From Department of Medicine, Huddinge,
Division of Gastroenterology and Hepatology
Karolinska Institutet, Stockholm, Sweden

EVALUATIONS OF MEASUREMENT TECHNIQUES IN FUNCTIONAL DYSPEPSIA:

Electrogastrography, satiety drinking test and ^{14}C-urea breath test

Shahab Abid

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Dedicated to my mother- Najma Abid,
sister- Farah Abid and wife- Fauziah Rabbani

ABSTRACT

The overall aim of this thesis was to evaluate the clinical usefulness of certain measurement techniques that may be helpful in the understanding of functional dyspepsia (FD).

Electrogastrography (EGG) parameters were evaluated and found unable to discriminate one specific motility disorder from other motility disorders of the gut. Moreover the relationship between the electrical activity of the stomach determined by EGG and contractile activity of the gastric antrum assessed by antro-duodenal manometry (ADM) were analyzed and no spatial correlation between EGG and ADM was observed. These results suggest that the clinical usefulness of EGG in the diagnosis of a motility disorder is generally poor and EGG and ADM measure two different aspects of gastric physiology.

The validity of ^{14}C urea breath test (UBT) was compared with histology and rapid urease test (RUT) for the diagnosis of *H. pylori* infection. Both sensitivity and specificity of ^{14}C UBT for the diagnosis of *H. pylori* infection were high. This study also indicated a very good concordance between UBT, histology, and RUT for the diagnosis of *H. pylori* infection. These observations suggest that ^{14}C UBT is a reliable test for diagnosis of *H. pylori* infection.

Satiety drinking tests were done in healthy volunteers by using drinking water at 100 ml/minute and nutrient drinks at 20 and 100 ml/minute. It was found that the maximum tolerated volume (MTV) determined by slow nutrient drinking (SND) was not influenced by body mass index, age and gender compared to rapid water and rapid nutrient drinking. Moreover there was more symptom generation after drinking nutrient liquid at a slow rate. These results suggest that SND is more meaningful in the assessment of MTV. This study also determined the normal values of MTV for satiety drinking tests in a Pakistani population.

The relationship between MTV and dyspepsia symptoms in patients with FD before and after the treatment was assessed. We found a significant correlation between dyspepsia symptom severity scores and MTV. Some of the individual symptom severity scores also correlated with MTV before and after treatment. The findings of

this study indicate that determination of MTV by SND can be a surrogate marker for symptoms severity in patients with FD.

This thesis concluded that the diagnostic usefulness of EGG is limited, that ^{14}C UBT is a reliable test for the diagnosis of *H. pylori* infection, that SND is a better test for the assessment of MTV compared to rapid water and nutrient drinking, and that MTV determined by SND may be useful for the assessment of response to treatment in patients with FD.

LIST OF PUBLICATIONS

1. **Abid S**, Lindberg G. Electrogastrography: Poor correlation with antro-duodenal manometry and doubtful clinical usefulness in adults. World J Gastroenterol 2007; 13:5101-5107

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4. **Abid S**, Azam Z, Siddiqui S, Quadri Z, Awan S, Jafri W, Lindberg G. Satiety drinking test volumes correlate to symptom severity scores in functional dyspepsia. Submitted.

The studies will be referred to by their Roman numeral I-IV

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LIST OF ABBREVIATIONS

FD	Functional dyspepsia
FGID	Functional gastrointestinal diseases
IBS	Irritable bowel syndrome
GERD	Gastroesophageal reflux disease
PDS	Postmeal distension syndrome
EPS	Epigastric pain syndrome
MRI	Magnetic resonance imaging
SPECT	Single photon emission computerized tomography
ADM	Antro-duodenal manometry
EGG	Electrogastrography
UBT	Urea breath test
SSRI	Selective serotonin reuptake inhibitor
TCA	Tricyclic antidepressants
MTV	Maximum tolerated volume
EGD	Esophago-gastro duodenoscopy
ANOVA	Analysis of variance
PPV	Positive predictive value
NPV	Negative predictive value
NERD	Non-erosive reflux disease
GI	Gastrointestinal
SND	Slow nutrient drinking
RND	Rapid nutrient drinking
RWD	Rapid water drinking
NDIRS	Non-dispersive isotope selective infrared spectrometers

1 INTRODUCTION

Dyspepsia is derived from the Greek words *dys* and *pepse*, which mean an upset stomach or indigestion. The term functional dyspepsia was coined by one of the most well-known medical writers, Walter Clement Alvarez (1884-1978). He was a physician who investigated the electrical activity of the stomach and was also the inventor of electrogastrography (EGG) - a new diagnostic technique.

1.1 Historical perspective

In the early 18th century dyspepsia was classified into dietary, moral or nervous dyspepsia (1). Sir Charles Darwin's illness in the 18th century provides an interesting link to functional dyspepsia. Charles Darwin's adult life was affected by repeated gastric symptoms which severely compromised his quality of life. Darwin intermittently suffered from a combination of symptoms: malaise, vertigo, muscle spasms, vomiting, abdominal colics, bloating, nocturnal intestinal gases, headaches, severe tiredness, nervous exhaustion, dyspnea, depression and many more. Several explanations were given for his illness, including diseases like Meniere's diseases, Chaga's disease, cyclic vomiting syndrome, nervous dyspepsia and most recently according to Barry Marshall *H. pylori* infection (2).

A German internist, Wilhelm Otto Leube (1842-1922), is remembered for his work with gastric and intestinal disorders notably his research in nervous dyspepsia (3). Leube believed that gastric irritation was often caused by the effects of food on the sensory nerves of the stomach. Leube performed extensive studies on indigestion and introduced a procedure known as 'intubation' to retrieve gastric contents for analysis. He also introduced 'test meals' of different types of food, which were later retrieved from patients via Leube's "gastric tube". This helped him to estimate the degree of digestion of the test meal, as well as the quantity and concentration of acid and pepsin in the patients' stomach. He developed the Leube-Rosenthal Meat Solution in collaboration with Isidor Rosenthal - a physiologist.

An upsurge in the subject of functional dyspepsia (FD) came after the re-discovery of *H. pylori* by Barry Marshall and Robin Warren as a cause of peptic ulcer disease in the

early 1980s (4). Later in the 1980s the Rome process evolved and elaborated symptom criteria for defining functional dyspepsia. Despite extensive research in the etio-pathogenesis of functional dyspepsia, FD remains a heterogeneous disorder of unknown etiology, unsatisfactory treatment, diverse manifestations, and unpredictable course.

1.2 The Rome process

The "Rome process" is an international effort to formulate symptom criteria for the diagnosis of functional gastrointestinal disorders (FGID). The latter include irritable bowel syndrome (IBS), functional dyspepsia (FD) and several other functional disorders of gastrointestinal tract. The Rome criteria are issued after passing through a consensus process, using the Delphi technique (5). The Rome process typically takes many months of work by investigators, organized into various committees. The committees work by mail and telephone conferences until the final meeting, which takes place in Rome, Italy.

1.2.1 Rome I

In 1991, the Rome committee defined dyspepsia as persistent or recurrent abdominal pain or abdominal discomfort centered in the upper abdomen (6). According to this definition, abdominal discomfort could be a post-prandial fullness, bloating, nausea, vomiting, belching or early satiety. Symptoms can occur continuously or intermittently. Rome criteria identified three categories of dyspepsia: (i) dyspepsia with an identified cause that if treated led to improvement, such as chronic peptic ulcer disease, reflux esophagitis, malignancy, or hepatobiliary disease; (ii) dyspepsia with identified abnormalities of uncertain significance like *H. pylori* infection and gastroparesis; and (iii) dyspepsia with no explanation identified. The term FD was used for dyspepsia with identified underlying mechanisms of uncertain significance or dyspepsia for which no explanation was available.

The definition of FD was further elaborated by dividing it into three subgroups; (i) ulcer-like dyspepsia with predominant pain symptoms localized to the upper abdomen, relieved by taking food or antacid or H_2 antagonist or proton pump inhibitors. (ii) dysmotility -like dyspepsia where three or more of the following symptoms; early

satiety, nausea, recurrent retching or vomiting, bloating without visible distension, or abdominal discomfort often aggravated by food. The latter subgroup excludes abdominal pain as the predominant symptom (iii) unspecified FD.

Moreover all dyspeptic symptoms should be present for at least three months. In the first version of the Rome criteria a fourth group (reflux-like dyspepsia) was also defined (7).This was deleted in the Rome 1991 paper.

