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**Direct Comparison of 99m-Techneium
Hepatoiminodiacetic Acid (HIDA)
Cholescintigraphy with
Ultrasonography Gallbladder Ejection
Fraction Assessment Following Fatty-
Meal Challenge – Correlation of Both
Techniques in Extrapolation Towards
Normal Values for Ultrasonographic
Measurements in Healthy Subjects.**

by
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A thesis submitted in fulfilment of the requirements for
the degree of
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*Dedicated to my loving wife for all her support and
patience.*

For my beloved children and parents

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DECLARATION

This thesis is submitted to the University of Warwick in support of my application for the degree of Doctor of Medicine.

It has been composed by myself and has not been submitted in any previous application for any degree.

The work presented (including data generated and data analysis) was carried out by the author except in the cases outlined below:

- Biliary scintigraphy study as part of the standard management pathway for the phase one subjects were carried out by nuclear medicine staffs.
- Image analysis of the biliary scintigraphy study were performed by nuclear medicine technologists and clinical scientists within the nuclear medicine department.

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ABSTRACT

Gallbladder dyskinesia is a functional gallbladder disease which is difficult to diagnose and normally relies on several clinical and biochemical findings including imaging in the form of biliary dyskinesia. Unfortunately, the method in performing biliary scintigraphy, especially with fatty meal stimulus varies widely with no standardised practice in place.

Ultrasound assessment of the gallbladder is a well-established method for gallbladder pathology but its use in the assessment of functional disorder remains limited.

This thesis aims to study the correlation between gallbladder ejection fractions obtained from biliary scintigraphy following fatty meal stimulus. The thesis also aims to assess gallbladder contraction in healthy volunteers to possibly established normal values.

The study has demonstrated a good correlation in the assessment of gallbladder contraction between biliary scintigraphy and ultrasound for patients with biliary symptoms following a fatty meal stimulus. As published literature for this aspect is limited, the findings have further enhanced the understanding of the subject and suggests the possibility of using ultrasound as a complement modality for biliary dyskinesia assessment.

Normal range were also obtained in the healthy volunteer cohort which is wide and consistent with the findings in the published literature.

ABBREVIATIONS

^{99m}Tc	Technetium-99m
ARSAC	Administration of Radioactive Substances Advisory Committee
BA	bile acids
CCK	cholecystokinin
CT	computed tomography
CURL	uncoupling of the receptor and ligand
FFA	free fatty acid
GBEF	gallbladder ejection fraction
GBVol	gallbladder volume
HIDA	hepatoiminodiacetic acid
IRMER	Ionising Radiation (Medical Exposure) Regulations
MBq	Mega-Becquerel
ml	millilitres
MMC	migrating motor complex
MR	magnetic resonance
REC	Research Ethics Committee
RME	receptor-mediated endocytosis
ROI	region of interest
SD	standard deviation
SO	Sphincter of Oddi

CHAPTER 1

BACKGROUND

1.1 Introduction

This chapter will discuss about the background for the thesis. The gallbladder anatomy and physiology will initially be presented followed by the definition and explanation of gallbladder functional disorder which includes biliary dyskinesia. Then, the different imaging modalities for assessment of biliary dyskinesia will be presented with focus on biliary scintigraphy and ultrasonography being the two main modalities used within this study. The final component for the chapter will introduce the rationale for the study.

1.2 Gallbladder Anatomy

Gallbladder is part of the biliary system which connects the liver to the duodenum for draining bile into the gastrointestinal system. The biliary system is also made up for intrahepatic biliary ducts and extrahepatic biliary ducts. The main function of the biliary system is to transport bile from the liver into the duodenum. The intrahepatic biliary ducts are formed from bile ductules that joined to form segmental ducts which subsequently merged to into the right and left hepatic ducts. The right and left hepatic ducts exit the liver at the porta hepatis and becomes part of the extrahepatic biliary system. The extrahepatic biliary system consists of the right and left hepatic ducts which combined to become the common hepatic duct. The common hepatic duct then forms the common bile duct upon merging with the cystic duct. The common bile duct

opens into the duodenum through the ampulla of Vater at the pancreatic head, which includes the Sphincter of Oddi (SO). Sphincter of Oddi is a functional muscular ring at the distal common bile duct which opens into the duodenum (Figure 1.1).

