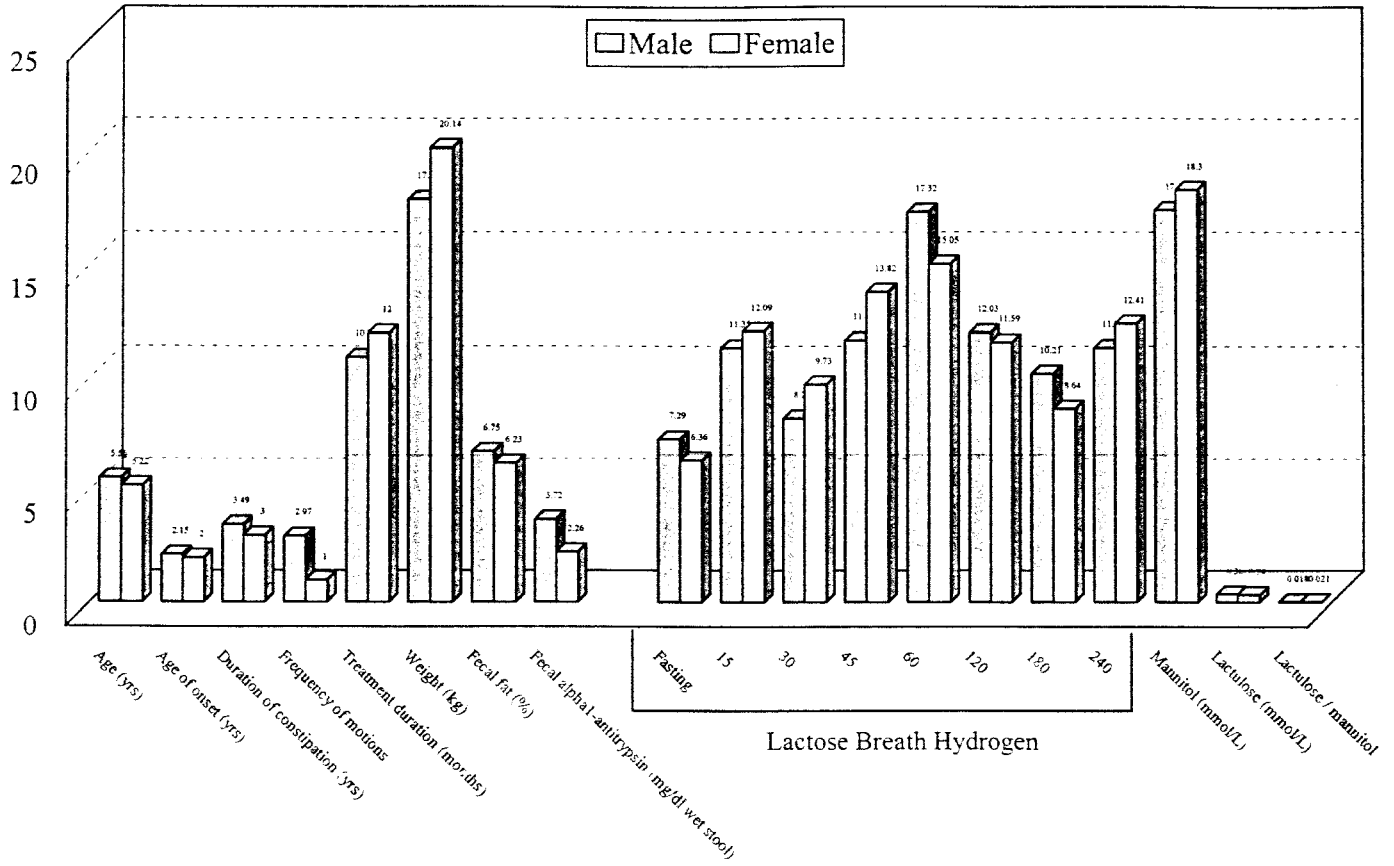


Fig. (7): Comparison between mean values of different parameters among male and female patients

- Table (13) and fig. (7) show that age was not different between male and female patients.
- Age of onset of constipation was significantly higher among male patients (2.15 ± 0.44), compared to female ones (2.00 ± 0.00).
- Frequency of motions per weak were significantly lower among female patients (once / week) compared to male ones (2.97 ± 0.17 / week).
- Weight was significantly lower among male patients (17.88 ± 5.45 kg) compared to female ones (20.14 ± 0.55 kg).
- However, height, fecal fat %, fecal a1-antitrypsin, lactose breath hydrogen readings, mannitol, lactulose and lactulose / mannitol ratio were not significantly different between male and female patients.

Table (14): Comparison of frequency of qualitative parameters between male and female patients

Parameter (%)	Male	Female	z	p
Straining	0.74 (74)	1.00 (100)	2.63	<0.01
Pain during defecation	0.56 (56)	0.96 (96)	2.67	<0.01
Blood per rectum	0.03 (3)	1.00 (100)	7.21	<0.001
Fecal soiling	0.03 (3)	1.00 (100)	7.21	<0.001
Obsessive toilet training	0.97 (97)	0.00 (00)	7.21	<0.001
Psychic problems	0.27 (27)	0.00 (00)	2.63	<0.01
Respiratory problems	1.00 (100)	0.86 (86)	2.21	<0.01
Anorexia	0.97 (97)	0.00 (00)	7.21	<0.01
Vomiting	0.03 (3)	1.00 (100)	7.21	<0.01
Urinary tract infection	0.03 (3)	0.86 (86)	6.36	<0.01
Anal inflammation	0.29 (29)	0.00 (00)	2.81	<0.01
Anal fissure	0.21 (21)	0.64 (64)	3.25	<0.001
Giardia lamblia in stool	0.71 (71)	0.86 (86)	1.37	>0.05
Entameba histolytica in stool	0.27 (27)	0.00 (00)	2.63	<0.01
Lactose malabsorption	0.29 (29)	0.14 (14)	1.37	>0.05

Table (14) and fig. (8) show that straining, pain, bleeding per rectum, fecal soiling, vomiting, urinary tract infection and anal fissure were significantly higher among female patients compared to male patients. On the contrary, obsessive toilet training, psychological troubles, anorexia, respiratory problems and anal inflammation were more common among male than female patients.

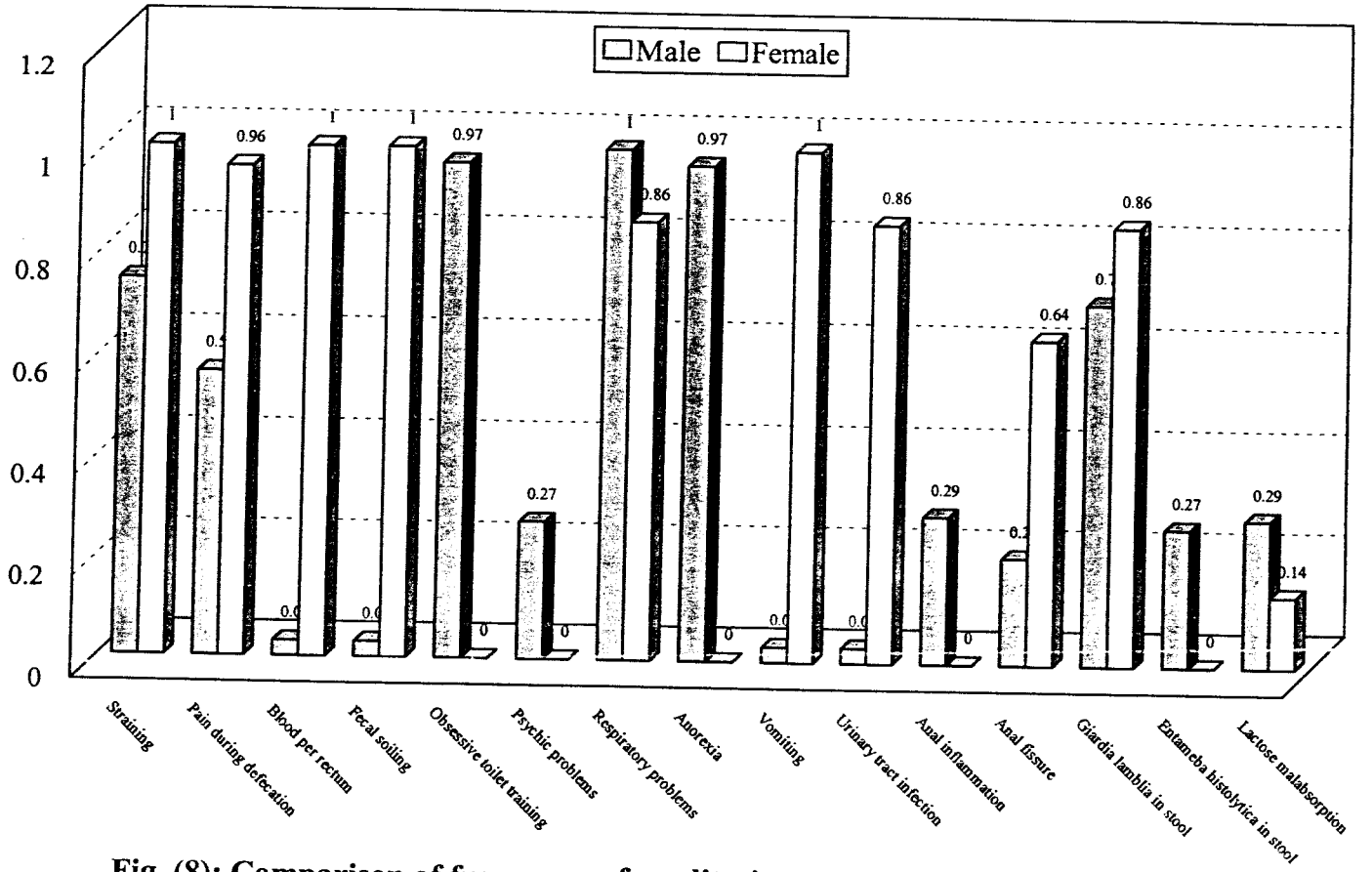


Fig. (8): Comparison of frequency of qualitative parameters between male and female patients

Table (15): Comparison of lactose malabsorption between male and female among controls

Parameter	Male	Female	z	p
Lactose malabsorption	0.065	0.080	0.224	>0.05

Table (15) and fig. (9) show that lactose malabosorption was not different between male and female controls.