1.2.2 Rome II

In 1999, the Rome committee partially changed the criteria for dyspepsia, although the definition of dyspepsia was not altered (8). The committee's main focus was to align one specific predominant symptom with underlying Rome I based sub-groups of FD as defined above. The purpose of categorizing sub-groups of FD by a predominant symptom was to target treatment towards the predominant symptom. Rome II also revised the reporting duration of all symptoms. It elaborated that the symptoms had to be present for at least 12 weeks over a 12 months period which need not to be consecutive.

Both Rome I and II criteria for FD received criticism. It was observed that patients were frequently unable to distinguish abdominal pain from discomfort. The word *predominant symptom* (Rome II) was ambiguous and classifying patients based on symptoms could not identify subgroups with homogeneous underlying patho-physiological mechanisms. The duration and time course specification for dyspepsia also deemed to be clumsy (9).

1.2.3 Rome III

In 2006, the Rome committee made several changes of the functional dyspepsia classification (10). The committee described a more elaborative localization of the pain in the epigastric area. The term abdominal discomfort was abandoned and the key concept was replaced by the terms post-prandial fullness and early satiety. Rome III changed the predominant symptom-based sub-groups of FD into new categories with several symptoms into each category. The term functional dyspepsia was abandoned for research purposes in favor of a more detailed classification of functional gastro-duodenal disorders Table 1.

Table1. Functional gastro-duodenal disorders according to the Rome-III classification (10).

B1. Functional dyspepsia
B1a. Postprandial distress syndrome
B1b. Epigastric pain syndrome
B2. Belching disorders
B2a. Aerophagia
B2b. Unspecified excessive belching
B3. Nausea and vomiting disorders
B3a. Chronic idiopathic nausea
B3b. Functional vomiting
B3c. Cyclic vomiting syndrome
B4. Rumination syndrome in adults

1.3 Epidemiology of functional dyspepsia

In clinical practice dyspepsia is a very common condition and up to one in 20 of all consultations in primary health care are for dyspepsia symptoms (11). A review by Agréus reported the prevalence of dyspepsia to range from 7-63 % while another review estimated it to be 2.5% - 41% (12, 13). This wide range is due to variability in the definition of dyspepsia. In a Swedish population based upper endoscopy study, the prevalence of FD was 16% and in another recently published population-based study showed that FD was present in 11% of the Italian general population (14, 15). The same study also documented that two distinct sub-groups of FD as described in Rome III exist in the general population.

Functional dyspepsia is a heterogeneous disorder with a fluctuation of symptoms and very little is known about its natural history. Symptoms of FD shift back and forth with gastro-esophageal reflux disease (GERD) and IBS. Studies have demonstrated both short-term and long-term fluxes of dyspeptic symptoms (12, 16). A study from Sweden noted some symptom fluctuation in the shorter term, but troublesome gastrointestinal complaints remained in approximately 90% of subjects over a 1-6 month period (17). In Japan, a 40 % prevalence of functional gastrointestinal diseases (FGID) was observed with overlapping functional bowel disorders (18).

1.4 Physiological dysfunctions in functional dyspepsia

FD is associated with physiological dysfunctions in the gastro-duodenal region. However, there is no uniform pattern of patho-physiological abnormalities found in all FD patients. Various mechanisms have been described.

1.4.1 Impaired gastric emptying

Studies have shown delayed gastric emptying occurs in approximately 20-50% of patients with FD (19, 20). In a meta-analysis of 17 studies, involving 868 patients with dyspepsia and 397 controls, significant delay of solid gastric emptying was present in almost 40% of patients with FD (21). However, some studies have documented a very weak association between delayed gastric emptying and dyspepsia symptoms (22, 23). In a subset of patients with FD, rapid gastric emptying has also been observed (24). Hence the relationship between FD and gastric emptying is not unified and remains controversial.

1.4.2 Increased visceral hypersensitivity

Increased perception of visceral stimuli has been considered as one of the major patho-physiological mechanisms in functional gastrointestinal disorders (25). Studies have demonstrated that a sub-set of patients with FD have enhanced sensitivity to isobaric gastric distension (26, 27). The exact site of hypersensitivity generated in these patients is however unknown. Two possible sites for generating hypersensitivity in these patients are the central nervous system and enteric nervous system especially visceral afferents.

In context with visceral hypersensitivity, several receptors have been identified as potential targets for future drug development. These include transient potential receptor vanilloid type I (TPRV1), voltage-gated sodium channels, ATP, acid sensing ion channel (ASICs), PAR-2, cannabinoid, prostaglandin, tachykinin, and 5HT-receptors (28).

1.4.3 Impaired gastric accommodation

Accommodation of the stomach is a vagus nerve-mediated reflex resulting in receptive relaxation of the proximal stomach after intake of a meal. Gastric accommodation

provides the meal with a reservoir and enables the stomach to handle intra-gastric volumes without a proportional increase of intra-gastric pressures. It is speculated that impaired accommodation of the proximal stomach during and after the ingestion of a meal may be accompanied by increase in intra-gastric pressure which activates mechanoreceptors in the gastric wall, thus inducing dyspeptic symptoms. Using a barostat, impaired gastric accommodation was demonstrated in 40% of patients with FD who presented with symptoms of early satiety and weight loss (29, 30).

1.4.4 Other putative pathogenetic mechanisms for FD

Bacterial gastroenteritis in adults may be a predisposing factor for FD in a subset of patients (31, 32).

Similarly untreated celiac disease is associated with intestinal inflammation. In untreated celiac disease, a high prevalence of delayed gastric emptying and of abnormalities on antroduodenojejunal manometery was found (33, 34). A gluten-free diet normalized gastric emptying rate (33), whereas persistence of motor abnormalities on antroduodenaojejunal manometry was associated with ongoing mucosal abnormalities suggestive of incomplete dietary adherence (34). Food intolerance has been debated for a long time as a factor causing functional dyspepsia. In patients with food intolerance, ingestion of a meal is associated with a rise in dyspeptic symptoms which is significant within 15 min after the start of the meal, and persists for at least 4 hours post-prandially (35). The underlying mechanism is unclear but sensorimotor dysfunction is a likely possibility (35, 36). Autonomic imbalance especially impaired vagal activity has also been implicated in the pathogenesis of FD (37).

1.5 Symptoms and patho-physiological mechanisms based categorization of FD

Attempts have been undertaken to classify patients with FD according to types or groups of symptoms that would reliably identify patients with different underlying patho-physiological mechanisms. This would help in directing therapy towards the underlying patho-physiology (38). However in clinical practice this classification showed great overlap between subclasses. Moreover a detailed analysis has revealed

that the predominant symptom does not reliably identify patho-physiological subsets (39, 40).

In a slightly different approach patho-physiology-based sub-groups of FD were formed and an attempt was made to find association between patho-physiological abnormalities and the dyspepsia symptoms (41). Thus, associations were demonstrated between delayed gastric emptying and symptoms of fullness, nausea, and vomiting (41). Moreover an association was also found between impaired fundic accommodation, early satiety and weight loss (41). A recent study on a large group of patients with FD in a tertiary care setting performed factor analysis of symptom patterns. This study showed that in patients with FD the presence of weight loss was strongly associated with early satiety and also with nausea and vomiting (42).

1.6 Etiologic factors associated with FD

Several factors have been suggested to play a significant role in the etiology of FD.

1.6.1 Genetic factors

There is increasing evidence that susceptibility to functional GI disorders is influenced by the presence of genetic factors (43). Homozygous GNB3 825C carrier status was found to be associated with unexplained predominantly upper abdominal symptoms (44). Recently, a study identified post-prandial distension syndrome (PDS) in Japanese male subjects with cholecystokinin intron carrier state (45). Similarly, another study from Japan showed association of IL-17F and MIF gene polymorphisms with the development of FD in *H. pylori*-infected patients (46).

1.6.2 Psychosocial disorders

A high score on psychomotor scale has been observed among patients with FD compared to normal individuals. Study has suggested that psychosocial stress, mood symptoms and coping style are the major predictors of FD (47). A recent population-based study found that anxiety was linked to uninvestigated dyspepsia and FD (14). The same study, however, did not find depression as an associated factor in patients with FD.