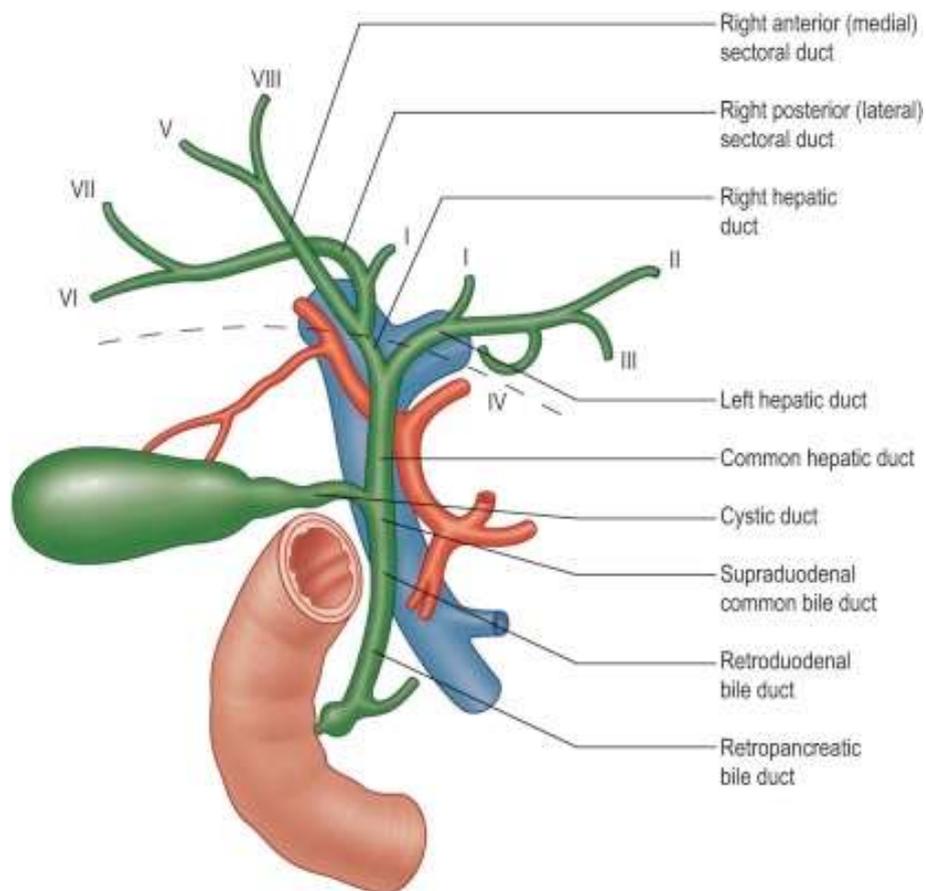


Figure 1.1 The overall arrangement of the intrahepatic and extrahepatic biliary tree. The segmental ducts often branch just before they enter the main ducts, or are multiple as they enter the main ducts, but for clarity are shown here as single ducts. Note that segment I often drains via both right and left hepatic ducts. The level of the liver parenchyma at the porta hepatis is shown by the dashed line. (Reprinted with permission from Elsevier®)

The gallbladder is a blind-ending diverticulum which connects to the cystic duct that has a pear-shaped appearance. The main role

of the gallbladder is to store and concentrate bile. The gallbladder is situated within the gallbladder fossa which is at the inferior surface of the liver. The gallbladder normally measures between 7 cm to 10 cm long with a resting capacity of approximately 25 ml and a capacity of up to 50 ml (1).

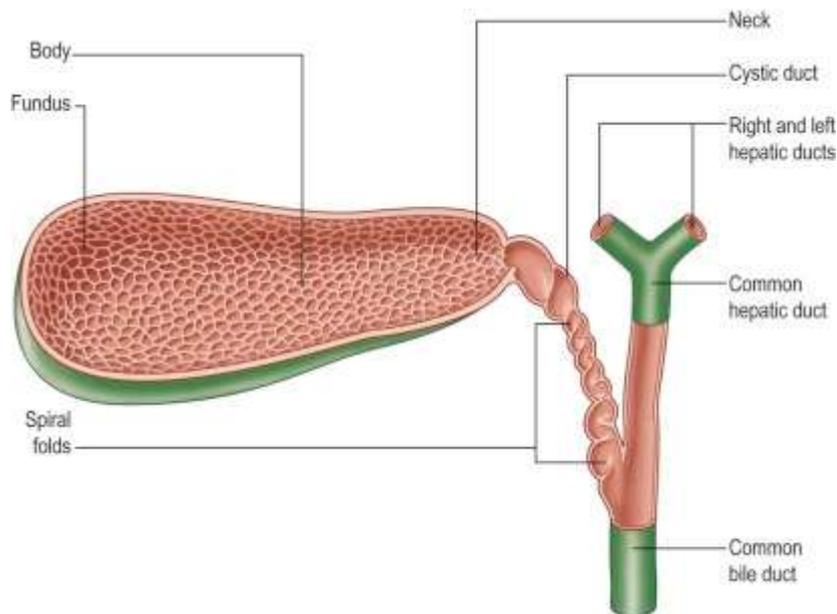


Figure 1.2 The interior of the gallbladder and bile ducts. (Reprinted with permission from Elsevier®)

The gallbladder is made up of three segments; fundus, body and neck. The bulbous end of the gallbladder away from the connection to the cystic duct is the fundus. The middle part of the gallbladder is the body which then connects to the neck of the gallbladder. The neck of the gallbladder is segment of the gallbladder which connects to the cystic duct. There are spiral folds of mucosa in the cystic duct known as the spiral valves of Heister which extends into the neck of the gallbladder. These folds function to prevent gallstones from entering the common bile duct (Figure 1.2).

The gallbladder can vary in shape and size, with variants recorded of double cystic ducts or septation within the gallbladder, dividing it into chambers. The gallbladder is a fibromuscular sac which shows only small amount of smooth muscle in its wall. The mucous membrane is lined with simple columnar epithelium tissue. This is then projected into folds which has a honeycomb appearance. The epithelial cells actively absorb water and solutes from the bile in order to concentrate the bile. The epithelial cells also secrete mucous, although the mucous secreting glands are only present in the neck.

The blood supply to the gallbladder is from the cystic artery which normally arises from the right hepatic artery. There are some variations noted to the origin of the cystic artery either from the left hepatic artery, common hepatic artery or even the gastroduodenal artery. Blood supply is then drained away from the gallbladder by multiple small veins in the gallbladder bed into the substance of the liver and eventually into the hepatic veins. Occasionally, cystic veins are present which drain into the portal vein, but these are uncommon and do not accompany the cystic artery. The lymphatic drainage of the gallbladder is connected to the lymph nodes in the porta hepatis, cystic node and eventually into the coeliac group of pre-aortic lymph nodes. The gallbladder has parasympathetic nerve fibres from the hepatic branch of the anterior vagal trunk and sympathetic fibres from cell bodies in the coeliac ganglia, which is with the preganglionic cells in the lateral horn of the spinal cord segments T7-T9. Some of the fibres from the gallbladder may run in the right phrenic nerve (C3-C5).

1.3 Gallbladder Physiology

As described within the anatomy section, the gallbladder can only accommodate between 25 ml to 50 ml of bile. However, the amount of bile volume secreted by the liver during fasting is many times greater than the capacity of the gallbladder. Nonetheless, the gallbladder can accommodate the large amount of secretion from the liver through its ability to concentrate bile. The concentration of bile salts, bile pigments and other large water-soluble molecules can be increased by a factor of 5 to 20 because of water and electrolyte absorption.

Bile is most frequently excreted from the gallbladder during meal times, coinciding with the digestion of food. Significant amount of bile can also be secreted during fasting with the migrating motor complex (MMC). Bile secretion from the gallbladder is coordinated with duodenal contractions, possibly through the cholinergic nerves. The stimulus for gallbladder contraction with meals is mainly hormonal. During digestion, food products, in particular, lipids will cause the release of cholecystokinin (CCK) from the mucosa of the duodenum. Cholecystokinin acts on its receptors within the smooth muscle of the gallbladder to initiate contractions and emptying of the concentrated bile from the gallbladder. Cholecystokinin also relaxes the Sphincter of Oddi at the same time to allow smooth drainage of bile into the duodenum (Figure 1.3).