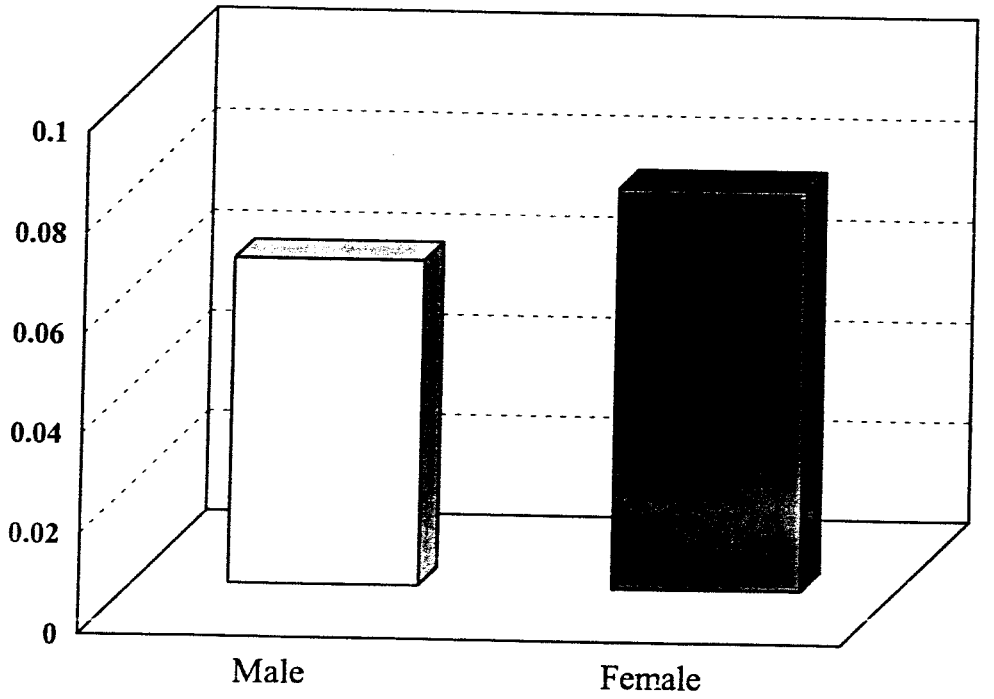


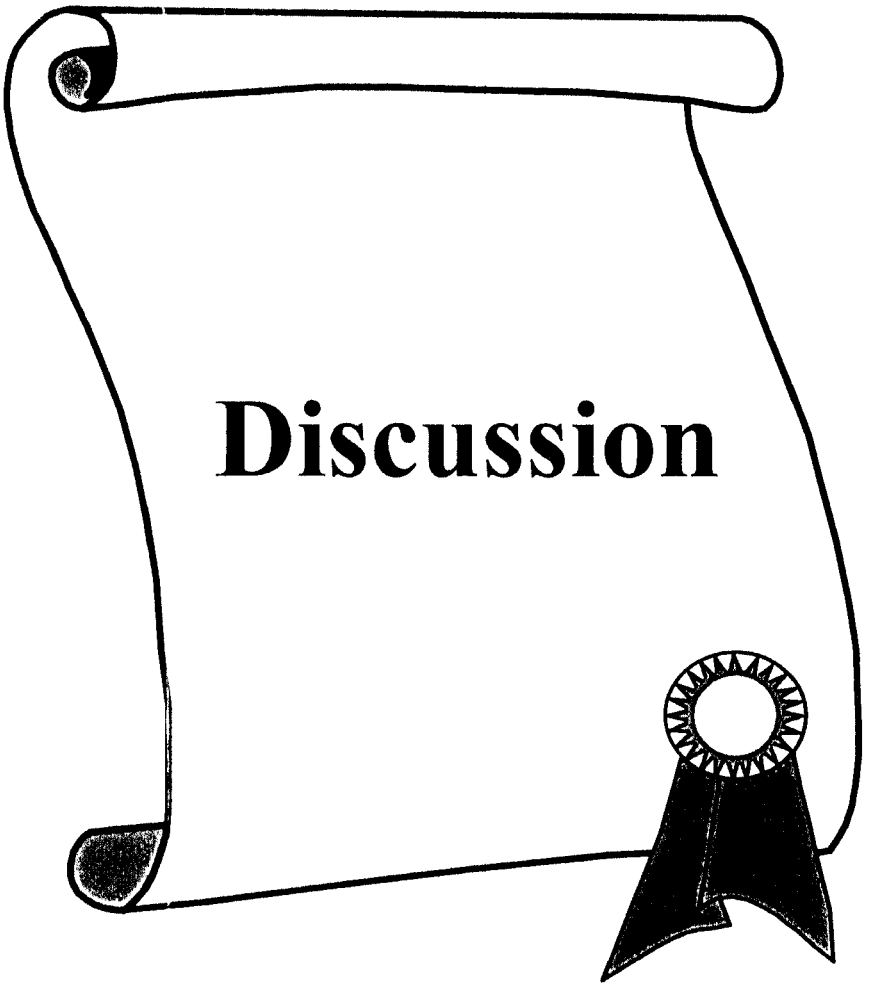
Fig. (9): Comparison of lactose malabsorption between male and female among controls

Table (16): Correlation matrix between clinical and laboratory parameters of patients

	Age (years)	Age - onset (years)	Duration (years)	Frequency (week)	Treatment duration (months)	Weight (kg)	Height (cm)	Fecal fat (%)	Fecal α_1-antitrypsin	Lactulose (mmol/dil)	Mannitol (mmol/L)	Lactulose / mannitol
Age (years)												
Age-onset (years)	0.72013*											
Duration (years)	0.97917*	0.58600*										
Frequency week	0.23791	0.26965	0.20444									
Treatment duration	0.34110*	0.27705*	0.47536*	0.08609								
Weight (kg)	0.85296*	0.50777*	0.87444*	0.26062	0.47465							
Height (cms)	0.95056*	0.75689*	0.92238*	.02995	0.22793	0.92272*						
Fecal fat (%)	-0.29325*	0.23187	0.27742*	0.21468	0.29023*	-0.34900*	-0.32079*					
Fecal α_1-antitrypsin (mg dil.wet stool)	0.28685*	0.25394	0.26362*	0.18241	0.00202	0.32311*	0.31440*	0.84746*				
Lactulose (mg dil.)	0.06314	0.13250	0.1106	0.25387	0.16293	0.21505	0.10530	0.19601	0.11945			
Mannitol (mmol/L)	0.20510	0.09287	0.2174	0.24901	0.20592	0.27802*	0.18665	0.15867	0.14278	0.15843		
Lactulose / mannitol	0.15482	0.17596	0.12973	0.08789	0.09454	0.13390	0.11391	0.03516	0.06317	0.42005*	0.82750*	

(*) = Significant with p-value < 0.05

- Table (11) shows that fecal fat was negatively correlated with age, weight and height.
- Fecal α_1-antitrypsin was correlated with age, weight and height as well.
- Mannitol was significantly correlated with the weight.



Discussion

DISCUSSION

Constipation is one of the most common abdominal symptoms of childhood with an estimated incidence varying from 0.3% to as high as 8% ***(Loening-Baucke, 1993)***.

Constipation accounts for approximately 3 percent of visits to pediatric ambulatory clinics and 20 to 25 percent of visits to pediatric gastroenterology clinics ***(Loening-Baucke, 1994 and Susan et al., 1999)***.

The management of constipation is problematic and requires considerable patience and effort on the part of the child and parents.

A variety of treatment programs, including pharmacologic ***(Perkin, 1977)***, behavioral, medical ***(Wright and Walker, 1978)*** dietary ***(Olness and Tobin, 1982)***, psychologic and surgical ***(Clayden and Lawson, 1976)*** as well as combinations of these, have been employed in the treatment of chronic constipation and encopresis.

Studies focused on the surgical or extra-intestinal complications of constipation but the problem of secondary insult to the gut mucosa was not assessed, hence the aim of this study was to evaluate the intestinal absorptive status as well as the integrity of the gut mucosal barrier.

In our study the mean age of patients was 5.4 ± 1.7 years ranging between 4 and 9 years, which was nearly the

same as reported by other studies as that by ***Agnarsson et al. (1990)***, the latter studied 136 constipated children whose mean age was 3.9 years.

Another study by ***Poenaru et al. (1997)*** who found that the mean age of their studied children was 5.4 ± 3.8 year. Similarly, ***de Araujosant'Anna and Calcado (1999)*** studied the prevalence of constipation among children and found that the ages of their studied group ranged between 8 and 10 years.

Age of onset of constipation in this study ranged between one and three years with a mean of 2.09 years. This denotes that the onset of constipation is early and this may be related to many factors. This age is the time of toilet training with its drawbacks if not conducted adequately. Simultaneously, this is the age of transition between infant feeding and adult feeding with the problem of either quantitatively inadequate or qualitatively inappropriate foods.

The frequency of motions per week were 2.2 ± 0.98. Duration of constipation in this study ranged between 2 and 6 years with a mean of 3.36 ± 1.37 years, however, the duration of treatment was relatively shorter ranging between 6 months and 2 years with a mean of 11.36 ± 6.23 months. This indicates that the parents of our populations do underestimate the problem of constipation or may be due to the insidious onset of such a problem so they do not present their kids to medical care except after a long duration or appearance of complications.

The duration of constipation in our study was nearly similar to that found in a study by ***Akkerman et al. (2000)*** where the mean duration of the illness among their studied constipated children was 4.2 years.

From the nutritional point of view, mean weight of constipated patients was 18.77 ± 4.37 kgs. When this weight was considered in the growth chart, it was equal to the 5th centile however individual cases on centile assessment showed that 32.14% were below the 5th centile i.e. undernourished, one case only was overweight above the 95th centile.

On the contrary, mean height (106.27 ± 11.18 cm) was just equal to the 10th centile, when the individual cases were assessed on height centile, we found that 26 cases were below the 10th centile and 2 cases were below the 5th centile and this denotes that underbuilt is a common finding in constipated children.

This is in agreement with ***Chemperek and Jeleniewski (2001)*** who found that constipated school children are usually candidates for either disturbed dietary habits or abnormal nutritional status.

Similarly, ***Robinson et al. (1990)*** found that children suffering from pica can have combined malnutrition, parasitic infestation and diarrhoea / constipation.

However, our results are contradictory to ***Rome et al. (1999)*** and ***Admidis et al. (2000)*** who found that feeding

habits with refined carbohydrates and little fibre can lead to constipation with increased body weight.

Similarly, ***Burkitt et al. (1980)*** demonstrated that high fibre diet treatment can be of hazard due to loss of large proportion of food energy in stool and can contribute to malnutrition observed in developing countries.

In this study, males were predominant than females representing 60.7%. This result was in consistent with the description in the literature as constipation in children is more frequent among boys than girls ***(DiLorenzo, 1996 and Akkermans et al., 2000)***. ***Loening-Baucke (1993)*** reported that constipation is more frequent among boys with male to female ratio 3:1 for children above the age of 5 years, while it was equally distributed among those below the age of 5 years. This is in disagreement with ***de Araujo Sant'Anna and Calcado (1999)*** who found that there is no gender difference in constipated school children.

However, ***Poenaru et al. (1997)*** found that there was a similar numbers of boys and girls (51.4% boys) among their studied patients, and this ratio was maintained both in the younger and older children.

Also, ***Agnarsson et al. (1990)*** found an equal numbers of males and females among their studied group (67 boys and 69 girls).

Straining effort during defecation (83.93%) and pain during defecation (76.79%) were the most common constipation associated symptoms however they were not always present.

Bleeding per rectum was found in 41.07% of the cases. This is in agreement with ***de Araujo Sant'Anna and Calcado (1999)*** who found that bleeding per rectum is a common finding in constipated children (50%) compared to non constipated ones.

This may be due to anal inflammation or anal fissures which were found in 17.86% and 37.5% of patients respectively. In fact, anal fissure and anal inflammation are recognized as complications of constipated patients. This is in agreement with ***Agnarsson et al. (1990)*** who found that anal fissure and perianal inflammation occurs in 26% of constipated children. Similarly, ***Lubowski (2000)*** demonstrated that anal fissure is a common finding in constipated children and that in the majority of the cases the etiology of the anal fissure is due to ischemia of anal mucosa due to anal sphincter spasm and that reminds that decrease in anal spasm are essential in treatment of these fissures.