1.6.3 Helicobacter pylori

Several attempts have been made to determine if there is a relationship between *H. pylori* infection and functional dyspepsia. However, no consistent difference in the prevalence or severity of individual dyspeptic symptoms and physiological dysfunction were found between *H. pylori* positive and negative subjects (23). Moreover, the effects of eradication of *H. pylori* on epigastric symptoms of dyspepsia are debatable. A Cochrane meta-analysis suggested a small but significant beneficial effect of eradicating *H. pylori* on symptoms associated with FD (48). Therefore, the influence of *H. pylori* on the pathogenesis and symptom generation in patients with FD remains unclear.

1.7 Measurement techniques in FD

FD is a diagnosis of exclusion referring to absence of typical symptoms of GERD, and a normal upper GI endoscopy. There is no specific test or a biomarker available for the diagnosis of FD. However certain measurement techniques have been used for the evaluation of pathological changes and physiological dysfunction in patients with FD. Most of these measurement techniques require a tertiary care setting and their feasibility for the daily clinical practice is doubtful.

1.7.1 Magnetic resonance imaging (MRI)

MRI is a non-invasive and radiation-free technique, which can be use for the assessment of gastric emptying of both solids and liquids. An initial validation study showed good agreement between MRI and single photon emission computerized tomography (SPECT) for both solid and liquid meal gastric emptying (49). It is now possible to assess the gastric motility index (calculated from the frequency, amplitude, velocity and moving distance of peristaltic waves in the gastric antrum) reliably with MRI (50). The reproducibility of MRI in the assessment of gastric emptying and antral motion has been established (51). A recent study demonstrated MRI to be a sensitive technique for simultaneous measurement of gastric emptying and gastric motility index (52). The prolonged procedure time and positioning of the patient however have some technical and general limitations.

1.7.2 Single photon emission computerized tomography (SPECT)

SPECT is a non-invasive technique considered to be a gold standard in the assessment of gastric emptying. This technique has been extensively validated for the measurement of gastric volumes (53). When tested for reproducibility by using same caloric meal the coefficient of variation was approximately 10%. Gastric volume estimation by SPECT is a surrogate marker of gastric accommodation as well. A new application of SPECT (with significant potential interest) is the simultaneous measurements of gastric emptying and volume (54)

In comparison with Barostat, SPECT is a non-invasive method. However, SPECT cannot provide information about gastric tone, nor about gastric sensory responses. The SPECT technique is promising for investigation of gastric volumes in health and disease and for the assessment of the effects of pharmacological agents on gastric volume and emptying (41).

1.7.3 Barostat measurement

The barostat technique is regarded as the gold standard for assessing gastric accommodation. A 1200 ml balloon is passed into the stomach and unfolded by inflation of 500ml air and then positioned in the proximal stomach. The bag is connected to a pump through a double lumen tube. The pump is computer-controlled and can maintain a constant pressure or a constant volume of the bag. Both gastric sensitivity and accommodation can be studied using the barostat (55, 56). However, the barostat technique is invasive, uncomfortable and stressful for the patient.

1.7.4 Ultrasonography

Ultrasonography is a non-invasive technique, which has been tested for the assessment of gastric emptying, antral motility and trans-pyloric flow. Assessment of gastric accommodation by ultrasonography is evolving and two-dimensional ultrasonography of the proximal stomach has been used to demonstrate volume changes after meal and its impairment in patients with FD (57-61). Recently real time ultrasound technique has been used for the assessment of trans-pyloric flow and duodeno-gastric reflux (62). Ultrasonography has the advantage of being widely available. However, ultrasonographic assessment of gastric motility disorders requires technical expertise and the interpretation is operator dependent.

1.7.5 Antro-duodenal manometry (ADM)

The gastric antrum and duodenum generate lumen-occluding pressure activities, which are suitable for manometric recordings because of relatively narrow lumen of antro-duodenal region compared to the body of stomach. ADM is an invasive test, involving intestinal intubation and fluoroscopy for accurate catheter placement. Currently, this technique is used to differentiate whether the documented slow gastric or small bowel transit is due to neuropathy, myopathy or undetected mechanical obstruction. ADM is also indicated for the diagnosis of intestinal pseudo-obstruction (63). The role of antro-duodenal manometry is limited because of the technical challenges involved in its performance and the expertise required for its interpretation (64).

1.7.6 Electrogastrography (EGG)

Electrogastrograpy was introduced early in the 20th century (65). This test measures the slow wave electrical activities trans-cutaneously from the gastrointestinal (GI) tract. EGG is non-invasive and practically feasible. EGG detects rhythm and power (amplitude) of gastric myoelectricity. Studies have shown a good correlation between cutaneous EGG recordings and myoelectrical signals recorded from gastric serosal leads (66).

Although feasible, there are major concerns regarding the clinical usefulness of EGG. The placing of electrodes, skin preparation, stomach distension/contraction and EGG filtering and recording systems all influence the obtained EGG parameters. Moreover, there are no standard recommendations for the position of the patient during recording, recording periods and test meals. Lack of standardization for the equipment used to conduct EGG and the software for analysis of wave forms also remains a challenge. It appears that an acceptable consensus is needed to define the abnormalities based on the EGG recording when it is used to assess stomach motor dysfunction (67, 68)

1.7.7 Satiety drinking test

The satiety drinking test has been proposed as a surrogate method for estimating gastric volumes. Several versions of this test are available that differ from each other by the nature of liquid use for drinking (water, nutrient drink or meat soup) and the speed of drinking, ranging from 15 to 100 ml/min (29, 69). A previously published study has suggested that a high-caloric, slowly administered drinking test compares favorably

with the barostat in predicting impaired gastric accommodation (69). Satiety drink test is inexpensive and non-invasive. However there is ongoing controversy whether the satiation test reflects exclusively gastric accommodation or a combination of accommodation, sensation, and emptying (70).

1.7.8 Breath tests

1.7.8.1 ^{13}C-octanoic acid breath test

Solid meal gastric emptying can be determined by ^{13}C-octanoic acid breath test. The nonradioactive isotope, ^{13}C is usually bound to a medium chain triglyceride (octanoic acid) which is incorporated into a standardized test meal. The ^{13}C-labeled substrate is ingested, emptied by the stomach, and absorbed in the proximal gut. This is a reproducible technique with a very low inter-individual variability (71, 72). The substrate that is commonly used to measure gastric emptying of solids by breath test is the ^{13}C-octanoic acid. The substrate is rapidly absorbed in the duodenum, metabolized in the liver where $^{13}CO_2$ is formed and then exhaled rapidly in the breath (73). Octanoic acid breath test is a safe and radiation-free technique. The ^{13}C-octanoic acid breath test gives an indirect measure of gastric emptying and results may depend upon several factors such as the patient's age, weight and the level of physical activity during the test. Results can also be influenced by catabolic states and intestinal bacterial overgrowth. However, a recently published study with a large sample size showed a very small effect of such factors on the estimation of gastric emptying by ^{13}C octanoic acid breath test (74).

1.7.8.2 Urea breath test (UBT)

Urea breath test is one of the most reliable non-invasive techniques for the diagnosis of *H. pylori* infection. It comes in two versions ^{14}C and ^{13}C isotopes of carbon; the former is radioactive while the latter is not. This test is based upon the property of *H. pylori* to produce urease, an enzyme that splits urea into ammonia and bicarbonate. The carbon is liberated and it is measured in the exhaled air. Sensitivity and specificity are greater than 90% (75). However, there are certain limitations of using UBT in patients who were taking antibiotics and PPI, which may give rise to false negative results (76-78).

1.8 Treatment of FD

A strong physician-patient relationship is of the utmost importance in treating individual patient with FD so that reassurance and education may be provided. Dietary modification, such as ingestion of a low fat diet and small frequent meals may be beneficial, but data is scarce. Empiric acid suppression with H_2-receptors angonist (H2RAs) or PPIs is likely superior to placebo in treatment of FD, but those patients with meal-related symptoms are least likely to respond. Acid reduction is effective in reducing symptoms up to 35% of patients with non-ulcer dyspepsia, possibly due to unrecognized non-erosive reflux disease (79).

1.8.1 *Helicobacter pylori* eradication

Test and treat strategy for associated *H. pylori* infection is recommended for patients with uninvestigated dyspepsia when there are no alarming features. Eradication of *H. pylori* is a preferable strategy for patients with uninvestigated dyspepsia in areas where prevalence of *H. pylori* is 20% or higher (80). A Cochrane meta-analysis has shown a small but significant benefit from eradication of *H. pylori* in patients with FD that will reduce the risk of symptoms by 10% and NNT required to improve the condition of one patient is 14 (48).