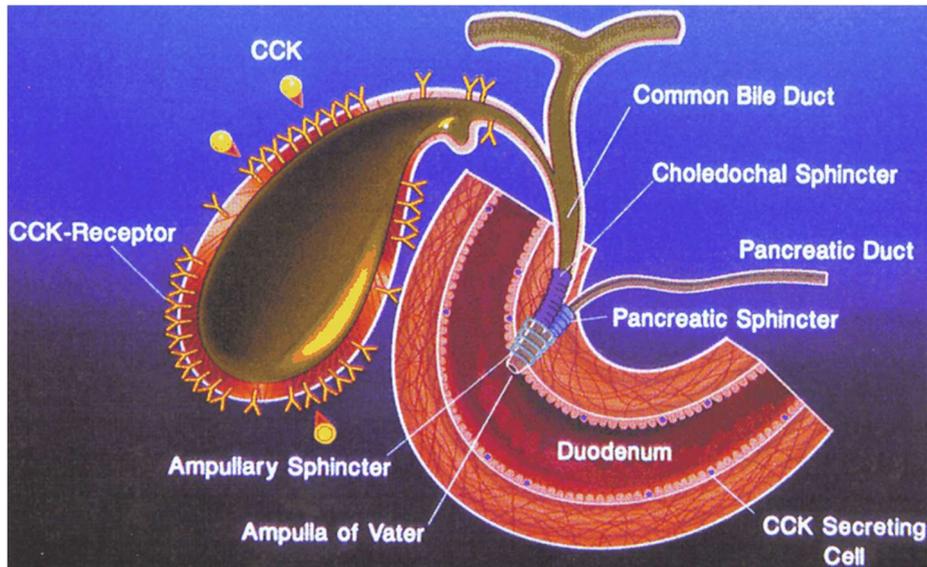


Figure 1.3 The relationship among the sphincter of Oddi, gallbladder, and cholecystikinin. The sphincter of Oddi consists of three components: choledochal sphincter, pancreatic sphincter, and ampullary sphincter. Gallbladder wall contains mainly stimulatory (contraction) and the sphincter of Oddi inhibitory (relaxation) receptors for cholecystikinin. CCK-secreting endocrine cells are distributed densely in the mucosa of the duodenum (Reprinted with permission from Springer Nature®).

Cholecystikinin is found in different organs within the body, but the highest concentration is seen within the upper intestinal tract. Cholecystikinin-secreting cells are found within the duodenum, jejunum and proximal ileum. There are no cholecystikinin-secreting cells within the oesophagus, stomach, distal ileum or the colon. As cholecystikinin is one of the main hormonal stimuli within this mechanism, a synthetic cholecystikinin is used to stimulate the gallbladder contraction, which is used, in-conjunction with hepatobiliary scintigraphy, to study the contraction of gallbladder.

1.4 Gallbladder Functional Disorder

Gallbladder dyskinesia has been classified as a functional biliary disorder based on the Rome IV criteria (2). There are however other multiple terms and nomenclature which have been used to describe gallbladder dyskinesia such as chronic acalculous cholecystitis, gallbladder spasm, cystic duct syndrome or biliary dyskinesia. Therefore, the true definition for gallbladder dysfunction can be difficult to accurately define.

Functional biliary disorder is a broader term which includes acalculous biliary pain, biliary dysmotility, sphincter of Oddi dysfunction, ampullary stenosis, and post-cholecystectomy syndrome. The diagnosis for functional biliary disorder will require the absence of stones, sludge, microlithiasis or microcrystals within the gallbladder or biliary tree. Therefore, the main issue regarding the definition of gallbladder dysfunction centres upon the absence of an anatomical cause such as gallstones with symptoms typical of biliary colic following food ingestion. There can be other associated symptoms such as nausea, vomiting, bloating, cramping, reflux or diarrhoea (3).

1.4.1 Epidemiology

Functional gallbladder disorder is rare when compared with other functional gastrointestinal disorders. This is shown in a survey completed by 5931 out of 6000 adults, of which 35% had functional gastrointestinal disorders. Only 0.2% within the overall sample had symptoms meeting the definition for functional gallbladder disorder (4). The true prevalence of the disease is not known. It has been noted that approximately 5% of patients with symptoms of biliary colic have been diagnosed with functional gallbladder disorder based on referrals for cholecystectomies in the past few decades. The referral for cholecystectomies due to

diagnosis of functional gallbladder disorders have increased to approximately 25% in recent years. (5). More recent data indicates that functional gallbladder disorder is a common indication for surgery and accounts for 2 to 5 percent in adults and up to 10 percent in children (6-8).

1.4.2 Definition

The hallmark for biliary dyskinesia is the absence of other aetiology contributing to complex biliary-type pain. According to the Rome IV criteria for functional gallbladder disorder diagnosis, the presence of biliary-type pain is required (2). Biliary pain is described as pain located in the epigastrium and/or the right upper quadrant with the following characteristics:

- builds up to a steady level and lasting 30 minutes or more
- occurs at different intervals (not daily)
- severe enough to interrupt daily activities or leading to accident and emergency attendance
- not significantly (<20%) related to bowel movements
- not significantly (<20%) relieved with postural changes or acid suppression

There are also supporting criteria which indicates pain maybe associated with

- nausea and vomiting
- pain radiating to the back and/or right infrasubscapular region
- pain resulting in waking from sleep.

The definition of biliary dyskinesia has changed over the last 20 years in order to better refine the diagnosis and reducing the number of patients undergoing surgery or invasive procedures.

Therefore, the final criteria for biliary dyskinesia within Rome IV requires the presence of biliary pain and absence of gallstones or other structural pathology. In addition, supporting criteria have been added to the Rome IV diagnostic criteria which includes low gallbladder ejection fraction on gallbladder scintigraphy with normal liver enzymes, conjugated bilirubin and amylase. The authors for Rome IV criteria have emphasized that gallbladder dyskinesia can occur in the presence of abnormal liver enzymes such as in fatty liver disease, hence the use of liver enzymes as supporting criteria in contrast to definite criteria. Similarly, low gallbladder ejection fraction is not specific for the diagnosis and hence not a definite required criterion (9).