Fecal soiling (encopresis) was not uncommon in our series (41.07%). ***Borowitz et al. (1996)*** demonstrated that fecal soiling is a common complication of constipation in children and that anal sphincter spasm demonstrated by manometry is highly correlated to the frequency of fecal soiling. On the contrary, ***de Araujo Sant'Anna and Calcado (1999)*** demonstrated that the prevalence of fecal soiling does not vary between constipated and non constipated patients. This may be a difference in the defining criteria of constipation.

Symptoms of upper gastrointestinal involvement were commonly present in the constipated children with anorexia in 58.93% and vomiting in 41.07%. Respiratory problems were found in 5.36% of cases. Urinary tract infection was found in 35.7% of cases, however, minority were symptomatic with dysuria being present in 5.36%. This is in agreement with ***Loening-Baucke (1997)*** who found that urinary symptoms are very frequent in children with functional constipation. In addition, ***Schlager (2001)*** demonstrated similar relation between recurrent urinary tract infection and constipation and he found that treatment of constipation an essential preventive tool for urinary tract infection.

Parasitic infestation were commonly found in our series with Giardia lamblia present in 76.7% and Entamoeba histolytica in 16.07%. In contradiction with our results, parasitic infestation with constipation are infrequently reported. An example were the report of ***Bassoti et al. (1993)*** who reported a case of constipation having giardiasis. However, the high prevalence of parasitic infestation in our locality may explain this difference.

Regarding the absorptive indices, the study showed that fecal fat (steatocrite percent) was significantly higher among constipated patients (6.76 ± 5.68) compared to controls (2.08 ± 0.84), with p-value < 0.001. This denotes that constipated children have a degree of steatorrhea.

Similarly, fecal α_1-antitrypsin as a measure of protein malabsorption and mucosal outpouring was significantly

higher in constipated children (3.14 ± 3.83) compared to controls (0.26 ± 0.08) with p-value < 0.001. Moreover, lactose malabsorption was encountered in 23.2% of constipated children compared to 7.1% of controls with a significant difference at a p-value < 0.01. The previous result demonstrate that there is a degree of malabsorption in constipated children. This can explain the underweight and height shown in some of these patients. In fact, a viscious circle might be established starting by deficient intake leading to constipation followed by malassimilation of food with resultant aggravation of malnutrition and constipation.

To the best of our knowledge, no previous studies were focused on the absorptive features in constipated patients. However, patients with celiac disease as a malabsorptive syndrome were occasionally found to present with constipation ***(Hungerford, 1996).***

Bode and Gudmand-Hoyer (1996) reported that 12% of celiacs present with constipation. Another report by ***Bassoti et al. (1993)*** found that one case of constipated patient having giardia lamblia infestation proved to be a case of Whipple disease.

In fact, another factor is the use of high fibre food that can impair the absorptive function.

Zoppi et al. (1982) demonstrated that use of wheat bran in constipated children can be complicated with a decrease in serum calcium, phosphorus and trace elements, as well as increase of fecal excretion of biliary salts with increase of the proteolytic fecal flora.

Regarding the gut permeability, it was clear that urinary lactulose excretion was significantly higher among constipated patients (0.35 ± 0.027) when compared to controls (0.25 ± 0.014). On the contrary, mannitol urinary excretion was diminished in constipated patients (18.19 ± 0.33) compared to controls (24.96 ± 2.02). Lactulose molecules are large molecules that cross the gut epithelial barrier from the area of junctions between cells. When the intercellular adherence showed dehiscence, the gut permeability to large molecules as lactulose is enhanced.

Mannitol is an example of low molecular weight carbohydrate. They can cross the gut epithelial barrier transcellularly. Whenever, the brush border surface area is diminished, the permeability to this sugar is diminished and never compensated by the enhanced intercellular permeability.

So, in our cases increase of lactulose permeability and decrease of mannitol gut permeability indicated that gut barrier is disrupted and intestinal surface area is diminished.

This is confirmed by demonstration of increased lactulose / mannitol ratio in constipated patients (0.02 ± 0.002) compared to controls (0.01 ± 0.001).

Ewe (1988) demonstrated that gut permeability may be altered in constipated children and laxatives may interfere with ion transport across gut membrane. However,

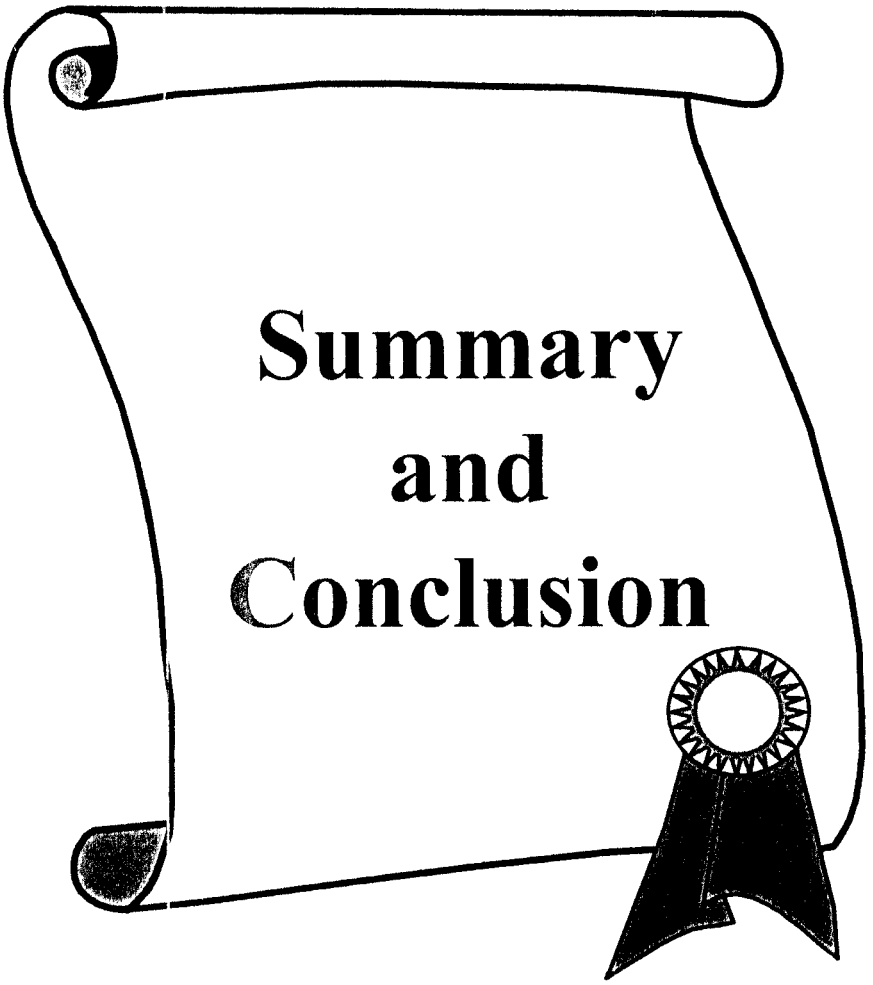
he assessed permeability to glucose and electrolytes which is not a very precise test.

From another view, many literature sources reported that cow milk allergy (cow milk protein intolerance) can present in some children by constipation as reported by ***Iacono et al. (1998).*** However, the studies did not delineate the cause-result pattern between constipation and cow milk allergy.

We can conclude that nutritional status is impaired in some of our constipated children. The absorption indices are somewhat impaired in our patients.

The interrelationship between malabsorption, undernutrition and constipation may be an egg-hen model.

Gut barrier is disrupted in cases with functional constipation but its clinical implications were not the target of this work.



Summary and Conclusion

SUMMARY AND CONCLUSION

Constipation is not only a common problem in pediatric practice, but also is a problem that is difficult to solve.

Most of the studies concerning complications of constipation focused in the surgical aspects. The absorptive faculties and integrity of the gut barrier were not assessed before in constipated children.

Hence, this work was devoted to assess small intestine absorptive function as well as integrity of the gut barrier in constipated children.

This work was conducted on 56 children with chronic constipation were selected among those attending the pediatric gastroenterology clinic, Children hospital, Ain Shams University. They were recruited in the period between June 1999 and December 2000. They were 34 males and 22 females. Their ages ranged between 4 years and 9 years with a mean of 5.90 ± 1.18 years.

Enrollment criteria:

1) Constipation more than 3 months duration.
2) Motions less than 3 times per week.

Exclusion criteria:

1) Cases with defined underlying surgical or medical cause of constipation.
2) Cases that recovered within the last 2 weeks.

A cohort of 56 health children were chosen as a control group.

All cases were subjected to the following:

1) Medical history taking with special emphasis on different gastrointestinal symptoms and characters of constipation.
2) Clinical examination including general, abdominal and anthropometric assessment. Those including measurement of weight, height and their centiles.
3) Laboratory investigations:
   a. Stool analysis
   b. Fecal α_1-antitrypsin as a measure of protein malabsorption.
   c. Fecal fat by semiquantitative steatocrite method.
   d. Lactose absorptive status by measurement of breath hydrogen after a lactose load using the quentron apparatus.
   e. Assessment of mannitol, lactulose and lactulose /mannitol ratio by capillary gas chromatographgy as a measure of gut mucosal permeability.

Controls were subjected to all the laboratory studies only.

The study results showed the following:

- Males are predominant (60.71%) in the studies cases.
- Straining and painful defecation are commonly encountered (83.93% and 76.79% respectively).
- Bleeding per rectum, encopresis (soiling) and anal fissure (41.07%, 41.07% and 37.3% respectively) are not uncommon among our series of constipated children.
- Anorexia and vomiting are commonly found among our patients (58.93% and 41.07% respectively).

- Obsessive toilet training was common among our cases (58.93%). However, psychological problems are uncommon (16.07%).
- Urinary tract infection is not uncommon (35.71%) but recurrent attacks of wheezy chest are rare finding (5.36%).
- Stool analysis demonstrated a common prevalence of giardia lamblia (76.79%) but entameba histolytical is rarely found (5.36% and 16.07% respectively).
- 23.2% of the patients were lactose malabsorpers.
- The age was not different between patients (5.47 ± 1.58) and controls (5.94 ± 1.18).
- Fecal fat by steatocrite method as a measure of fat malabsorption was significantly higher among patients (6.76 ± 5.68) than controls (2.08 ± 0.84).
- Similarly, fecal α_1-antitrypsin as a measure of protein-loosing enteropathy was significantly higher among patients (3.14 ± 3.83) compared to controls (0.26 ± 0.08).
- Urinary excretion of lactulose and mannitol after oral dose of each shows that urinary lactulose is significantly higher in patients (0.35 ± 0.027) than controls (0.25 ± 0.014). However, urinary mannitol is lower in patients (18.19 ± 0.33) compared to the controls (24.96 ± 2.02). Moreover, the lactulose / mannitol ratio as a parameter of enhanced intestinal permeability is significantly higher in patients (0.02 ± 0.002) compared to controls (0.01 ± 0.001).
- The different readings of lactose breath hydrogen testing were not different between cases and controls.
- There was no gender difference between patients and controls.