1.8.2 Prokinetics

Experience with the use of prokinetics in functional dyspepsia is limited. Domperidone which is a D_2 antagonist that acts peripherally to increase antral motility; therefore, less potential for central side effects exists. One trial comparing metoclopramde and domperidone demonstrated no significant difference (81). Domperidone is not freely available worldwide. There are some other emerging prokinetic agents such as itopride which is D_2 receptor antagonist with acetylcohlinesterase inhibitory and low central activities. However the clinical usefulness of itopride in FD patients is controversial (82). Erythromycin a motilin agonist stimulates gastric and duodenal motility by acting on smooth muscles and enteric nerves. Erythomycin may promote gastric emptying but does not improve dyspeptic symptoms. There are issues of tachyphylaxis and impairment of meal induced gastric accommodation with the use of erythromycin (83).

1.8.3 Antidepressants

Selective serotonin reuptake inhibitors (SSRI) increase the amount of serotonin within the synapse by inhibiting the serotonin reuptake transporter in both the CNS and ENS. This leads to increased availability of serotonin to the enteric nerves, which may alter sensorimotor functions of the gut. Almost no data regarding the efficacy of SSRIs in the treatment of patients with FD exist.

Tricyclic antidepressants (TCA) block serotonin and nor-epinephrine reuptake pumps. Hence, TCA have the potential to stimulate serotonin and noradrenergic neurons and thus affect sensorimotor functions of the gut, as well as analgesic pathways. Very few studies with TCAs in the treatment of FD have been completed. Amytriptiline- a TCA has shown decreased perception of symptoms compared to placebo in patients with FD but it was not associated with decreased perception to gastric distension (84).

1.8.4 Other agents for treatment of functional dyspepsia

Visceral hypersensitivity is increasingly recognized as a patho-physiologic mechanism of FD. Possible therapeutic targets include certain receptors involved in perception such as opioid, neurokinin and vanilloid receptors. Drugs targeting receptors involved in pain perception, are under development, yet no conclusive data is available to support their use at present (85).

2 AIMS OF THE STUDY

The overall aim of this project was to evaluate the clinical usefulness of certain measurement techniques that may aid in the understanding of FD.

Study I

To evaluate the relationship between the electrical activity of the stomach determined by electrogastography and antral contractile activity measured by antro-duodenal manometry and to study whether the underlying motility disorder could be predicted from EGG parameters.

Study II

To determine the validity and cost of microdose ^{14}C-UBT in comparison with histology and rapid urease test for the diagnosis of *H. pylori* infection.

Study III

To compare the maximum tolerated volume (MTV) of taking water and a nutrient liquid at two different rates of drinking and to assess the effects of age, gender and body mass index on MTV.

Study IV

To find out if there is a relation over time between changes in gastric accommodation determined by a satiety drinking test and changes in symptom severity scores in patients with functional dyspepsia after four weeks of treatment.

3 MATERIAL, METHODS, AND STUDY DESIGN

Study I was a retrospective analysis done on a preexisting large data set of patients with motility disorders who underwent a combined antro-duodenal manometry and EGG test for the evaluation of their disease. Study II, III, and IV were prospectively designed. Materials, methods and study designs are summarized in Table 2.

Table 2. Summary of material, methods, and study design.

Study	**I**	**II**	**III**	**IV**
No. of participants	148	94	42	132
Design	Retrospective Observational	Prospective Experimental validation	Prospective Experimental	Prospective Interventional
Data source and Method	Medical records	Functional dyspepsia patients who underwent endoscopy, urea breath test, rapid urease test, and gastric biopsy with histology	Healthy volunteers Satiety drinking tests	Functional dyspepsia patients, who underwent endoscopy, urea breath test, satiety drinking test. These patients received proton pump inhibitor and/or anti-*H. pylori* treatment. Urea breath test and satiety drinking test were repeated after treatment
Data analysis	Descriptive and statistical analysis	Descriptive and statistical analysis	Descriptive and statistical analysis	Descriptive and statistical analysis

3.1 Study I

The data was collected between 1994-2001. A total of 148 patients were evaluated for EGG parameters and 144 for correlations between EGG and ADM parameters. There were 21 EGG variables and 8 ADM variables that were analyzed and compared. A single channel EGG was used and Polygram version 6.40 was applied for data analysis. Similarly ADM was performed by a flexible 6-8 channel catheter connected to a pneumo-hydraulic pump. Data logging was done with a Polygraph 12HR and the

Polygram (Medtronic Synectics, Stockholm, Sweden) software. The protocol included a 3-hour fasting period followed by a test meal (500 kcal standardized mixed meal) and a 2-hour post-prandial recording.

3.2 Study II

Consecutive patients with dyspeptic symptoms, undergoing upper GI endoscopy were enrolled. During upper GI endoscopy, gastric biopsies were taken for histopathological examination and rapid urease test. A ^{14}C-UBT was done by asking the patient to swallow 37 kBq (1 uCi) of an encapsulated form of ^{14}C-urea/citric acid composition (Helicap, Noster System AB, Stockholm, Sweden) with water after endoscopy. Breath samples were collected with a special dry cartridge system 10 minutes after swallowing the UBT capsule. Results were expressed both as counts per minute (CPM) and as grade (0: not infected; CPM < 25, 1: equivocal; CPM 25-50, 2: infected; CPM > 50), which was suggested by the manufacturer according to the counts obtained from the cartridges. Grades 0 and 1 were considered negative for the detection of *H. pylori*.

3.3 Study III

This study comprised 42 healthy adult volunteers who were free from any gastrointestinal disease, had no abdominal complaints, and no history of abdominal surgery. They were subjected to satiety drinking tests on three different occasions. On the first occasion, subjects were also interviewed and their gender, age, height, weight, and BMI were recorded. On subsequent visits the other two drinking tests were taken. The study subjects drank plain water at a rate of 100 ml/min in one test (RWD = rapid water drinking). In the other two tests, study subjects drank a nutrient drink at a rate of 100 ml/min (RND = rapid nutrient drinking) or 20 ml/min (SND = slow nutrient drinking). During satiety drinking tests the study subjects scored their satiety level using a graphic rating scale that combined verbal descriptors on a scale graded from 0 to 5. Study subjects were instructed to stop drinking when they reached a score of 5. Symptoms of bloating, nausea, and abdominal pain were graded on a visual analogue scale (0-100) 30 min after cessation of drinking.

3.4 Study IV

Patients with FD were enrolled after initial screening by a Rome-III based questionnaire. All patients underwent a diagnostic esophago-gastro-doudenoscopy (EGD). Patients with gastric or duodenal erosions as well as those with ulcers, polyps or malignancy were excluded. Similarly, patients with predominant symptoms of GERD or endoscopic evidence of esophagitis were excluded. Patients with abnormal complete blood count, liver function tests, creatinine or abnormal upper abdominal ultrasound investigation were also excluded and so were patients who had received antibiotics in the last two weeks (e.g. clarithromycin, amoxicillin or metronidazole) or who had been treated in the past for *H. pylori* infection or received non-steroidal anti-inflammatory drugs (NSAIDs). A ^{14}C-urea breath test (UBT) was done in all patients after EGD. Patients were considered to have *H. pylori* infection if they had both positive UBT and evidence of *H. pylori* on histology. A satiety drinking test using nutrient drink at a rate of 30 ml/min was done in all patients before and 10 days after completion of four weeks' treatment. Dyspeptic symptom severity was scored using a global overall symptom scale (GOS) at the same timepoints as the drinking test.

4 STATISTICAL METHODS

Descriptive analyses were done in all four studies and results were expressed as mean ± SD. In study I, logarithmic transformation was done for some data that was not normally distributed. Specific statistical methods applied in each study are described in Table 3. Description and rationale of some statistical tests used in the analysis of the studies presented in this thesis are described below.

4.1 Student's t-test

Student's t-test is applied to analyze research questions involving two means. The t-test assesses whether the means of two groups are *statistically* different from each other. Student's t-test can be use to assess the differences of the means between two independent samples (unpaired t-test) or between two measurements in the same sample (paired t-test). In study IV, the assessment of MTV was done before and after treatment. Since the same study subjects were compared for assessment, the paired t-test was used.

4.2 Mann-Whitney U-test

This is one of the best known non-parametric tests for comparing two independent samples of observations if the normality assumptions are not satisfied. In study I the data for EGG variables was transformed to logarithmic values in order to make variables more normally distributed. Some EGG variables in study I (such as percentages of normogastria, bradygastria, tachygastria and dominant frequency) were not normally distributed and these were analyzed with Mann-Whitney U-test.