1.4.3 Pathophysiology

The pathogenesis for biliary dyskinesia is not completely clear. In the absence of an anatomical cause, it was felt that the dyskinesia is a form of functional disorder. The theories behind the pathogenesis includes poor contraction of the gallbladder wall to secrete bile, although the reason for the poor contraction is not known. The second hypothesis suggests the lack of coordination between the gallbladder contraction and sphincter of Oddi relaxation. The last hypothesis suggests that bile cannot flow through the common bile duct because of a functional obstruction or non-occluding narrowing of the biliary tract (10).

Merg et al demonstrated a higher incidence of chronic cholecystitis in patients with gallbladder dyskinesia as compared to normal subjects leading to the theory of an abnormality within the smooth muscle of the gallbladder wall causing impaired gallbladder contraction (11). Further in-vitro studies have also shown that patients with gallbladder dyskinesia have a higher

incidence of absence of normal function and reduced contractile response to cholecystokinin (CCK) and electrical field stimulation (12).

There are other associations seen with the presence of gallbladder dyskinesia symptoms with regards to motility disorders in other organs of the gastrointestinal tract. Gallbladder dyskinesia is observed more commonly in patients with slow-transit constipation (13) or achalasia (14). Furthermore, there are also associations with constipation and gastroparesis in children population (15, 16). Therefore, this raises the possibility of an underlying motility disorder as the cause of biliary dyskinesia and even suggests a similar cause to the associated gastrointestinal motility disorder.

Ruffolo et al have also demonstrated significant overlap between gallbladder dyskinesia and Sphincter of Oddi (SO) dysfunction (17). The authors could only conclude from their study that gallbladder dyskinesia and Sphincter of Oddi dysfunction may exist independently but can co-exist in patients with biliary pain and an acalculous gallbladder. A recent study by Szepes et al has demonstrated an improvement of the gallbladder ejection fraction on patients who had sphincterotomy for suspected Sphincter of Oddi dysfunction based on manometry results (18).

1.4.4 Clinical Evaluation

Biliary dyskinesia is essentially a diagnosis of exclusion after successfully establishing biliary type pain which is compatible with the set criteria illustrated above. Therefore, a focus history is required such as queries regarding the relationship to food, bowel

movement, duration or time of the day. Therefore, a classic biliary colic description is characteristic of biliary dyskinesia in the absence of gallstones or other structural pathologies. In addition, alternative sources of pain need to be excluded which is listed as below:

- Irritable bowel syndrome
- Peptic ulcer disease
- Chronic constipation
- Gastroesophageal reflux disease
- Cirrhosis/end stage liver disease
- Coronary artery disease
- Costochondritis/musculoskeletal disorder

To complement history taking and clinical examination, blood tests should also be performed to exclude any structural causes or alternative sources of pain as described above. The most common blood tests performed relates to liver enzymes to establish liver function, amylase to assess for pancreatitis and bilirubin in conjunction with liver enzymes to assess for biliary obstruction.

1.5 Investigation of gallbladder function

There are a wide variety of investigations performed to assess the gallbladder and bladder function, most of which are imaging in perspective. A more detailed focus will be made towards imaging tests which allows gallbladder functional assessment.

1.6 Biliary Scintigraphy

Biliary scintigraphy is a method of imaging the biliary pathway with nuclear medicine imaging techniques. The essence of nuclear medicine imaging involves the use of radioisotopes. Radioisotopes are elements that will spontaneously produce radiation. It is through these radiation in which an imaged can be formed from the scanner. As the radioisotope is not specified for any process within the body, there is also a need to develop a radiopharmaceutical agent to assess the process to be investigated which can then be linked to the radioisotope. For the purpose of biliary scintigraphy, in order to assess the biliary system, a radiopharmaceutical agent will have to be taken up by the liver hepatocytes and be secreted into the biliary system and excreted into the bowel and can be instilled into the body either by injection, ingestion or inhalation. Injection is the most commonly used method of administration in nuclear medicine due to the quick and reliable delivery method. Once such a radiopharmaceutical agent is identified, it can then be linked to the radioisotope. Subsequently, imaging of the radiation produced by the radioisotope linked to the correct radiopharmaceutical agent will allow qualitative and quantitative analysis of the process.

Biliary scintigraphy is a well-established method used in the diagnosis of biliary dyskinesia. This is evident by the listing of biliary scintigraphy as a supporting criterion within the Rome IV criteria for the diagnosis of biliary dyskinesia (2). Biliary scintigraphy has been in use over the last four decades.

Initially, this was performed using Iodine-123 and Iodine-131 radioisotope which is linked with Rose Bengal as the radiopharmaceutical agent which was introduced by Taplin et al (19). There was subsequent introduction of hepatoiminodiacetic acid (HIDA) radiopharmaceuticals which is linked with Technetium-99m (^{99m}Tc) as the radioisotope in the 1980s that gradually became a substitute to Rose Bengal.

Chiotellis et al demonstrated that Rose Bengal has a slower blood clearance when compared to the hepatoiminodiacetic acid radiopharmaceuticals (20). There is earlier accumulation of the hepatoiminodiacetic acid radiopharmaceuticals within gallbladder 5-10 minutes earlier than Rose Bengal. The group has also demonstrated further biological characteristics more suitable for rapid serial imaging in hepatoiminodiacetic acid radiopharmaceuticals.

The radiopharmaceutical chemical structure is based on two lidocaine analogues bichelated to the Technetium-99m with different benzene ring structures; producing the different forms of HIDA radiopharmaceutical agents' availability on the market.

Loberg et al first reported the development of ^{99m}Tc -HIDA radiopharmaceuticals in the 1970s (21). The first ^{99m}Tc -HIDA radiopharmaceutical, Dimethyl IDA (lidofenin [Technescan; Mallinckrodt]) was first approved by the Food and Drug Administration in 1982 (22). Unfortunately, there is suboptimal image quality and diagnostic utility of this radiopharmaceutical in patients with high serum bilirubin levels above 5.0 mg/dL. Therefore, this product was subsequently removed and no longer available in the market.