- Lactose malabsorption is significantly more frequent in patients (23.2%) compared to controls (7.1%).
- The age, fecal fat%, fecal α_1-antitrypsin, breath hydrogen readings, mannitol, lactulose and lactulose / mannitol ratio were not significantly different between cases and controls.
- Age was not different between male and female patients.
- Age of onset of constipation was significantly higher among male patients (2.15 ± 0.44) compared to female ones (2.00 ± 0.00).
- Frequency of motions per week significantly lower among female patients (once/week) compared to male ones (2.97 ± 0.17 / week).
- Weight was significantly lower among male patients (17.88 ± 5.45 kg) compared to female ones (20.14 ± 0.55 kg). However, height, fecal fat%, fecal α_1-antitrypsin, lactose breath hydrogen readings, mannitol, lactulose and lactulose / mannitol ratio were not significantly different between male and female patients.
- Straining, pain, bleeding per rectum, fecal soiling, vomiting, urinary tract infection and anal fissure were significantly higher among female patients compared to male patients. On the contrary, obsessive toilet training, psychological troubles, anorexia, respiratory problems and anal inflammation were more common among male than female patients.
- Lactose malabsorption was not different between male and female controls.
- Fecal fat was negatively correlated with age, weight and height.

- Fecal α_1-antitrypsin was correlated with age, weight and height as well.
- Mannitol was significantly correlated with the weight.

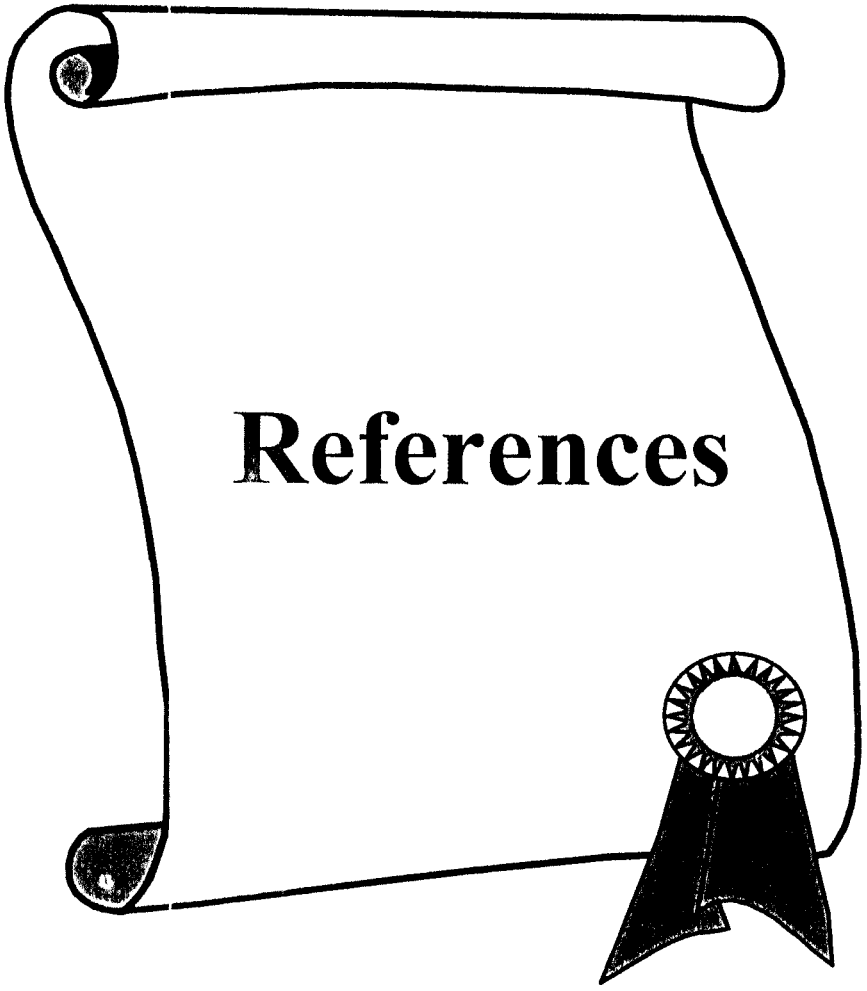
We can come to the conclusion that constipation is not only a disease of the colon but the rest of the gut may be involved in one way or other. So that a degree of malabsorption is present in a large proportion of constipated children. Unfortunately, the gut barrier was found to be significantly disrupted in such patients, however the clinical impact of this disruption was not assessed.


Recommendations

RECOMMENDATIONS

1) Absorptive indices should be investigated in constipated children especially in those with undernutrition.

2) Further clinical study should be conducted to assess the clinical impact of gut barrier disruption on the immunological and allergic system.



References

REFERENCES

Abrahamian FP, Lloyd-Still JD. Chronic constipation in childhood: a longitudinal study of 186 patients. J Pediatr Gastroenterol Nutr 1984; 3: 460 – 7.

Adamidis D, Roma-Giannikou E, Karamolegou K, Tselalidou E, Constantopoulos A. Fiber intake and childhood appendicitis. Int J Food Sci Nutr 2000 May; 51 (3): 153 – 7. Related Articles, Books, Link Out.

Agnarsson U, Warde C, McCarthy G, Evans N. Perianal appearances associated with constipation. Arch Dis Child 1990; 65 (11): 1231 – 4.

Akkermans LMA, Redekop WK, Taminiau JA Buller HA. Megarectum in constipation. Arch Dis Child 2000; 83 (52 – 58).

Alexander KC, Leung MBBS, Paul YH, Chan MD, Helen YH, Cho MD. Constipation in childhood. Am Fam Physican 1996; 54: 611-8.

Alpers DH. Digestion and absorption of carbohydrates and proteins. In Johnson LR (ed.) Physiology of the gastrointestinal tract, ed. 2, New York, Raven Press, 1987; pp. 1469 – 1488.

Amerongen MH, Weltzin RW, Mack JA, et al. M-cell mediated antigen transport and monoclonal IgA antibodies for mucosal immune protection. Ann N Y Acad Sci 1992; 664: 18-26.

Ariel I, Hershlag A, Lernau OZ, et al. Hypoganglionosis of the myenteric plexus with normal Meissner's plexus: A new variant of colonic ganglion cell disorders. J Pediatr Surg 20: 90, 1985.

Atisook K, Madura JL. An oligopeptide permeates intestinal tight junctions at glucose-elicited dilatations. Implications for oligopeptide absorption. Gastroenterology 1991; 100: 719 – 24.

Auricchio S, Stellato A De Vizia. Development of brush border peptidases in human and rat small intestine during fetal and neonatal life. Pediatr Res 1981; 15: 991-5.

Auricchio S. Developmental aspects of brush border hydrolysis and absorption of peptides. In: Lebenthal E. (ed.): Textbook of Gastroenterology and Nutrition in Infancy. New York, Raven Press, 1981; 375 – 384.

Axelsson I, Jakobsson I, Lindberg T, Poleberger S, Benediktsson B, Raiha ON. Macromolecular absorption in preterm and term infants. Acta Paediatr Scand 1989; 78: 532 – 7.

Bannister JJ, Davison P, Timms JM, et al. The effect of stool size and consistency on defecation. Gastroeterology 1987; 92: 1305.

Bassotti G, Pelli MA, Ribacchi R, Miglietti M, Cavalletti ML, Rossadivita ME, Giovenali P, Morelli A. Giardia lamblia infestation reveals underlying Whipple's disease in a patient with long standing constipation. Am J Gastroenterol 1993 Mar; 86 (3): 371 – 4. Related Articles, Books, Link Out.

Bellman M. Studied on encopresis. Acta Pediatr Scan 1966; 170 (suppl): 1.

Benninga MA, Buller HA, Taminiau JA. Biofeedback training in chronic constipation. Arch Dis Child 1993; 68: 126 – 9.

Bhan MK, Khoshoo V, Chowdhary RMD, Jain R, Eaj P, Jayashree S, Kumar R. Increased fecal α_1 antitrypsin in children with persistent diarrhea associated with enteric pathogens. Acta Pediatr Scand 1989; 78: 265 – 267.

Bjarnason I, Peters TJ, Veall N. A persistent defect in intestinal permeability in celiac disease demonstrated by a ^{51}Cr-labelled EDTA absorption test. Lancet 1983; 1: 323 – 5.

Bode S, Gudmand-Hoyer E. Symptoms and haematologic features in consecutive adult celiac patients. Scand J Gastroenterol 1996 Jan; 31 (1): 54 – 60. Related Articles, Books, Link Out.

Bond JH, Levitt MD. Fate of soluble carbohydrate in the colon of rates and man. J of Clinical Investigation 1976; 57: 1158 – 1164.

Bond JH, Levitt M. Investigation of small bowel transit time in man utilizing pulmonary H_2 measurements. J of Laboratory and Clinical Medicine 1975; 85: 546 – 555.

Borowitz SM, Sutphen J, Ling W, Cox DJ. Lack of correlation of anorectal manometry with symptoms of chronic childhood constipation and encopresis. Dis Colon Rectum 1996 Apr; 39 (4): 400 – 4. Related Articles, Books, Link Out.

Brandt LJ. The colon. In Brandt LJ (ed): Gastrointestinal disorders of the elderly. New York, Raven Press, 1984, p. 261.

Brazelton TB. A child oriented approach to toilet training. Pediatrics 1962; 29: 121.

Brown BB. New mind, new body. New York. San Francisco. 1974, Harper & Row publishers.

Burkitt D, Morley D, Walker A. Dietary fibre in under- and overnutrition in childhood. Arch Dis Child 1980 Oct; 55 (10): 803 – 7. Related Articles, Books.

Cacchiatra S, Coremans G, Staiano A, et al. Gastrointestinal transit time and anorectal manometry in children with fecal soiling. J Pediatr Gastroenterol Nutr 1984; 3: 545-550.

Caprini G, Burlina A, Olivieri D. Evaluation of rapid tests for fat malabsorption. Serum triglycerides test and serum turbidity test. Ital J Gastroenterol 1982; 14: 80 – 5.

Carpenter G, Wahl MI. The epidermal growth factor family. In: Sporn MB, Roberts AB, eds. Peptide growth factors and their receptors. I. New York: Springer-Verlag, 1991; 69-171.

Casanova JE, Apodaca G, Mostav KE. An autonomous signal for basolatarela sorting in the cytoplasmic domain of the polymeric immunoglobulin receptor. Cell 1991; 66: 65 – 75.

Castassi C, Cardinali E, D'Angelo G, Coppa GV, Glorgl PL. Reliability of random fecal α_1-antitrypsin

termination on non dried stools. Journal of Pediatrics 1986; 9 (3): 500 – 502.

Cavataio F, Iacono G, Montalto G, et al. Gastroesophageal reflux associated with cow's milk allergy in infants: which diagnostic examinations are useful ? Am J Gastroenterol 1996; 91: 1215 – 20.

ChadwickVS, Phillips SF, Hofmann AF. Measurements of intestinal permeability using low molecular weight polyethylene glycols (PEG 400). II. Application to normal and abnormal permeability states in man and animals. Gastroenterology 1977; 73: 247 – 51.