4.3 Log-rank test

This test is sometimes called the Mantel-Cox test. It is used for comparing the survival distributions of two samples. It is a non-parametric test widely used in clinical trials to establish the efficacy of a new treatment compared to a control treatment when the

measurement is the time to an event (such as time from initial treatment to a heart attack). In study III, the log-rank test was used for comparing maximum tolerated volumes between male and female groups. The event in study III was the achievement of complete satiety that prevents further drinking of nutrient liquid. Instead of time we have the amount of nutrient drink that was ingested before reaching the maximum satiety. Since a specified amount of nutrient liquid per time unit was taken, volume becomes a surrogate marker for time and it was feasible to use the log-rank test.

4.4 Analysis of variance (ANOVA)

One-way ANOVA is a statistical test which compares the means of several groups and therefore generalizes students two sample t-test to more than two groups. In study III, ANOVA was used to compare the three different satiety drinking tests and symptoms score that the study subjects developed.

4.5 Pearson's correlation

In statistics, the Pearson's product-moment correlation coefficient (sometimes referred to as PMCC, and typically denoted by 'r' is a measure of the correlation (linear dependence) between two variables X and Y, giving a value between +1 and −1 inclusive. It is widely used in science as a measure of the strength of linear dependence between two variables. A key mathematical property of the Pearson correlation coefficient is that it is invariant to changes in location and scale, i.e. changing the scale of either the X or the Y variable will not change the size of the correlation coefficient

The Pearson's correlation test was extensively applied in study I, III and IV. In study I, ADM variables were compared with EGG variables. Similarly, in study III, the MTV measured by three drinking tests were compared and the relationship between MTV with age and BMI was assessed by using Pearson's correlation.

In study IV, Pearson correlation was extensively utilized in order to understand the relationship between dyspepsia symptom severity scores and MTV. Correlations

between dyspepsia symptoms and MTV were analyzed at baseline and after 4 weeks of treatment.

Table 3. Summary of overall statistical methods applied in various studies.

Function	**Statistical method**	**Study**			
		I	**II**	**III**	**IV**
Frequency distribution using mean with standard deviation, median with range and percentages	Descriptive statistics	X	X	X	X
Comparing means normally distributed data	Student's t-test			X	
Comparing ranks non-parametric	Mann-Whitney U-test	X			
Comparing survival distribution in two samples	Log-rank test			X	
Survival proportion of the groups	Kaplan-Meier plot			X	
Comparing means of several groups	Analysis of variance			X	
Measure of correlation linear dependence	Pearson's product moment correlation	X		X	X
Explore the relationship between dependent and independent variables.	Multiple linear or logistic regression	X		X	X
Reliability of a investigational test for diagnosis	Specificity, sensitivity, negative and positive predictive values		X		
Adjusting p-values for multiple comparisons	Bonferroni's test				X
Agreement between observations	Cohen's kappa		X		

4.6 Multiple regression analysis

Regression analysis is applied to understand which among the independent variables are related to the dependent variable, and to explore the forms of these relationships. When three or more measurement variables are available one of the measurement variables is the dependent (Y-axis) variable. The rest of the variables are the independent (X-axis) variables. The purpose of a multiple regression is to find an equation that best predicts the Y variable as a linear function of the X variables. We have used logistic regression in study I to determine EGG variables that were of independent value for predicting underlying diagnoses.

Multiple regressions were also used in study III and IV in order to evaluate the effects of various factors such as age, gender, weight and BMI on MTV.

4.7 Sensitivity, specificity, and negative and positive predictive values

The accuracy of a diagnostic test or procedure has two aspects. The first is the test's ability to detect the condition it is testing for, thus being positive in patients who actually have the condition; this is the sensitivity of the test. If a test has high sensitivity it has low false negative rate. Sensitivity is the proportion of positive that are correctly identified by the test. The other aspect of the accuracy is the test's ability to identify those who do not have the condition. This is called the specificity of the test. If the specificity of a test is high it has a low false positive rate. Specificity is the proportion of negatives that are correctly identified by the test.

The positive predictive value of a test is the percentage of patients with a positive test result who actually have the condition. The negative predictive value of a test is the percentage of patients with a negative test who do not have the condition. In Study II all these tests were used to evaluate the validity of ^{14}C-UBT in the diagnosis of *H. pylori* infection.

4.8 Cohen's kappa

Agreement between two observations can be tested by a simple kappa statistic, it is denoted by κ which measures the level of agreement beyond that exepected by chance. When κ is zero the agreement is no better than chance expected agreement. Agreement less than chance returns a negative κ-value. A value of kappa ranging from 0.61-0.80 is considered a good agreement, > 0.81 – 0.90 very good and > 0.91 excellent (86).

4.9 Bonferroni's correction

When several comparisons are made the probability of obtaining significance by chance is increased and the probability of making a Type-I error increases. One way to

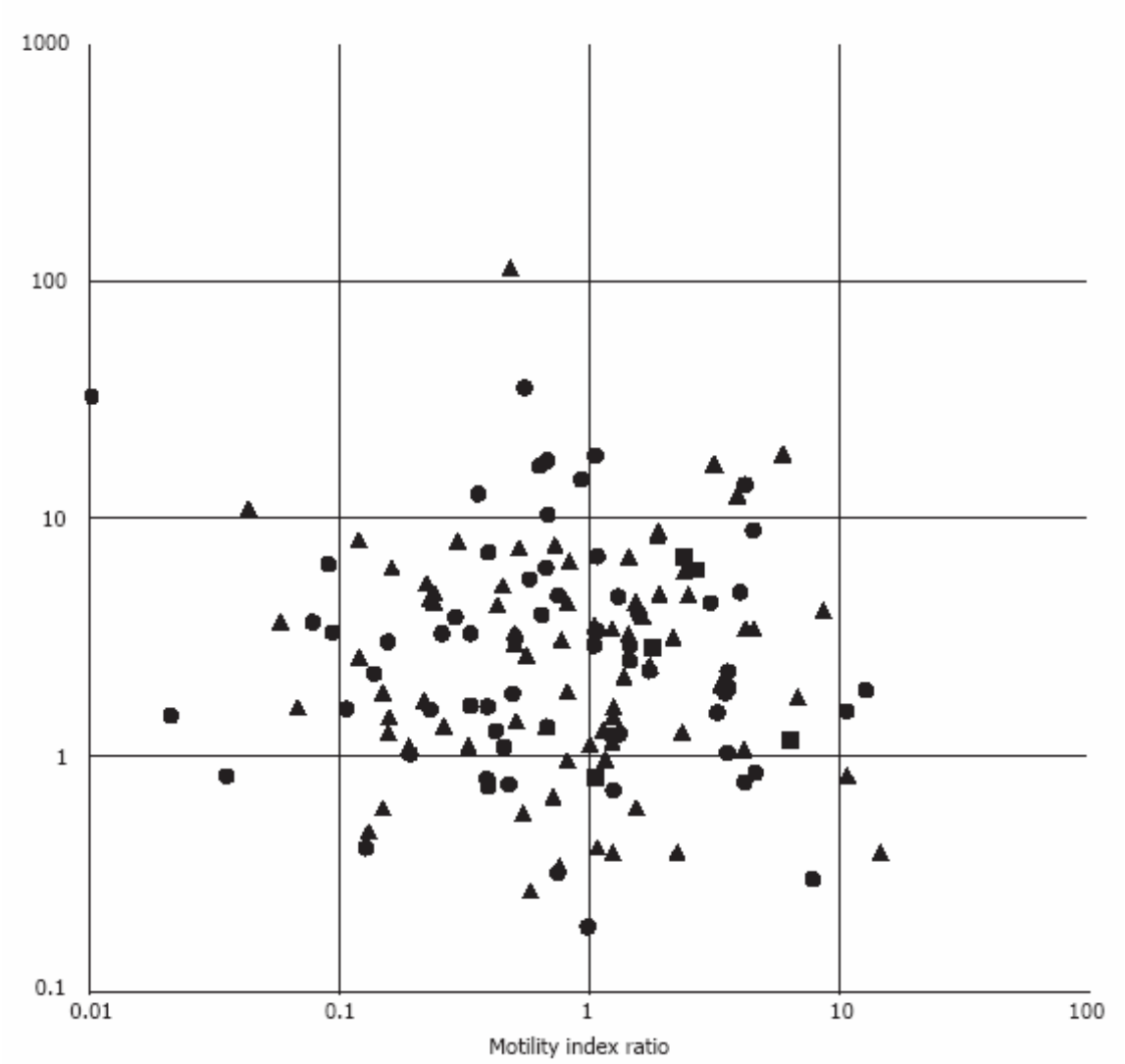
compensate for multiple comparisons is to decrease the alpha level by dividing the alpha level with the number of comparisons made. In study IV, We used Bonferroni's correction for multiple comparisons when analyzing correlations between MTV and individual symptom severity scores.