^{99m}Tc -disofenin (diisopropyl-IDA [Hepatolite; Pharmalucence]) was approved for clinical usage in 1986. This radiopharmaceutical provided diagnostic quality images even with bilirubin levels of 25-30 mg/dL. Subsequently, in 1993, ^{99m}Tc -mebrofenin (bromotriethyl-IDA [Choletec; Bracco]) was approved, which had higher liver extraction (98% vs. 89%) and more rapid biliary clearance than ^{99m}Tc -disofenin (half-life of 19 min vs. 17 min) (22).

As mentioned previously, different forms of HIDA radiopharmaceutical agents have different rates of hepatic uptake and clearance. ^{99m}Tc -mebrofenin (bromotriethyl-Iminodiacetic acid) is currently the most commonly used HIDA radiopharmaceutical agent due to its high liver extraction and more rapid biliary clearance pharmacokinetics. The pharmacokinetic mechanism of ^{99m}Tc -HIDA agents can be divided into 6 functional phases:

- Blood transport
- Uptake by the hepatocyte

- Transit through the hepatocyte and secretion into the bile canaliculi
- Flow through the intrahepatic and extrahepatic ducts
- Entry into the gallbladder
- Final discharge into the small intestine

Small amounts of the ^{99m}Tc -HIDA agents which are not taken up by the liver (2%) are excreted in the urine.

Blood transport

The blood transport mechanism for the ^{99m}Tc -HIDA are through binding with serum albumin to form an albumin- ^{99m}Tc -HIDA-complex (23). The binding to the serum albumin enhances the hepatic delivery and hepatocyte uptake into the liver. This also reduces renal clearance of ^{99m}Tc -HIDA. Therefore, patients in a state of hypoalbuminaemia will have a reduced hepatic delivery and increased renal excretion. The albumin- ^{99m}Tc -HIDA-complex will leave the sinusoidal space through the fenestrae of the endothelial cells and enters the perisinusoidal space of Disse, a space unique for liver capillaries. ^{99m}Tc -HIDA then dissociates from the albumin in the space of Disse close to the basolateral border of the hepatocyte (Figure 1.4).

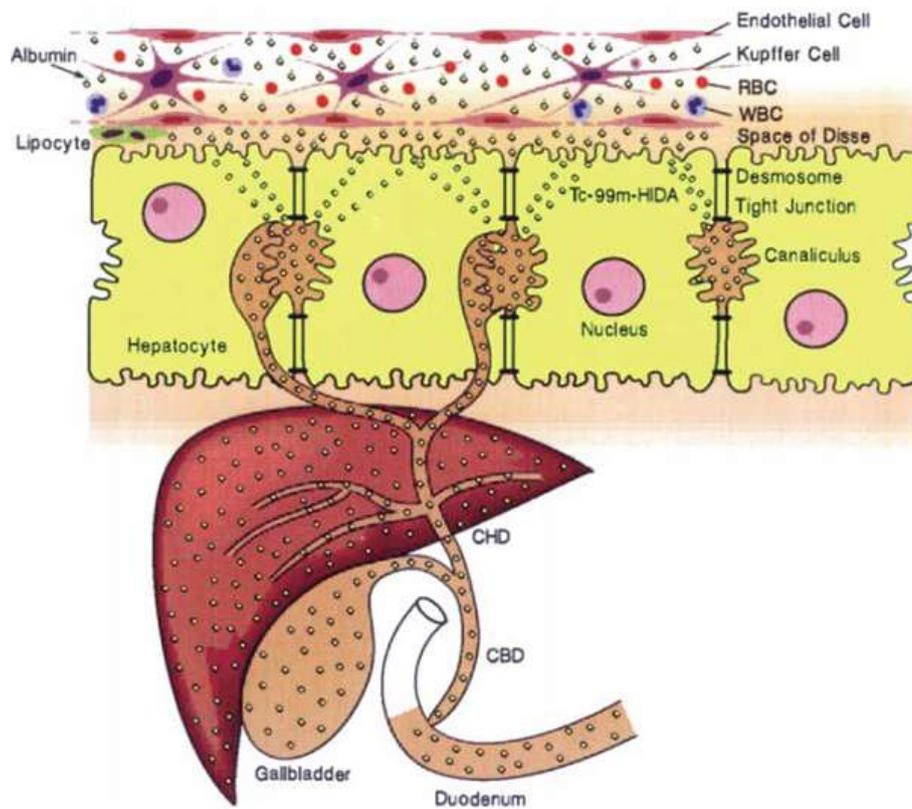


Figure 1.4 Albumin delivers the radiotracer to the space of Disse where the dissociation takes place. Tc-99m HIDA is taken up by the hepatocyte and secreted into bile canaliculi in free form where it mixes with the hepatic bile and serves as an ideal in vivo tracer for imaging of the entire hepatobiliary tree (Reprinted with permission from Springer Nature®).

Hepatocyte Uptake

The liver concentrates three types of organic anions which are bile acids, free fatty acids and non-bile acids cholephils (which includes ^{99m}Tc -HIDA). It has been hypothesized that the mechanism of ^{99m}Tc -HIDA uptake into the hepatocytes is through the receptor-mediated endocytosis (RME) pathway (24).

There are receptor proteins (ligandin) which are located along the basolateral border and along the walls of the coated pits, which are invaginations of the basolateral membrane into the hepatocyte cytosol. Once dissociated, ^{99m}Tc -HIDA will attach to its specific receptor which is ligandin. The ^{99m}Tc -HIDA which is bounded with ligandin will cluster in the coated pit (Figure 1.5). The coated pit then forms a coated vesicle after separation from the surface membrane and results in ^{99m}Tc -HIDA getting internalised within the hepatocyte. The vesicle then becomes an endosome by losing the protein covering.