Chemperek E, Jeleniewski M. Digestive system diseases and style of nutrition among secondary school students. Pol Merkuriusz Lek 2001 Mar; 10 (57): 153 – 5. Related Articles, Books, Link Out.

Chung YC, Young SK, Shadchehr A, Carrido A, MacGregor IL, Sleisenger MH. Protein digestion and absorption in human small intestine. Gastroenterology 1979; 76: 1415 – 21.

Ckane RK. Intestinal absorption of sugars. Physiol Rev 1960; 40: 787 – 825.

Clark J, Russel G, Fitzgerlad J. Serum betacarotene, retinal, and alpha tocopherol levels during mineral oil therapy for constipation. J Pediatr Gastroenterol Nutr 1985; 141: 1210.

Clayden GS. Constipation and soiling in childhood. Br Med J 1976; 515 – 517.

Clayden GS, Lawson JO. Investigation and management of long-standing chronic constipation in childhood. Arch Dis Child 1976; 51: 918 – 923.

Clayden GS. Management of chronic constipation. Arch Dis Child 1992; 67: 340 – 344.

Cobden I, Dickinson RJ, Rothwell J, Axon ATR. Intestinal permeability assessed by excretion ratio of two molecules: results in celiac disease. BMJ 1978; ii: 1060 – 3.

Colombo C, Maiavacca R, Ronchi M, Consalvo M, Giunta A. The steatocrit: A simple method for monitoring fat malabsorption in patients with cystic fibrosis. J Pediatr Gastroenterol Nutrition 1987; 6: 926 – 30.

Corazziari E, Cucchiara S, Staiano A, Romaniello G, Tamburrini O, Torsoli A, et al. Gastrointestinal transit time, frequency of defecation, and anorectal manometry in healthy and constipated children. J Pediatr 1985; 106: 379 – 382.

Cornell R, Walker WA, Isselbacher KJ. Small intestinal absorption of horseradish peroxidase. A cytochemical study. Lab Invest 1971; 25: 42 – 8.

Crossly J, Elliot RB. Simple method for diagnosing protein losing enteropathies. B Med J 1977; 1: 428.

Cummings JH. Fermentation in the human large intestine: evidence and implication for health. Lancet 1983; 1: 1206 – 9.

DAN Poenaru, Nancy Roblin, Mary Bind, Sharon Duce et al. The pediatric bowel management clinic:

Initial results of a multidisciplinary approach to function constipation in children. Journal of Pediatric Surgery 1997; 32: 843 – 48.

Davidson LA, Lönnerdal B. Fecal α_1-antitrypsin in breast-fed infants is derived from human milk and is not indicative of enteric protein loss. Acta Pediatr Scand 1990; 79: 137 – 44.

Dawson DJ, Lobley RW, Burrows PC, Notman JA, Mahon M, Holmes R. Changes in jejunal permeability and passive permeation of sugars in intestinal biopsies in celiac disease and Crohn's disease. Clin Sci 1988; 74: 427 – 31.

de Araujo Sant'Anna AM, Calcado AC. Constipation in school children at public schools in Rio de Janeiro, Brazil J Pediatr Gastroenterol Nutr 1999; 29 (2): 190 – 3.

Denny-Brown D, Robertson G. An investigation of the nervous control of defecation. Brain 1935; 58: 256 – 310.

Di Lorenzo C, Flores AF, Reddy SN, Hyman PE. Use of colonic manometry to differentiate causes of intractable constipation in children. J Pediatr 1992; 120: 690 – 5.

Di Lorenzo C. Approach to the child with constipation and incopresis. In: Rudolph AM, Hoffman JIE, Rudolph CD (eds.): Rudolph's Pediatrics. 20th ed., Preintice Hall International Inc., P.1038, 1996.

Dinari G, Rosenbach Y, Zahavi I, Sivan Y, Nitzan M. Random fecal α_1-antitrypsin in children with intestinal disorder. UDC 1984; 971 – 972.

Dohil R, Roberts E, Jones KV, Jenkins HR. Constipation and reversible urinary tract abnormalities. Arch Dis Child 1994; 70: 56 – 7.

Donatelle EP. Constipation: pathophysiology and treatment. Am Fam Physician 1990; 42: 1335- 42.

Doumeng C, Maroux S. Aminotripeptidase cytosol enzyme from rabbit intestinal mucosa. Biochem 1979; 177: 801 – 808.

Drummey GD, Benson JA, Jones CM. Microscopical examination of the stool for steatorrhea. N Engl J Med 1961; 264: 8 5 – 7.

Dupont C, Barau E, Molkhou P. Modifications of intestinal permeability to large molecules during oral provocation tests in children with cow's milk sensitive enteropathy and atopic dermatitis. In: Harms HK, Wahm U, eds. Food allergy in infancy and childhood. Berlin: Springer-Verlag, 1989; 147-55.

Dupont C, Moreno JL, Barau E, Bargaoui K, Thiane E, Plique O. Effect of diosmectite on intestinal permeability changes in acute diarrhea: a double blind placebo controlled trial. J Pediatr Gastroenterol Nutr 1992; 14: 413 – 9.

Emery Y, Descos L, Meunier P, et al. Constipation terminale par asynchronisme abdomino-pelvien; analyse des données étiologiques, cliniques, manométriques, et des resultants thérapeutiques après reeducation par biofeedback. Gastroenterol Clin Biol 1988; 12: 6.

Ewe K. Intestinal transport in constipation and diarrhoea. Pharmacology 1988; 36 Suppl 1: 73 – 84. Related Articles, Books, Link Out.

Fälth-Magnusson K, Kjellman NIM, Magnusson KE, Sundqvist T. Intestinal permeability in healthy and allergic children before and after sodium-cromoglycate treatment assessed with different-sized polyethyleneglycols (PEG 400 and PEG 1000). Clin Allergy 1984; 14: 277 – 86.

Ferraris RP, Yasharpour S, Lloyd KCK, Mirzayan R, Diamond JM. Luminal concentrations of glucose in the gut under normal conditions. Am J Physiol 1990; 259: G822-37.

Fischbach W, Becker W, Hössner J, Koch W. Fecal α_1-antitrypsin and excretion of = III indium, in assessment of disease activity in chronic inflammatory bowel disease. Gut 1987; 28: 386 – 93.

Feurle GE. Diagnostic value of fecal fat concentration. Gastroenterology 1985; 88: 857 – 8.

Fitzgerald JF. Constipation in children. Pediatr Rev 1987; 8: 294 – 302.

Fleisher DR. Diagnosis and treatment of disorders of defecation in children. Pediatr Ann 1976; 5: 71–101.

Ford RPK, Menzies IS, Walker-Smith JA, Phillips AD, Turner MW. Intestinal sugar permeability: relationship to diarrhoeal disease and small bowel morphology. J Pediatr Gastroenterol nutr 1985; 4: 568 – 74.

Ford RPK, Walker-Smith JA. Food hypersensitivity and gut permeability. Front Gastrointest Res 1986; 13: 69 – 81.

Forget P, Sodoyez-Goffaux F, Zappitelli A. Permeability of the small intestine to ^{51}Cr-EDTA in children with acute gastroenteritis or eczema. J Pediatr Gastroenterol Nutr 1985; 4: 393 – 6.

Forman J, Baluarte HJ, Gruskin AB. Hypokalemia after hypertonic phosphate enemas. J Pediatr 1979; 94: 149.

Freckner B. Function of the anal sphincters in spinal man. Gut 1975; 16: 638 – 644.

Frömter E, Diamond J. Route of passive ion permeation in epithelia. Nature (New Biol) 1972; 235: 9 – 13.

Ganapathy V, Leibach FH. Peptide transport in intestinal and renal brush border membrane vesicles. Life Science 1982; 30: 2137 – 46.

Ganong WF. Gastrointestinal function: digestion and absorption. In Ganong WF (ed.) Review of Medical Physiology (ed. 16), Lange Medical Book, pp 427-430. 1993.

Gilger MA, Wagner ML, Barrish JO, et al. New treatment for rectal impaction in children: An efficacy, comfort, and safety trial of the pulsed-irrigation enhanced-evaculation procedure. J Pediatr Gastroenterol 1994; 18: 29.

Goldstein R, Blondheim O, Levy E. The fatty meal test; an alternative to stool fat analysis. Am J Clin Nutr 1983; 38: 763 – 8.

Hamilton I, Hill A, Bose B, Bouchier IAD, Forsyth JS. Small intestinal permeability in pediatric clinical practice. J Pediatr Gastroenterol Nutr 1987; 6: 697 – 701.

Hauri PP. Biofeedback and self-control of physiological functions: clinical applications. Int J Psychiatr Med; 1975; 6: 955.

Heitlinger LA, Lebenthal E. Disorders of carbohydrate digestion and absorption. Ped Clin North America 1988; 35 (2): 239.

Higgins PR, Weiner HL. Suppression of experimental autoimmune encephalomyelitis by oral administration of myelin basic protein and its fragments. J Immunol 1988; 140: 440 – 5.

Hill RE, Herez A, Corey ML, Gilday DL, Eng B, Hamilton JR. Fecal clearance of alpha-1 antitrypsin: a reliable measure of enteric protein loss in children. J Pediatr 1981; 99: 416.

Hinton JM, Lennard-Jones JE, Young AC. A new method for studying gut transit times using radio opaque markers. Gut 1969; 10: 842 – 847.

Hodges S, Ashmore SP, Patel HR, Tanner MS. Cellobiose: mannitol differential permeability in small bowel disease. Arch Dis Child 1989; 64: 853 – 5.

Holschneider A. Elektromanometrie des enddarmes: Diagnostik der inkontinenz und chronischen obstipation. Edition 2. Munich, Urbana und Schwarzenberg 1983; p. 113.

Hungerford C. Constipation can be a sign of coeliac disease. Aust Fam Physician 1996 May; 25 (5): 802 – 3. Related Articles, Books, Link Out.

Iacono G, Carroccio A, Montalto G. Usefullness of steatocrit test in monitoring malabsorption in cystic fibrosis. Ital J Gastroenterol 1998; 20: 255.

Iacono G, Carroccio A, Cavataio F, Montalto G, Cantarero MD, Notarbartolo A. Chronic constipation as a symptom of cow milk allergy. J Pediatr 1995; 126: 34 – 9.

Iacono G, Carroccio A, Cavataio F, Montalto G, Mancuso C, Balsama V, Notarbartolo A. Statistical test: normal range and physiological variation in infants. Journal of Pediatric Gastroenterology and Nutrition 1990; 11: 53 – 57.

Iacono G, Cavataio F, Montalto G, et al. Intolerance of cow's milk and chronic constipation in children. N Engl J Med 1998; 339: 1100 – 4.

Ian R, Sanderson W, Allan Walker. Uptake and transport of macromolecules by the intestine. Intestinal Immunology and Food Allergy 1995; 34: 19 – 30.

Ingebo KB, Heyman MB. Polyethylene glycol-electrolyte solution for intestinal clearance in children with refractory encopresis. Am J Dis Child 1988; 142 – 340.

Inman LR, Cantey JR. Specific adherence of *Escherichia coli* (strain RDEC-1) to membranous (M) cells of the Peyer's patch in *Escherichia coli* diarrhea in the rabbit. J Clin Invest 1983; 71: 1-8.