5 RESULTS

5.1 Study I

The study population included 148 patients for comparing EGG parameters among various diagnosis groups and 144 patients for assessing the correlation between EGG and ADM parameters. The median age of the patients was 45 (range 17-76) years and 122 (82%) were females. There were 52 patients (35%) with IBS, 22 (15%) with enteric dysmotility, i.e. abnormal small bowel motor activity but no bowel dilatation, 26 (18%) with slow transit constipation, 11 (7%) with chronic intestinal pseudo-obstruction, 13 (9%) with functional dyspepsia with or without gastroparesis and 24 (16%) with other diagnoses, including various systemic and neurological diseases.

A correlation matrix between 21 EGG parameters and 8 ADM parameters was analyzed. Only a few and weak correlations were found between EGG and ADM. The comparison between the EGG response (ratio between postprandial and fasting power) and the ADM response to ingestion of a 500 kcal test meal (ratio between postprandial and fasting motility index) revealed no correlation ($r = -0.07$, $P = 0.44$), Figure 1. The ability of EGG to identify diagnostic groups was tested by comparing EGG parameters for each group against all other patients included. In general, the discriminatory power of EGG was low. Only patients with slow transit constipation showed a reasonable number of differences in postprandial EGG parameters compared to those in all other diagnosis groups.



■ = excellent; ● = good; and ▲ = fair quality.

Figure 1. Scatter plot of motility index ratio and power ratio in 144 patients (logarithmic scales).Symbols indicate different qualities of the EGG record:

5.2 Study II

Ninety-four consecutive patients with dyspeptic symptoms were enrolled for this study. They underwent ^{14}C-UBT, rapid urease test, and gastric histology. There were 60 (64%) men and the mean age of study group was 40.8 ± 12.8 years. *H. pylori* infection was diagnosed by histology in 66 (70%) patients and by rapid urease test in 61 (65%) patients. UBT detected active *H. pylori* infection in 63 (67%) patients.

In comparison with histology, UBT had a sensitivity of 92% (95% CI: 87%-95%) and a specificity of 93% (95% CI: 79%-99%). The positive predictive value (PPV) of ^{14}C-UBT was 97% (95% CI: 91%-99%) and the negative predictive value (NPV) was 84% (95% CI: 72%-89%) compared with histology (Table 4). These results showed that UBT had an accuracy of 93% compared to histology. Kappa test showed concurrence between the UBT diagnosis and histological diagnosis of *H. pylori* infection with value of 0.80 ($p<0.001$).

In comparison with rapid urease test, the sensitivity and specificity of UBT was 98% (95% CI: 93%-99%) and 91% (95% CI: 80%-94%). PPV and NPV were 95% (95% CI: 89%-97%) and 97% (95% CI: 86%-99%), respectively. UBT has an accuracy of 96% compared with rapid urease test for the diagnosis of *H. pylori* infection (Table 4). The result of the Kappa test was 0.88 ($p< 0.001$), which indicates a very good concurrence between rapid urease test and ^{14}C-UBT.

Four patients with histological evidence of *H. pylori* infection had negative results with UBT and rapid urease test. At the time of this study, the cost of gastroscopy was 90 USD while the cost of histology and rapid urease test was 20 USD and 5 USD respectively in our institute. Therefore, the overall cost of *H. pylori* diagnosis by histology was 110 USD and 95 USD by rapid urease test. The cost of UBT was only 15 USD in our institute.

Table 4. Sensitivity and specificity of ^{14}C UBT against histopathology and rapid urease test for *H. pylori* diagnosis (n=94).

UBT Compared to	**Sensitivity (95% CI)**	**Specificity (95% CI)**	**PPV (95% CI)**	**NPV (95% CI)**	**Accuracy**
Histopathology	92% (87-95)	93% (79-99)	97% (91-99)	84% (72-89)	93%
Rapid urease test	98% (93-99)	91% (80-94)	95% (89-97)	97% (86-99)	96%

UBT: urea breath test PPV: Positive predictive value: NPV: negative predictive value; CI: confidence interval

5.3 Study III

Among 42 healthy volunteers MTV estimated by rapid water drinking was 1595 ±405 ml in males and 1327 ±308 ml in females ($p<0.05$). In rapid nutrient drinking (RND), the average volume tolerated by males was 945 ±376 ml, whereas females tolerated 760 ±174 ml ($p<0.05$). In slow nutrient drinking (SND) the tolerated amount of liquid was 692 ±184 ml in males and 594 ±131 ml in females ($p=0.051$). Both males and females tolerated significantly larger amounts of plain water at a rapid rate compared to the drinking of nutrient liquid either at a rapid or a slow rate.

Kaplan-Meier plots demonstrated that in rapid nutrient drinking test, fewer calories were required to reach the maximum tolerance in females compared to males (Figure 2a). However no significant difference in calorie requirement was observed between females compared to males on slow nutrient drinking test (Figure 2b).

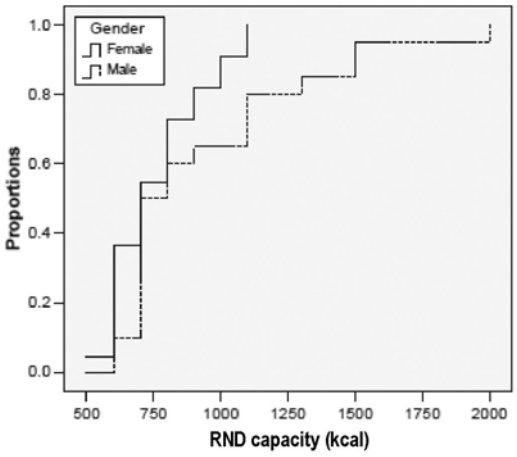


Figure 2a. Kaplan-Meier plot of rapid nutrient drinking (100 ml/min) to maximum satiety in males and females. Females needed a lower kcal load to reach maximum satiety; $p<0.03$ (log-rank test). RND = rapid nutrient drinking.

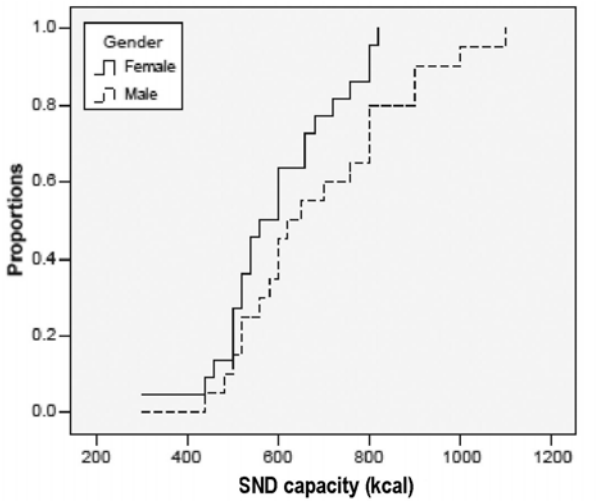


Figure 2b. Kaplan-Meier plot of caloric intake using slow nutrient drinking (20 ml/min) to reach maximum satiety in males and females, $p<0.051$ (log-rank test). SND = slow nutrient drinking.

Multiple regression analysis showed no influence of age, BMI, or gender on maximum tolerated volumes in SND, while RND showed a gender difference but no influence of BMI and age. In contrast to this, RWD showed influence of BMI, age and gender over maximum tolerated volume.

Self-assessment of symptoms (maximum score 300) 30 minutes after cessation of drinking indicated that SND was associated with significantly higher symptom scores than the other drinking tests. Females experienced more symptoms, especially nausea and bloating, than did males after each of the three drinking tests.

5.4 Study IV

A total of 132 patients were enrolled and 128 completed the study. The median age of the patients was 38 (range 19-65) years and 65 (49%) were males. *H. pylori* were present at inclusion in 59 (46%) patients. Among *H. pylori* positive patients 50/59 (85%) achieved successful eradication.