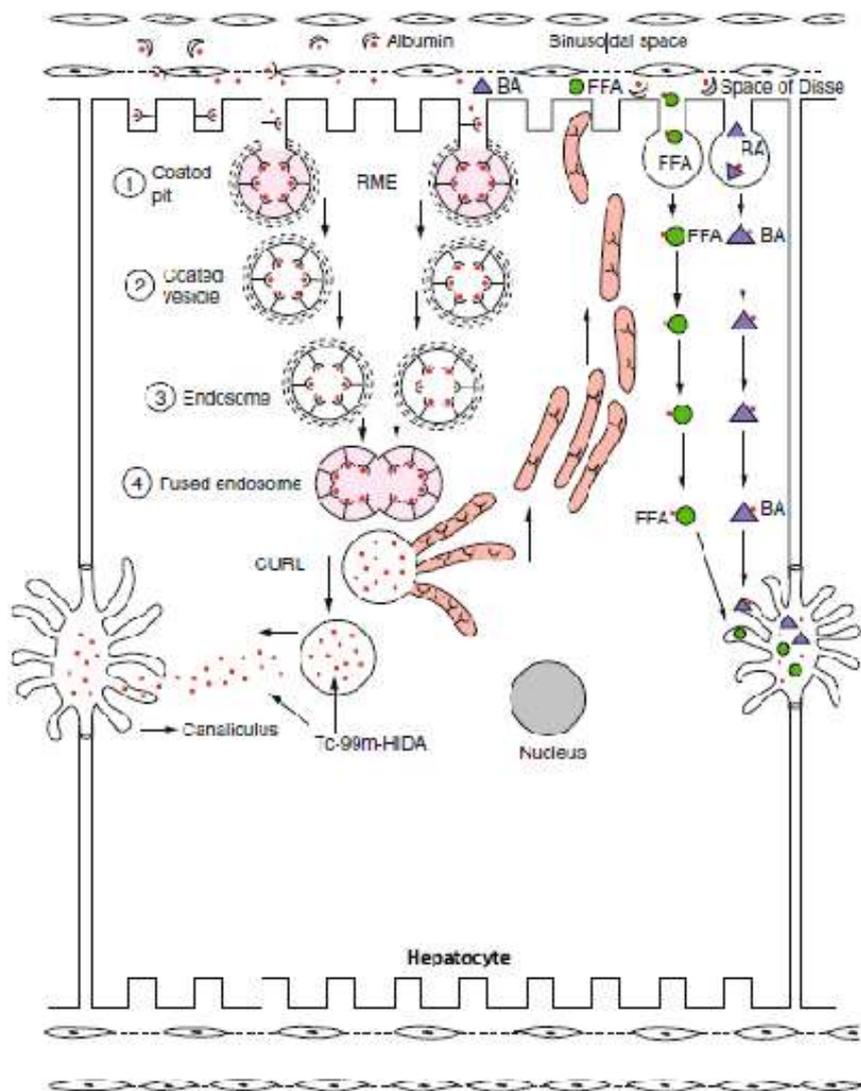


Figure 1.5 Schematic representation of receptor-mediated endocytosis for uptake and excretion of Tc-99m HIDA by the hepatocyte. Primary uptake occurs via receptor-mediated endocytosis (*RME*). After detaching from albumin in the space of Disse, the radiotracer attaches to the ligand in receptors within the coated pits [1], which are invaginations of the basolateral border of the hepatocyte. A coated vesicle [2] is formed when it separates from the surface membrane. The coated vesicle rapidly loses its clathrin coat, forming an endosome [3]. Two endosomes combine to form a fused endosome [4]. Hydrogen is pumped into the fused intra-vesicular space, initiating uncoupling of the receptor and ligand (*CURL*). Ligand enters the bile canaliculi, and the receptor moves to the surface for recycling. Tc-99m HIDA in addition uses free fatty acid (*FFA*) and bile acid (*BA*) pathways for uptake and

excretion in free form into bile canaliculi (Reprinted with permission from Springer Nature®).

Transit Through the Hepatocyte and Secretion into Bile Canaliculi

^{99m}Tc-HIDA are secreted into bile canaliculi without undergoing any conjugation during their transit through the hepatocyte (21). The exact mechanism by which ^{99m}Tc-HIDA is transported through the hepatocyte and then secreted into the bile canalicular lumen is not understood but the mechanism is believed to be similar to those of non-cholephil organic anions, free fatty acids and bile acids involving both vesicular and receptor-ligand transport. After entering the canaliculi, ^{99m}Tc-HIDA mixes thoroughly with the hepatic bile which would then allow delineation of the entire hepatobiliary tree on imaging. The assessment of liver time-activity curve measures the rapidity of uptake and excretion of ^{99m}Tc-HIDA.

Flow Through Intrahepatic and Extrahepatic Ducts

There is immediate in vivo bile radiolabelling once the ^{99m}Tc-HIDA enters the bile canaliculi which then enables the delineation of the entire intrahepatic and extrahepatic bile ducts. Therefore, the bile within the ducts is radiolabelled as the hepatic bile passes through the ducts.

Gallbladder Storage

Approximately 70% of the secreted hepatic bile during fasting hours enter the gallbladder, while the rest enters the duodenum directly (25). It would normally take approximately six hours for a completely emptied gallbladder to refill to its full capacity.

Radiolabelled hepatic bile with ^{99m}Tc -HIDA will accumulate in the gallbladder allowing the imaging of the gallbladder with biliary scintigraphy. The gallbladder is then stimulated to contract and discharge bile into the duodenum upon the arrival of food into the small intestine, whereby bile salts facilitate digestion and absorption of nutrients into the blood stream.

Final Discharge into the Duodenum

Cholecystokinin (CCK)-secreting cells in the mucosa of the duodenum and jejunum will release endogenous cholecystokinin into the circulation upon arrival of food into the duodenum from the stomach. It usually takes about 6-26 minutes (mean 16 minutes) after a meal for serum CCK levels to rise above the threshold to induce contraction and emptying of the gallbladder (26). Bile emptying is normally maintained for approximately 1-2 hours post-meal. CCK also stimulates water secretion by cholangiocytes lining the bile ducts as well as increasing the bile flow by stimulating the smooth muscle contractions of the bile ducts (27). In addition, CCK also stimulates bowel peristalsis to facilitate the bile excretion from the gallbladder into the small bowel. CCK also prevents duodeno-gastric bile reflux by causing contraction of the pyloric sphincter of the stomach.