Isolauri E, Majamaa H, Arvola T, Rantala I, Virtanen E, Arvilommi H. *Lactobacillus casei* strain CG reverses increased intestinal permeability induced by cow milk in suckling rats. Gastroenterology 1993; 105: 1643 – 50.

Issenman RM, Hewson S, Pirhonen D, Taylor W, Tirosh A. Are chronic digestive complaints the result of abnormal dietary patterns ? Diet and digestive complaints in children at 22 and 40 months of age. Am J Dis Child 1987; 141: 679 – 82.

Juby LD, Dixon MF, Axon ATR. Abnormal intestinal permeability and jejunal morphometry. J Clin Pathol 1987; 40: 714 – 8.

Kaiserlian D, Vidal K, Revillard JP. Murine enterocytes can present soluble antigen to a specific class-11 restricted CD4+ T cells. Eur J Immunol 1989; 19: 1513 – 6.

Keuzenkamp-Jansen CW, Fijnvandraat CJ, Kneepkens CMF, Douwes AC. Diagnostic dilemmas and results of treatment for chronic constipation. Arch Dis Child 1996; 75: 36 – 41.

Khouri ET. Sudan stain of fecal fat; A new report. Gastroenterology. 1989.

Kiely EM, Chopra R, Corkery JJ. Delayed diagnosis of congenital anal stenosis. Arch Dis Child 1979; 54: 68-70.

King CE, Snook LB, Toskes PP. The ^{190}C Triolein breath test. Is it ready for clinical use in its present form ? Gastroenterology 1982; 82: 110.

Klein PD, Klein ER. Application of stable isotopes to pediatric nutrition and gastroenterology: measurement of nutrient absorption and digestion using ^{13}C. J Pediatr Gastroenterol and Nutr 1985; 4: 9-19.

Koch TR. Constipation. In Gastroentrology by eds: Haubricl W.S.; Schaffner F and Ber KJE WB Saunders Company, Philadelphia 5th Ediction P.102. 1995.

Koldovsky O, Britton J, Davis D, et al. The developing gastrointestinal tract and milk-borne epidermal growth factor. In: Mestecky J, et al., eds. Immunology of milk and the neonate. New York: Plenum Press, 1991; 99 – 106.

Koletzko S, Stringer DA, Cleghorn GJ, et al. Lavage treatment of distal intestinal obstruction syndrome in children with cystic fibrosis. Pediatrics 1989; 83: 727.

Kotter DP, Holt PR, Rosensweig NS. Modification of the breath hydrogen test: increase sensitivity for the detection of carbohydrate malabsorption. Lab Clin Med 1982; 100: 798 – 805.

Kreek MH, Schaefer RA, Hahn EF, Fishman J. Naloxon, a specific opoioid antagonist reverses chronic idiopathic constipation. Lancet 1983; 1: 261 – 262.

Lanfranchi GA, Bazzocchi G, Brignola C, Campieri M, Labo'G. Different patterns of intestinal transit time and anorectal motility in painful and painless chronic constipation. Gut 1984; 25: 1352 – 1357.

Lennard Jones JE. Constipation, fecal impaction and laxative abuse. In: Bouchier IAD, Allan RN, Hodgson JIF, Keighley MRB, eds. Textbook of gastroenterology. London: Bailliere Tindall, 1984; 793 – 811.

Lennard Jones JE. Coloproctology and the pelvic flood: Constipation. London, Butterworths, 1985; pp. 350 – 377.

Leonard A, Rappaport MD, Melvin D, Levine MD. The prevention of constipation and encopresis: A developmental model and approach. Pediatr Clinics of North America 1986; 33: 859-67.

Leung AK. Encopresis. Contemp Pediatr 1986; 2(4):20 – 5.

Levine MD, Bakow H. Children with encopresis: A study of treatment outcome. Pediatrics 1976; 58: 845.

Levine MD. Children with encopresis: A descriptive analysis. Pediatrics 1975; 56: 412 – 416.

Levitt MD, Bond JH. Gastrointestinal gas. In Jonson LR, ed. Physiology of the gastrointestinal tract. New York Raven Press, 1981; 1301 – 16.

Levitt MD. Production and excretion of hydrogen gas in man. N Engl J Med 1969; 281: 122 – 127.

Lifshitz CH, Irving CS, Marks LM, Nichols BL, Finegold MS, Klein PD. Polyethyleneglycol polymers of low molecular weight as probes of intestinal permeability: II. Application to infants and children with intestinal disease. J Lab Clin Med 1986; 107: 290 – 8.

Lifshitz CH. Intestinal permeability. J Pediatr Gastroenterol Nutr 1985; 4: 520 – 2.

Lloyd-Still JD. Symptoms and signs of gastrointestinal disease. In: Kelley VC, ed. Practice of pediatrics. Philadelphia: Harper & Row, 1987: 1-14.

Loening-Baucke V. Anorectal manometry: Experience with strain gauge pressure transducers for the diagnosis of Hirschsprung's disease. J Pediatr Surg 1983; 18: 595.

Loening-Baucke V. Sensitivity of the sigmoid colon and rectum and in children treated for chronic constipation. J Pediatr Gastroenterol Nutr 1984; 3: 454.

Loening-Baucke V, Cruikshank B. Abnormal defecation dynamics in chronically constipated children with encopresis. J Pediatr 1986; 108: 562.

Loening-Baucke V. Factors responsible for persistence of childhood constipation. J Pediatr Gastroenterol Nutr 1987; 6: 915-22.

Loening-Baucke V. Factors determining outcome in children with chronic constipation and fecal soiling. Gut 1989; 30: 999.

Loening-Baucke V. Persistence of chronic constipation in children after biofeedback treatment. Dig Dis Sci 1991; 36: 153.

Loening-Baucke V. Elimination disorders. In Greydanus DE, Wolraich ML (eds): Behavioral Pediatrics. New York, Springer-Verlag, 1991, p 280.

constipated children with encopresis. J Pediatr 1990; 116: 214 – 22.

Lowery SP, Srour JW, Whitehead WE, et al. Habit training as treatment of encopresis secondary to chronic constipation. J Pediatr Gastroenterol Nutr 1985; 4: 397 – 401.

Lubowski DZ. Anal fissures. Aust Fam Physician 2000 Sep; 29 (9): 839 – 44. Related Articles, Books.

Lucas A, Gibbs JAH, Lyster RLJ, Baum JD. Creamatocrite: a simple clinical technique for estimating fat concentration and energy value of human milk. Br Med J 1978; 1: 1018 – 20.

Madara JL. Pathobiology of the intestinal epithelial barrier. Am J Pathol 1990; 137: 1273 – 81.

Marie Fallon B, Bill O'Neill. Constipation and diarrhoea. Clin Rev 1997; 315: 1293-6.

Martelli H, Devroede G, Arhan P, et al. Mechanisms of idiopathic constipation: outlet obstruction. Gastroenterology 1978; 75: 623 – 631.

Maxton DG, Bjarnason I, Reynolds AP, Catt SD, Peters TJ, Menzies IS. ^{51}Cr-labelled ethylene-diaminetetraacetate, L-rhamnose and polyethylene glycol 4000 as probe markers for assessment in vivo of human intestinal permeability. Clin Sci 1986; 71: 71 – 80.

McClung HJ, Boyne LJ, Linsheid T, et al. Is combination therapy for encopresis nutritionally safe ? Pediatrics 1993; 91: 591 – 594.

Meier-Ruge W. Epidemiology of congenital innervation defects of the distal colon. Virchows Arch A Pathol Anat 1992; 420: 171.

Menzies IS. Absorption of intact oligosaccharide in health and disease. Biochem Soc Trans 1974; 2: 1042- 7.

Meunier P, Louis D, Jaubert de Beaujeu J. Physiologic investigation of primary chronic constipation in children: comparison with the barium enema study. Gastroenterology 1984; 87: 1351-7.

Meunier P, Marechal JM, Jaubert de Beaujeu M. Rectoanal pressures and rectal sensitivity studies in chronic childhood constipation. Gastroenterology 1979; 77: 330-6.

Meunier P, Rochas A, Lambert R. Motor activity of the sigmoid colon in chronic constipation: comparative study with normal subjects. Gut 1979; 20: 1095 – 1101.

Miller TL, Wolin MJ. Fermentations by saccharolytic intestinal bacteria. Am J Clin Nutr 1979; 32: 164 – 72.

Monar D, Taitz LS, Urwin OM, et al. Anorectal manometry results in defecation disorders. Arch Dis Child 1983; 58: 257 – 61.

Montalto G, Carroccio A, Iacono G, Soresi M, Notarbartolo A. Steatocrit test in monitoring malabsorption in cystic fibrosis. Int J Pacreatol 1988; 3 (2): 265.

Muller DPR. Disorders of lipid absorption. Clin Gastroenterology 1982; 11: 119 – 40.

Nathavitharana KA, Lloyd DR, Raafat F, Brown GA, McNeish AS. Urinary mannitol: lactulose excretion ratios and jejunal mucosal structure. Arch Dis Child 1988; 63: 1054 – 9.

Navarro J, Sonsino E, Boige N, et al. Visceral neuropathies responsible for chronic intestinal pseudo-obstruction syndrome in pediatric practice: Analysis of 26 cases. J Pediatr Gastroenterol Nutr 1990; 11: 179.

Nixon SE, Mawer GE. The digestion and absorption of protein in man II. The form in which digested protein is absorbed. Br J Nutr 1970; 24: 241-58.

Noren O, Dabetsteen E, Sjostrom H, Joseffson L. Histological localization of two dipeptidases in the pig small intestine and liver using immunoflourescence. Gastroenterology 1977; 72: 87 – 92.

O'Regan S, Yazbeck S, Schick E. Constipation, bladder instability, urinary tract infection syndrome. Clin Nephrol 1985; 23: 152 – 4.

Olatawura MO. Encopresis: a reviw of thirty-two cases. Acta Pediatr Scand 1973; 62: 358 – 64.

Olness K, Tobin J. Chronic constipation in children: can it be managed by diet alone ? Postgrad Med 1982; 72: 149 – 54.

Pang KY, Bresson JL, Walker WA. Development of the gastrointestinal mucosal barrier: evidence for structural differences in microvillus membranes from newborn and adult rabbits. Biochem Biophys Acta 1983; 727: 201 – 8.

Pappenheimer JR, Reiss KZ. Contribution of solvent drag through intercellular junctions to absorption of nutrient by the small intestine of the rat. J Membr Biol 1987; 100: 123 – 36.

Pappenheimer JR. Paracellular absorption of glucose, creatinine and mannitol in normal animals: relation to body size. Am J Physiol 1990; 259: G290-9.

Parks AG, Porter NH, Melzak J. Experimental study of the reflex mechanisms controlling the muscles of the pelvic floor. Dis Colon Rectum 1962; 5: 407-414.

Partin JC, Hamill SK, Fischel JE, et al. Painful defecation and fecal soiling in children. Pediatrics 1992; 89: 1007.

Peng JH, Turner MW, Strobel S. The generation of a tolerogen after the ingestion of ovalbumin is time-dependent and unrelated to serum levels of immunoreactive antigen. Clin Exp Immunol 1990; 81: 510 – 15.