MTV determined at baseline was 393 ±202 ml in *H. pylori* negative group compared to 381 ±237 ml in *H. pylori* positive group, the difference in MTV was not significant between the groups. On repeat testing following treatment, MTV showed a mean

increase of 188 ±102 ml from baseline (<0.001) in *H. pylori* positive patients and an increase of 56 ±77 ml from baseline (p<0.01) in *H. pylori* negative patients. While a decrease of 10 ±34 ml in MTV was noted in patients in which *H. pylori* eradication was unsuccessful.

A significant inverse relationship was found between baseline MTV and the overall symptom score (maximum 56), (r = -0.44) in the whole group. Moreover, MTV at baseline showed significant correlations (after Bonferroni correction at p<0.05) with 4/8 symptoms (epigastric pain: r = 0.45, p<0.00625; epigastric burning: r = 0.43, p<0.00625; postprandial fullness: r = 0.56, p<0.00625; and early satiety: r = 0.50, p<0.00625; but not with upper abdominal bloating, excessive belching, vomiting, or nausea.

A significant correlation was also observed between the overall change in MTV and change in the overall symptom score following treatment for the whole group; (r=-0.61, p<0.001, Figure 3). In sub-group analysis, strong relationships were found between the change in MTV and the change in the total symptom score among *H. pylori* positive and *H. pylori* negative patients, (r=-0.60, p<0.001) and (r=-0.52, p<0.001) respectively.

We found significant correlations (after Bonferroni correction at p<0.05) between the change in MTV and changes in individual symptom scores for 4/8 symptoms (abdominal pain, abdominal burning, post-prandial fullness and early satiety). However, changes in the severity of belching, bloating, nausea and vomiting did not show a significant correlation with the change in MTV. On multivariate analysis age and weight were found to be independent predictors for MTV but not BMI and *H. pylori* status.

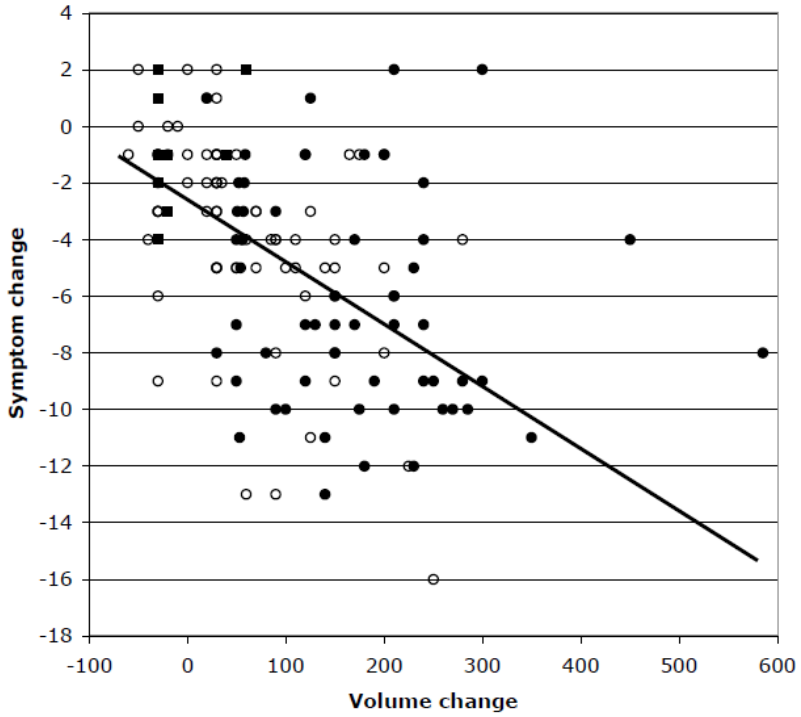


Figure 3. Scatter plot of the changes in maximum tolerated volume and symptom severity after treatment in patients with functional dyspepsia. Regression line is shown for all values (r=-0.61, p<0.001). ○ *H. pylori* negative patients, ● *H. pylori* positive patients with successful eradication, ■ *H .pylori* positive patients with unsuccessful eradication.

6 METHODOLOGICAL CONSIDERATIONS

6.1 Inherent disadvantages of retrospective study design

Study I was a retrospective analysis of patient and laboratory records. A retrospective study uses existing data that have been recorded for purposes other than research. Therefore the correlation of EGG and ADM findings with patient's symptoms (potentially useful information) was not possible because of lack of uniform symptom recording in the data set. Moreover there was no control group of healthy individuals to compare with and it was not possible to add such a group because of retrospective nature of the study design.

6.2 Potential of enhancing alpha error

During hypothesis testing an alpha error is made when it is concluded that a result is positive while actually it is not i.e. a false positive conclusion. In Study I we compared 22 EGG and 8 ADM variables among various diagnostic groups of motility disorders. Such large number of variables could have enhanced the probability of obtaining significant differences between the groups (an increase of alpha error). We used logistic regression to account for interdependence of variables and to arrive at variables with an independent value for predicting diagnoses. A Bonferoni correction could have been applied to the correlation matrix but already without such a correction correlations were few and of small size.

6.3 Rate of drinking the nutrient liquid

In study III, MTVs were estimated in healthy volunteers through satiety drinking tests on three occasions using different rates of drinking either water or nutrient drink. The rate of slow nutrient drinking was 20 ml/min (1 kcal/ml), slightly different from the previously published study in which 15 ml/min (1.5 kcal/ml) was used (41). However, the caloric value of the nutrient drink in our study (study III) was 20 kcal/min, which is close to 22.5 kcal/ml used in the latter study.

6.4 Potential effects of lack of blinding and recall bias

Although not the main aim, study IV also evaluated symptoms among *H. pylori* negative and *H. pylori* positive patients with FD before and after treatment. Since the treatment was not administered in a blinded and controlled fashion a biased response on improvement in symptoms among *H. pylori* positive patients after treatment can not be ruled out. The blinding is particularly important when the response criteria are subjective, such as alleviation of pain. In study IV, apparent high rate of symptom improvement in patients with FD in which *H. pylori* was successful eradicated compared to patients without *H. pylori* infection could be due to open label administration of anti *H. pylori* treatment.

Symptom evaluation was done by using global overall symptom score (GOS) applying two days' symptom recall. It would have been more appropriate if a symptoms diary had been kept for a more precise evaluation of symptom improvement and fluctuation. Keeping a diary of symptoms can minimize the recall bias very effectively (87).

7 DISCUSSION

This thesis is based on evaluation of certain measurement techniques that may be helpful in the management of patients with FD. My thesis clarifies several ambiguities regarding clinical usefulness and practicality of these procedures.

7.1 Clinical usefulness of EGG

Previous studies have demonstrated EGG changes in various motility disorders such as diabetic neuropathy, systemic sclerosis, functional dyspepsia, Parkinson's disease, intestinal pseudo-obstruction, and after gastric surgery (88-93). Using EGG, the relationship between gastric motility and reflux symptoms in patients with non-erosive reflux disease (NERD) were analyzed before and after the use of a prokinetic drug (94). In that study it was found that gastric dysmotility, assessed by EGG was a significant feature in a subset of NERD patients and that dysmotility improved after prokinetic treatment. EGG in that study provided a non-invasive technique for evaluating the response to treatment objectively along with symptom improvement. Similarly, in a recent paper EGG was used for evaluating patients with diabetic gastroparesis (95). It was observed that patients with a cyclic symptom pattern had more EGG abnormalities and more prolonged delays in gastric emptying compared to patients without cyclic symptoms. These studies support the application of EGG in the evaluation of patients with motility disorders.

In Study I we found variability in several EGG parameters among patients with various motility disorders. However, these parameters could not be linked to a specific disorder and thus the discriminatory value of EGG in the diagnosis of various motility disorders was questionable. Study I demonstrated some EGG parameters that could discriminate slow transit constipation from other motility disorders of the GI tract. Another study has reported that healthy normal individuals can also depicting certain abnormalities on EGG recording (96). Therefore the careful interpretation of EGG findings is required.

7.2 Correlation between EGG and ADM

A direct comparison of EGG and ADM on a large number of patients with motility disorders (Study I) revealed no spatial correlation between the two techniques. This indicates that EGG and ADM measure different aspects of gut motor activity. A "one-to-one" correlation between gastro-duodenal motility parameters and EGG was also not found in a recently conducted study (90).

7.3 Efficacy of ^{14}C-UBT

The high sensitivity and specificity observed for ^{14}C-UBT (study II) in the diagnosis of *H. pylori* infection is comparable to histology and rapid urease test. Our results are corroborated by a study from Japan, which showed a high sensitivity (96.6%) and specificity (100%) of ^{14}C-UBT in the diagnosis of *H. pylori* infection compared to histology, rapid urease test, and PCR (97).