1.6.1 Effect of Food Intake on Uptake and Excretion of ^{99m}Tc -HIDA

Post-prandial state will alter the physiological functional parameters of the ^{99m}Tc -HIDA biliary scintigraphy study whereby the time to peak hepatic uptake of ^{99m}Tc -HIDA decreases compared to studies performed 6-10 hours post-fasting (Figure 1.6).

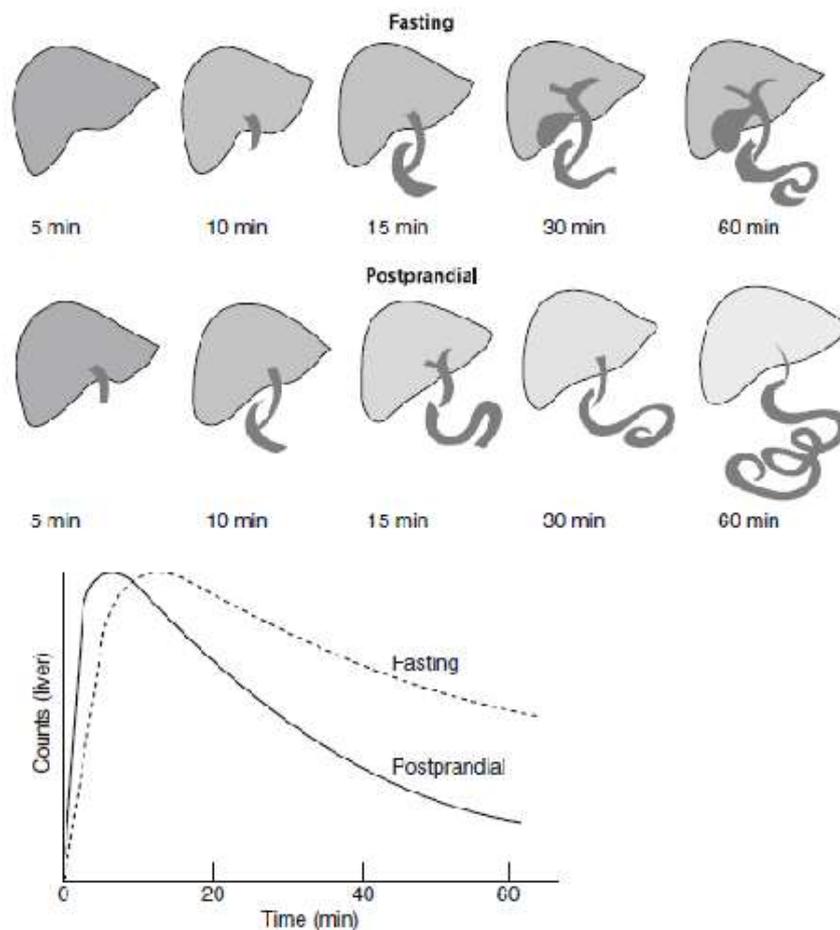


Figure 1.6 Effect of feeding on the kinetics of Tc-99m-HIDA: In the fasting state (top), increased tonus of the sphincter of Oddi diverts more of the hepatic bile into the gallbladder than intestine. There is residual radioactivity in the liver at 60 min. In the post-prandial state (bottom), there is more rapid hepatic uptake and excretion, shifting the peak of the curve to an earlier time, and very little residual radioactivity remains in the liver beyond 15 min. The gallbladder does not fill in because of a lax sphincter (Reprinted with permission from Springer Nature®).

This is in effect due to increased post-prandial liver blood flow and faster extraction of ^{99m}Tc -HIDA by the liver (28). Within the immediate post-prandial state, there is release of endogenous secretin, cholecystokinin and other gastrointestinal hormones causing increased in ductal bile flow. This enables a much more rapid clearance of ^{99m}Tc -HIDA from the liver parenchyma. Consequently, the gallbladder also does not fill because the presence of cholecystokinin during the post-prandial state will

induce gallbladder contraction. Therefore, for gallbladder assessment, it is essential to maximise hepatic bile entry into the gallbladder by ensuring a fasting state. During fasting, the epithelium of the gallbladder wall absorbs water from the lumen through widely opened lateral intercellular spaces between the columnar cells of the mucosa. There is selective absorption of water as bile salts, bile pigments, cholesterol and other bile constituents are not absorbed, hence resulting in higher concentrate of solutes. This process is known as concentration of the gallbladder (29). After fasting, there is gradual increased in mean pressure within the gallbladder to the common bile duct and distally within the Sphincter of Oddi. The hepatic bile flow will follow the path of least resistance and enters the gallbladder. Nonetheless, fasting longer than 24 hours can have an adverse effect on gallbladder filling because of the formation of bile sludge, which decreases water absorption through the wall.

1.6.2 Biliary Scintigraphy Pharmacological Intervention

The biliary scintigraphy technique is variable between institutions although this is a widely standardised practice for the assessment of biliary dyskinesia. This has led to the introduction of biliary scintigraphy guidelines from national nuclear medicine societies (30). The biliary scintigraphy technique will be described more in detail within the methodology. However, within the context of biliary scintigraphy in the assessment of biliary dyskinesia, it has been described as above the need for gallbladder stimulation in order to assess the gallbladder contraction by measuring the gallbladder ejection fraction.

1.6.3 Synthetic Cholecystokinin

One of the main methods to stimulate gallbladder contraction is the use of a synthetically-prepared C-terminal octapeptide of cholecystokinin which is known as Sincalide (Kinevac; Bracco Diagnostics). Sincalide functions like the endogenous cholecystokinin which causes an increased in bile secretion, gallbladder contraction and relaxation of the Sphincter of Oddi which will results in bile drainage into the duodenum.

There has been several different Sincalide infusion protocol over the years making result for gallbladder ejection fraction analysis variable, therefore causing a reduced validity of the study. The short infusion duration of over 3-5 minutes that was initially used in the 1990s was subsequently shown to have a wide variation in the measured and calculated gallbladder ejection fraction even within healthy subjects. This meant that a normal reference value could not established with such a short infusion duration (31). A subsequent multicentre trial evaluating healthy subjects has established that a 60-minute infusion duration at 0.02 µg/kg demonstrated the least variable results with clinically useful reference values determined (32). This method was subsequently incorporated into the Society of Nuclear Medicine and Molecular Imaging (SNMMI) Practice Guidelines for Hepatobiliary Scintigraphy (30) as well as within a consensus recommendation of an interdisciplinary panel made up of gastroenterologists, surgeons and nuclear medicine physicians (33).