Perkin JM. Constipation in childhood: A controlled comparison between lactulose and standardized senna. Curr Med Res 1977; 4: 540 – 3.

Perman JA. Clinical application of breath hydrogen measurements. Can J Physiol Pharmacol 1991; 69(1): 111-5.

Phuapradit P, Narany A, Mendonca P, Harris DA, Baum JD. The steatocrit : a simple method for estimating stool fat content in newborn infants. Arch Dis Children 1981; 56: 725-5.

Poenura D, Roblin N, Bird M, Duce S, Groll A, Pietak D, Spry K, Thompson J. Approach to functional constipation in children. J Pediatr Surg 1997; 32 (6): 843 – 848.

Poenura D, Roblin N, Bird M, Duce C, Groll A, Pretak D, Spry K, Thompson J. The pediatric bowel management clinic: Initial results of a multidisciplinary approach to functional constipation in children. J Pediatr Surg 1997; 32(6): 843 – 848.

Preston DM, Adrian TE, Christofides ND, Lennard-Jones JE, Bloom SR. Positive correlation between symptoms and circulating motilin, pancreatic polypeptide and gastrin concentrations in functional bowel disorders. Gut 1985; 26: 1059-64.

Rintala RM, Kokkonen LH, Lindahl H, Saariola H. Does disordered upper gastrointestinal motility predisposes to Helicobater Pylori colonization of the stomach in children. J Pediatr Surg 1994; 29(6): 734 – 37.

Roberton DM, Paganelli R, Dinwiddie R, Levinsky RJ. Milk antigen absorption in the preterm and term neonate. Arch Dis Child 1982; 57: 369-72.

Robinson BA, Tolan W, Golding-Beecher O. Childhood pica. Some aspects of the clinical profile in Manchester, Jamaica. West Indian Med 1990 Mar; 39 (1): 20 – 6. Related Articles, Books.

Roma E, Adamidis D, Nikolara R, Constantopoulos A, Messaritakis J. Diet and chronic constipation in children: the role of fiber. J Pediatr Gastroenterol

Nutr 1999 Feb; 28 (2): 169 – 74. Related Articles, Books, Link Out.

Sanderson IR, Walker WA. Uptake and transport of macromoleules by the intestine: possible role in clinical disorders (an update). Gastroenterology 1993; 104: 622-39.

Scharli AF. Defecation and continence: some new concepts. Dis Colon Rectum 1970; 13: 81-107.

Schlager TA. Urinary tract infections in children younger than 5 years of age: epidemiology, diagnosis, treatment, outcomes and prevention. Paediatr Drugs 2001; 3 (3): 219 – 27. Related Articles, Books, Link Out.

Schrander JJ, van den Bogart JP, Forget PP, Schrander-Stumpel CT, Kuijten RH, Kester AD. Cow's milk protein intolerance in infants under 1 year of age: a prospective epidemiological study. Eur J Pediatr 1993; 152: 640- 4.

Shouler P, Keighley RB. Changes in colorectal function in severe idiopathic chronic constipation. Gastroenterology 1986; 414-20.

Shulman RJ, Lifschitz CH, Langston C, Gopalakrishna GS, Nichols BL. Human milk and the rate of small intestinal mucosal recovery in protracted diarrhea. J Pediatr 1989; 114: 218-24.

Sicinski P, Rowinski J, Wasrchol JB, et al. Poliovirus type 1 enters the human host through intestinal M cells. Gastroenterology 1990; 98: 56 – 8.

Silk DBA. Peptide transport. Clin Sci 1981; 60: 607-15.

Simister N, Mostov KE. An Fc receptor structurally related to MHC class I antigens. Nature 1989; 333: 184-7.

Simister NE, Rees AR. Isolation and characterization of an Fc receptor from neonatl rat small intestine. Eur J Immunol 1985; 15: 733-8.

Simons K, Wandinger-Ness A. Polarized sorting in epithelia. Cell 1990; 62: 207 – 10.

Sjostrom H, Noren O, Joseffson L. Purification and specificity of pig intestinal prolidase. Biochem Biophysic Acta 1973; 327: 457-70.

Solomons NW, Viteri F. Breath hydrogen during sleep. Lancet 1976; 2: 636.

Sondheimer JM. Helping the child with chronic constipation. Contemp Pediatr 1985; 12 – 28.

Sondheimer JM, Gervaise EP. Lubricant versus laxative in the treatment of chronic functional constipation of children: A comparative study. J Pediatr Gastroenterol Nutr 1982; 1 (2): 223-6.

Staiano AM, Cucchiara S, Andreotti MR, Minella R, Manz G. Effect of Cisapride on chronic idiopathic constipation in children. Dig Dis Sci 1991; 36: 733 – 6.

Strobel S, Brydon WG, Ferguson A. Cellobiose/mannitol sugar permeability test complements biopsy histopathology in clinical investigation of the jejunum. Gut 1984; 25: 1241-6.

Strobel S. Immunologically mediated damage to the intestinal mucosa. Acta Pediatr Scand Suppl 1990; 365: 46 – 57.

Susan C, Leech Kieran, McHugh, Peter B, Sullivan. Evaluation of a method of assessing fecal loading on plain abdominal radiographs in children. Pediatr Radiol 1999; 29: 255-258.

Suzuki H, Amano S, Honzumi M, et al. Rectoanal pressure and rectal compliance in constipated infants and children. Z Kinderchir 1980; 29: 330-6.

Swanwick T. Encopresis in children: A cyclical model of constipation and fecal retention. Br J Gen Pract 1991; 41: 514.

Targan SR, Kagnoff MF, Brogan MD, Shananhan F. Immunologic mechanisms in intestinal disease. Ann Intern Med 1987; 106: 853-70.

Tedesco FJ, Di Piro JT. American College of Gastroenterologic's Committee on FDA-related matters, laxative use in constipation. Am J Gastroenterol 1985; 80: 303.

Teitelbaum DH, Drongowski RA< Chamberlain JN, Coran AG. Long-term stooling patterns in infants undergoing primary endorectal pull-through for Hirschsprung's disease. J Pediatr Surg 1997; 32 (7): 1049-52.

Terry F, Hatch MD. Encopresis and constipation in children. Pediatr Clinics of North America 1988; 35: 257-80.

Ukaham SO, Cooper BT. Small intestinal permeability as an indicator of jejunal mucosal recovery in patients with celiac sprue on a gluten free diet. J Clin Gastroenterol 1985; 7: 232 – 6.

Unanue ER. Antigen presenting function of the macrophage. Annu Rev Immunol 1984; 2: 395 – 428.

Wald A, Chandra R, Chiponis D, et al. Anorectal function and continence mechanisms in childhood encopresis. J Pediatr Gastroenterol Nutr 1986; 5: 346-51.

Wald A, Chandra R, Gabel S, et al. Evaluation of biofeedback in childhood encopresis. J Pediatr Gastroenterol Nutr 1987; 6: 554.

Walker-Smith A, Skyring AP, Mistillis SP. Use of radioactive CrCl in the diagnosis of protein losing enteropathy. Gut 1967; 8: 166.

Walker-Smith JA. Dietary protein intolerance. In: Walker-Smith JA, ed. Diseases of the small intestine in childhood. London: Butterworth, 1988; 144-84.

Watkins JB, Klein PD, Schoeller DA. Differentiation of fat malabsorption in children using ^{13}C labeled lipids: Triolein Trioctanoin and palmitic acid breath tests. Gastroenterology 1982; 82: 911-7.

Weaver LT. Bowel habit from birth to old age. J Pediatr Gastroenterol Nutr 1988; 7: 637 – 40.

West PS, Levin GE, Griffin GE, Maxwell JD. Comparison of simple screening tests for fat malabsorption. British Medical J 1981; 382.

Wheeler PG, Menzies IS, Creamer B. Effect of hyperosmolar stimuli and celiac disease on the permeability of the human gastrointestinal tract. Clin Sci Mol Med 1978; 54: 495-501.

Wolf JL, Rubin DH, Finberg R, et al. Intestinal M cells: a pathway for entry of reovirus into the host. Science 1981; 212: 471-2.

Woodruff C, Fabacher D, Latham C. Fecal α_1-AT and infant feeding. J Pediatr 1985; 106: 228.

Wrenn K. Fecal impaction. N Engl J Med 1989; 321: 658-62.

Wright L, Walker C. A simple behavioral treatment program for psychogenic encopresis. Behav Res Ther 1978; 16: 209-12.

Wyman JB, Heaton KW, Manning AP, et al. The effect on intestinal transit and the feces of raw and cooked bran in different doses. Am J Clin Nutr 1976; 29 (12): 1474-9.

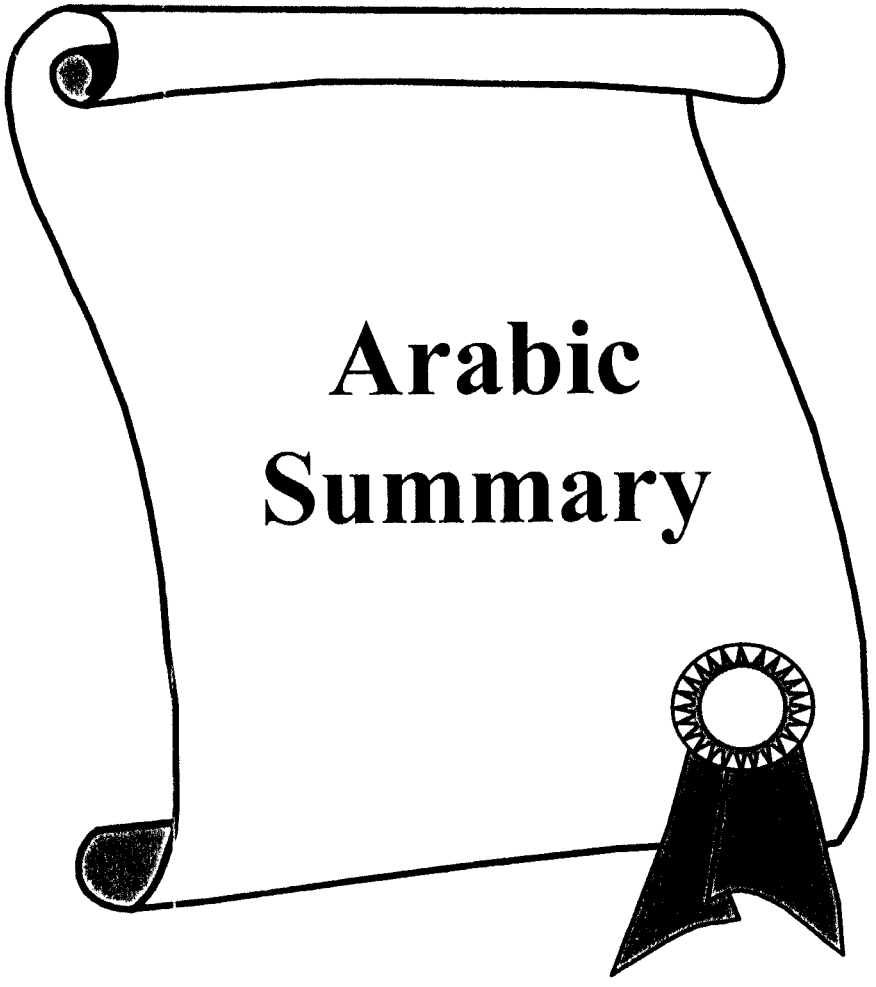
Younoszai MK, Tolaymat N. Chronic functional constipation in infants and children. In: Lebenthal E, ed. Textbook of gastroenterology and nutrition in infancy. 2nd ed. New York: Raven Press, 1989; 1311-26.