A study has suggested using baseline and a 30-minute breath sample after ingesting 75 mg urea for the diagnosis of *H. pylori* infection (98). In study II, only one-time test i.e. 10 minutes after ingestion of a urea capsule was done yielding a high sensitivity and specificity. One-time ^{14}C-UBT test could therefore be more practical and convenient for the patients.

There are concerns about the radiation hazards of using radioactive ^{14}C-isotope of carbon in UBT. A recently published study has described that a very minimal emission of beta radiation takes place during ingestion of ^{14}C, which is even less than the daily background radiation (99).

False positive results of UBT have been reported due to contamination of oral cavity and stomach by urease producing non-*H. pylori* bacteria (100,101). Therefore, in study II while validating the ^{14}C sensitivity and specificity it would have been better if thorough mouth cleansing had been done before performing UBT.

Another issue related to the accuracy of UBT for the diagnosis of *H. pylori* infection is the possibility of false negative results of UBT in patients who have been taking proton pump inhibitors or H_2-antagonists (102). In this era of indiscriminate use of PPIs and

their availability over the counter, a random ^{14}C-UBT for the diagnosis of *H. pylori* (without abstinence from PPI) must be interpreted with caution.

Consensus guidelines have recommended that it is appropriate to eradicate *H. pylori* if found in patients with dyspeptic symptoms as a "test-and-treat" strategy, particularly in areas with a high prevalence of gastric carcinoma (103-105). At the primary care level, a reliable and portable test is required for the diagnosis of *H. pylori* infection. Findings from Study II and review of the literature suggest that ^{14}C-UBT is efficacious and probably one of the best non-invasive tests for the diagnosis of *H. pylori* infection especially for developing countries.

7.4 Comparison of three satiety drinking tests

Important observations were made in Study III when healthy volunteers were subjected to three different satiety drinking tests. It was found that slow nutrient drinking (SND) was associated with more symptom generation than the other two tests. Moreover, it was noted that the MTV achieved by study subjects during SND was not influenced by age and BMI as compared to rapid nutrient drinking (RND) and rapid water drinking (RWD). A previously published study has also demonstrated that caloric nutrient liquid used in satiety drinking test is more symptom provoking than water (106).

In Study III, weak correlations were observed between MTV and BMI in all three satiety drinking tests. However, on multiple regression analysis, BMI showed significant influence over the MTV values obtained after the RWD test. These observations are in agreement with a previous study, which has demonstrated that the BMI did not influence MTV beyond its association with age and gender (107). These observations indicate that SND is probably a better method of performing satiety drinking test because it is less influenced by BMI or age and is associated with more symptoms.

7.5 Normal values of satiety drinking tests

Study III had an added value: normal values of MTV by satiety drinking tests using slow (20 ml/min) and rapid (100 ml/min) nutrient drinking and water drinking at 100

ml/min for this region were obtained. The study also demonstrated that MTV determined by rapid water drinking was comparable to published values from the Western world (29, 41, and 69,108).

7.6 MTV as a surrogate marker for symptom severity in dyspepsia

A significant correlation between MTV and dyspepsia symptom scores was observed in Study IV in patients with FD. Additionally, some individual dyspepsia symptom scores also correlated well with MTV. These observations suggest that MTV estimated by SND satiety drinking test can be used as a surrogate marker for symptom evaluation in patients with FD. To our best knowledge, no other study has described MTV as a surrogate marker for the severity of dyspepsia symptoms.

7.7 Relationship between age and MTV

A weak but inverse correlation was observed between age and MTV in healthy volunteers as well as in patients with FD in study III and IV respectively. This inverse relationship between age and MTV is quite in contrast to Western data that has indicated an increase in MTV with the increase in age (107). The nature of this inverse relationship is unclear and no other study has demonstrated such effect of age on MTV using satiety drinking tests. Interestingly, a recently published study has shown that symptoms of pain and nausea are inversely correlated with age following a standardized nutrient challenge (109).

The satiety drinking test was first applied more than a decade ago in an attempt to have a simple non-invasive test for the assessment of gastric accommodation. A significant correlation has been observed between gastric accommodation measured by barostat and total calories consumed during a nutrient drink test administered at 15 ml/min (41, 69). However, in another study using nutrient drink at 100 ml/min, no such relationship was noted between caloric consumption during drinking test and gastric accommodation estimated by barostat (29). Others have found no relationship between gastric accommodation assessed by SPECT and a satiety drinking test (110). Only a modest relationship was found between gastric emptying and satiety drinking test (111). Moreover, there is evidence that certain psychosocial factors might influence the

outcome of satiety drinking tests (112). Therefore, it remains questionable what the satiety drinking test actually measures. In view of this ambiguity, the terms "slow nutrient tolerance test" and "nutrient challenge test" have been suggested to replace “satiety drinking test”. The nutrient challenge test assesses global stomach sensation rather than just being the descriptor of single motor phenomena (113).

8 CONCLUSIONS

This thesis concluded that

EGG is a technique for evaluation of gastric motility whose discriminatory power to diagnose a motility disorder is very limited. The results of study I and review of the literature did not support any practical clinical utility of this technique in patients with gastrointestinal motility disorders. Therefore EGG will most likely remain a research tool only, in years to come.

^{14}C-UBT is a reliable, highly sensitive, specific and economical test for the diagnosis of *H. pylori* infection. The amount of radiation exposure is negligible. Studies are required to determine its sensitivity in patients who are currently taking PPI or other acid lowering agents.

Satiety drinking test is a simple and inexpensive technique for evaluation of patients with FD. Use of a slower rate for nutrient drinking is better and yields a more meaningful measurement of MTV compared to nutrient drinking at a rapid rate or water drinking.

Changes in satiety drinking test volumes correlated well with changes in symptom severity in patients with FD. Therefore satiety drinking test might be useful as an adjunct to symptom score for objective assessment of clinical outcome in patients with FD.

9 FUTURE PERSPECTIVES

The electrogastrography technique is still evolving. Recent studies have focused on understanding the gastric slow wave changes demonstrated by EGG in response to proximal gut surgery, and the evaluation of certain novel pharmacotheraputic agents (93,114). A new device has recently been introduced (which is applied through laparoscope) for investigating the effect of gastric surgical procedure on gastric slow wave activities (115). However the clinical usefulness of EGG remains unproven.

Breath tests using the radioactive carbon isotope ^{14}C were developed 3-4 decades ago for measuring exocrine pancreatic function, intestinal absorption, and liver function. With increasing awareness of radiation hazards ^{14}C has been replaced by a stable, non-radiating carbon isotope, ^{13}C in these breath test. For studies in children and pregnant women, and for repeated studies in adults, the use of stable isotopically labeled substrates is preferable and safe. High resolution mass spectrometry is the gold standard for measuring the slight mass difference of one neutron between ^{13}C-labelled carbon dioxide and the naturally most common carbon dioxide with ^{12}C. The development of non-dispersive isotope selective infrared spectrometers (NDIRS) opened up a lower-priced analytical alternative with adequate precision (116,117). Operation and handling of NDIRS is easy, even for non-experienced users. At the push of a button, breath samples can be analyzed within about 60 seconds. This enables not only the performance, but now also the analysis of ^{13}C-breath tests in primary care settings. Measurement of gastric emptying, orocecal transit time and carbohydrate assimilation are some potential areas of further application of ^{13}C breath test by using NDIRS.

Satiety drinking tests have the potential for clinical usefulness in routine practice especially for interventional trials as an adjunct to other evaluation techniques and symptom severity scores. In a recent study the role of endocannabinoid system in the regulation of motility and sensitivity of stomach was evaluated using nutrient liquid challenge test (118). In another study, water load test was used to evaluate symptoms in response to gastric distension before and after PPI therapy (119). These studies reflect the clinical application of a simple measurement technique, which is inexpensive and helpful in the evaluation of patients with FD and other functional proximal gut

disorders. However, there is a need to standardize the technique for uniform application in terms of the choice of the drink and rate of ingestion.

SmartPill™, is a wireless capsule that continuously measures the temperature, pH and pressure in the GI tract. This capsule may be a useful technique in the future for determination of transit times in different parts of the GI tract without radiation and much invasiveness (120). Likewise, MRI (non-invasive and free from radiation hazard) is a promising technique for the assessment of gastric emptying and antral wall motion (51).

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