Zaslavsky C, Avila EL, Araujo MA, et al. Constipacao intestinal da infancia-um estudo de prevalencia. Rev AMRIGS 1988; 32: 100-2.

Zempsky WT, Rosenstein BJ. The cause of rectal prolapse in childen. Am J Dis Child 1988; 142: 338-9.

Ziskind A, Gellis SS. Water intoxication following tap water enemas. Am J Dis Child 1958; 96: 699.

Zoppi G, Gobio-Casali L, Deganello A, Astolfi R, Saccomani F, Cecchettin M. Potential complications in the use of wheat bran for constipation in infancy. J Pediatr Gastroenterol Nutr 1982; 1 (1): 91 – 5. Related Articles, Books, Link Out.



Arabic Summary

الملخص العربى

يعتبر الإمساك المزمن من الأمراض ذائعة الانتشار بين الأطفال حيث يمثل نسبة تصل الى ١٥% من حالات اضطرابات الجهاز الهضمى. ومما يزيد الأمر سوءا تعدد أسباب الإمساك وصعوبة علاجه.

وقد اختصت معظم الدراسات بالبحث فى أسباب الإمساك المزمن وكذلك نمط الحركية المعوية واضطرابها فى هذه الحالات غير أن القليل من الدراسات قد ركزت على مضاعفات الإمساك الجراحية فقط مثل الشرخ أو السقوط الشرجى.

ولم تجرى أبحاث فى السابق – على حد ما وصلنا من معلومات– لمناقشة الحالة الامتصاصية وحالة الحاجز المعوى فى مثل هذه الحالات. ولما كانت بعض الدراسات الحديثة قد أشارت الى علاقة حساسية اللبن البقرى بالإمساك كان الهدف من هذا البحث هو دراسة درجة سلامة الحاجز المعوى وكذلك وظائف الامتصاص فى الأطفال المصابين بالإمساك المزمن.

الحالات والوسائل:

تم إجراء هذا البحث على ٥٦ طفل من المصابين بالإمساك المزمن الغير جراحى السبب من بين المترددين على عيادة الجهاز الهضمى بمستشفى الأطفال جامعة عين شمس، وقد أجريت الدراسة فى الفترة من يونيو ١٩٩٩م وحتى ديسمبر ٢٠٠٠م واشتملت على ٣٤ ذكرا و ٢٢ أنثى، وقد تراوحت الأعمار بين ٤-٩ سنوات بمتوسط ٥,٩ ± ١,١٨ سنة.

شروط الانضمام الى البحث:

١) إمساك أكثر من ثلاثة أشهر.

٢) عدد مرات تبرز أقل من ثلاث مرات أسبوعيا.

شروط الاستبعاد من البحث:

١) وجود سبب جراحى أو طبى للامساك.

٢) الحالات التى قد تحسنت خلال الأسبوعين السابقين للبحث.

وكذلك تم اختيار عدد ٥٦ طفل من الأصحاء لهم نفس التوزيع السنى والنوعى كمجموعة ضابطة.

وقد تم إجراء ما يلى لجميع الحالات:

١) تاريخ طبى مفصل مع التركيز على أعراض الجهاز الهضمى ومؤشرات أى اضطرابات نفسية فى الطفل أو الأهل.

٢) فحص سريرى شامل وبخاصة كشف البطن والقياسات الجسمية.

٣) فحوصات معملية واشتملت على مايلى:

أ) تحليل براز كامل

ب) قياس نسبة مضاد التربسين فى البراز كمقياس لعصر امتصاص البروتينات.

ج) قياس الدهون فى البراز.

د) قياس هيدروجين النفس بعد جرعة من اللبن المحتوى على اللاكتوز كمقياس لامتصاص السكريات.

هـ) قياس سكرى اللاكتيولوز والمانيتول ونسبتهما فى البول بعد جرعة منهما بالفم كمقياس لسلامة الحاجز المعوى.

وقد أثبتت الدراسة ما يلى:

- كانت نسبة الذكور أكثر من الإناث بين مرضى الإمساك.
- كان النزيف الشرجى والتبرز اللاإرادى والشرخ الشرجى من الأعراض الشائعة بين هؤلاء المرضى.
- وقد لوحظ كذلك أن نسبة الأطفال الذين تعرضوا لتدريب التبرز بطريقة قاسية قد وصلت الى ٥٨,٩% بين الأطفال المصابين بالإمساك.

- وعلى الرغم من ذلك كانت المشاكل النفسية مثل اضطرابات الأبوين موجودة فى ١٦% فقط من الحالات.
- ومما تبين أيضا أن التهابات المسالك البولية كانت كثيرة الحدوث فى حالات الإمساك المزمن (٣٥,٧%) بينما كانت نوبات حساسية الصدر نادرة الحدوث (٥,٣%).
- وتبيـــن من هذه الدراسة أيضا أن طفيل الجيارديا كان موجودا بنسبة ٧٧% من حالات الإمساك.
- ومـمـــا يخـــص دراســـة الامتصاص فقد ثبت أن نسبة الدهنيات وكذلك نسبة مضاد التربسين فى البراز كانت أعلى بين حالات الإمساك مقارنة بالمجموعة الضـــابطة. وكذلـــك كانت نسبة الحالات المصابة بسوء امتصاص السكريات أعلى بين مرضى الإمساك (٢٣%) مقارنة بالمجموعة الضابطة (٧%).
- وثبت من البحث أيضا أن الحاجز المعوى يعانى من اضطراب وعدم اكتمال فى حالات الإمساك المزمن.

ونخلـــص مـــن هذا البحث الى أن الإمساك ليس مرضا قولونى فقط ولكنه يكـــون مصـــحوبا باضطرابات فى الأجزاء الأخرى من المعى ومن ذلك اضطراب الامتصـــاص والحاجـــز المعوى والذى من الممكن أن يكون له مساوئ على حالة الطفـــل سواء فى الوقت الحالى أو المستقبل ولذا ننصح بإجراء اختبار للامتصاص فـــى حـــالات الإمساك وبخاصة فى المرضى الذين يعانون نقص فى الحالة الغذائية وننصح كذلك باستكمال البحوث الخاصة بعلاقة اضطراب الحاجز المعوى بأمراض الحساسية والمناعة فى مرضى الإمساك.

المستخلص

أجري هذا البحث علي ٥٦ طفل من المصابين بالأمساك المزمن الغير جراحي السبب .

وكان الهدف من هذا البحث هو دراسة درجة سلامة الحاجز المعوي وكذلك وظائف الأمتصاص في الأطفال المصابين بالأمساك المزمن .

وقد أجريت للمرضي بحوث سريرية وبخاصة كشف الباطنة وفحوصات معملية .

وخلصت الدراسة الي أن الأمساك ليس مرض قولوني فقط ولكنه يكون مصاحبه بأضطرابات في الأعضاء الأخري من المعي ومن ذلك أضطراب الأمتصاص والحاجز المعوي والذي من الممكن أن يكون له مساوئ علي حالة الطفل سواء في الوقت الحالي أو المستقبل .

الكلمات الكاشفة

قياس نسبة مضاد الترپسين في البراز

قياس الدهون في البراز

قياس هيدروجين التنفس

قياس سكري الاكتيولوز والمانيتول ونسبتيهما في البول

أضراب الأمتصاص – سلامة الحاجز المعوي

جامعة عين شمس
معهد الدراسات العليا للطفولة

شكر

أشكر السادة الأساتذة الذين قاموا بالأشراف
وهم :

١- الأسم / أ.د حامد محمود شتلة — الوظيفة / أستاذ طب الأطفال محافظ الشرقية .

١- الأسم / أ.د جمال سامي علي — الوظيفة / أستاذ بقسم الطبي بمعهد الدراسات العليا للطفولة .

١- الأسم / أ.د مصطفي عبد العزيز هدهد — الوظيفة / أستاذ مساعد طب الأطفال – طب عين شمس .

١- الأسم / أ.د داليا حلمي فرج — الوظيفة / أستاذ الباثولوجيا الأكلينيكية – طب عين شمس .

ثم الأشخاص الذين تعاونوا معي في البحث

١) د.
٢) د.
٣) د.

وكذلك الهيئات الآتية

١)
٢)
٣)

جامعة عين شمس

معهد الدراسات العليا للطفولة

رسالة ماجستير / دكتوراه

أسم الطالب : عصام الدين فهمي الخولي

عنوان الرسالة : الحاجز المعوي ودلالات الأمتصاص في الأطفال المصابين بالأمساك وعلاقتها بالمتغيرات النفسية .

أسم الدرجة : (ماجستير / دكتوراه)

لجنة الأشراف

١- الأسم / أ.د حامد محمود شتلة — الوظيفة / أستاذ طب الأطفال محافظ الشرقية .

١- الأسم / أ.د جمال سامي علي — الوظيفة / أستاذ بقسم الطبي بمعهد الدراسات العليا للطفولة .

١- الأسم / أ.د مصطفي عبد العزيز هدهد — الوظيفة / أستاذ مساعد طب الأطفال – طب عين شمس .

١- الأسم / أ.د داليا حلمي فرج — الوظيفة / أستاذ الباثولوجيا الأكلينيكية – طب عين شمس .

تاريخ البحث : ١٦ / ٣ / ١٩٩٩

الدراسات العليا

ختم الإجازة :

موافقة مجلس الكلية

٢٦ / ٣ / ٢٠٠٢

أجيزت الرسالة بتاريخ ٢٨ / ١ / ٢٠٠٢

موافقة مجلس الجامعة

/ /

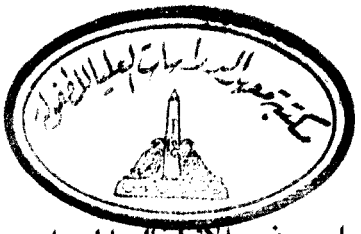
جامعة عين شمس
معهد الدراسات العليا للطفولة
قسم الدراسات الطبية

صفحة العنوان

أســـم الطالـــب : عصام الدين فهمي الخولي
الدرجة العلمية : دكتوراه في دراسات الطفولة
القسم التابع له : الدراسات الطبية
أســـم المعـــهد : معهد الدراسات العليا للطفولة
الجامـــعـــــــة : عين شمس
سنـــة التخـــرج :
سنة المـــنـــــح :

شروط عامة
يوضع شعار الجامعة علي الغلاف الخارجي

معهد الدراسات العليا للطفولة
قسم الدراسات الطبية



الحاجز المعوى ودلالات الإمتصاص فى الأطفال المصابين بالإمساك وعلاقتها بالمتغيرات النفسية

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رسالة للحصول على درجة دكتوراة الفلسفة
فى دراسات الطفولة للطبيب

عصام الدين فهمى الخولى
ماجستير طب الأطفال – كلية الطب جامعة عين شمس

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جامعة عين شمس

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أستاذ الباثولوجيا الأكلينيكية - كلية الطب
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