Unfortunately, due to production problems, this drug is intermittently not commercially available during recent years. This meant that the use of Sincalide for routine biliary scintigraphy is not reliable and many centres have tried to find alternatives.

1.6.4 Fatty Meal

As described above, the endogenous cholecystokinin within the body will be released following stimulation by food ingestion. Therefore, a fatty meal has been used as an alternative to Sincalide infusion. It has been suggested that a fatty meal is preferable to Sincalide for evaluating gallbladder contraction because it is more physiologic and less expensive. As the use of fatty meal relies on normal physiologic response of the subject, this can be variable, especially in the presence of other intervening factors such as delayed gastric emptying (gastroparesis). Endogenous cholecystokinin is produced and released from the small bowel. Therefore, in the presence of delayed gastric emptying, there will be slow transit of meal into the small bowel and delayed release of the endogenous cholecystokinin which will affect the gallbladder response. Patients with delayed gastric emptying can sometimes experience similar symptoms as patients with biliary dyskinesia and hence confounding the assessment of gallbladder ejection fraction by biliary scintigraphy with fatty meal stimulant.

A variety of fatty meals such as lactose-free fatty meal (34), a corn beef and cheese sandwich with milk (35), dried egg yolk (36), lipomul (37), corn oil emulsion (38), chocolate (39), yoghurt and chocolate (40), Humana (41), whipped cream (42), half and half milk (26, 43) and whole milk (44, 45) have been tried. Fatty meal standardisation has been difficult amongst different institutions. Nonetheless, studies have shown that a minimum 10 g of fat is required to sufficiently stimulate the gallbladder to contract (46). Reference values depend on the composition and size of the meal as well. There have been mixed reports on the reliability of producing reference values in healthy subjects as well as differentiating the gallbladder ejection fractions between

healthy subjects and patients. A systematic review of fatty meal options for biliary scintigraphy has demonstrated wide variation of the calculated normal values on healthy volunteer (47). Nonetheless, the conclusion has mentioned that the commercial fatty meal products offered the highest quality of normal values, likely representing the consistency of meal composition and size.

1.6.5 Morphine

Intravenous morphine is used when the gallbladder is not seen by 60 mins with ^{99m}Tc -HIDA in the clinical setting of acute cholecystitis (48). Morphine constricts the sphincter of Oddi and increases the pressure within the sphincter and the common bile duct, forcing the hepatic bile to enter the gallbladder.

When the gallbladder is not noted by 60 minutes but is noted with morphine administered after 60 minutes, a positive diagnosis of abnormal gallbladder function can be suggested. The major advantage of intravenous morphine is that it enables either the confirmation or the exclusion of the diagnosis of acute cholecystitis to be made within 90 minutes.

1.6.6 Treatment and Outcomes

Cholecystectomy is the main treatment for biliary dyskinesia. However, the outcome for the treatment of biliary dyskinesia following cholecystectomy have not been well-defined (49, 50). Most of the outcome studies are retrospective and uses Sincalide for gallbladder stimulation. The hallmark prospective study in 1991 demonstrated good outcome with cholecystectomy to identify patients with biliary dyskinesia the use of Sincalide biliary scintigraphy (51). A meta-analysis has also demonstrated benefit with cholecystectomy for biliary dyskinesia although reports significant heterogeneity observed in sensitivity analyses (52). A more recent prospective study has also shown good outcome with cholecystectomy (53). Nonetheless, larger studies, including ideally a randomised controlled trial are needed.

1.7 Ultrasonography

Ultrasonography is a form of imaging method which does not involve radiation. This form of technique uses ultrasound waves which are transmitted into the human body through a probe. The ultrasound waves that travel into the human body will be reflected at different depths and the probe will also detect the reflected ultrasound waves. The machine will generate an image from the detected ultrasound scan. Ultrasound imaging is a very safe technique, as it has no radiation involvement. Furthermore, it is portable and inexpensive and an excellent form of imaging to obtain dynamic live images.

Ultrasonography is an excellent form of imaging technique to visualise the gallbladder for the detection of acute cholecystitis (54-57) or gallstones. It can also visualise the common bile duct to assess for any biliary obstruction. However, ultrasonography is very dependent on the operator which can be variable. Therefore, one of the disadvantages is interobserver variability. In the past, the use of ultrasonography for the assessment of biliary dyskinesia has been attempted. Unfortunately, the evidence for this technique has been limited. Furthermore, there are also reported interobserver variability (58-60).

Most of the studies using ultrasonography for assessment of biliary dyskinesia have been with Sinchalide as the gallbladder stimulant. There have been other studies using ultrasonography in the detection of gallbladder contractility with fatty meal stimulus in a variety of conditions such as patients with spinal cord injury (61), diabetes mellitus (62), post-gastrectomy (63), achalasia (64),

Barrett's oesophagus (65), pregnancy (66) and chronic kidney disease (67).

The use of three-dimensional ultrasound probe remains less common. Therefore, most of the studies were performed on two-dimensional ultrasound probes measuring the longitudinal, transverse and anterior-posterior diameters and calculated the volumes using a standard correction factor by assuming an ellipsoid gallbladder shape.

1.7.1 Gallbladder Volume Calculation by Ultrasonography

The initial method for gallbladder volume calculation was described and validated by Everson et al group using the sum-of-cylinders method (68). The sum-of-cylinders method is however tedious and time consuming, although it has been used effectively in several gallbladder volume studies (69-72). The sum-of-cylinders method requires the measurement of multiple transverse diameter of each gallbladder slices 1 mm thick and 1 cm apart with the help of a grid oriented at 90° to the longitudinal gallbladder axis. The calculation of gallbladder volume by the sum-of-cylinders method were made using the formula derived from the Everson et al group (68) as below:

$$V = \sum_{n=i}^n \pi \frac{d_i^2 h_i}{4}$$

where n is the number of cylinders, d_i the diameter of individual cylinders, and h_i the height of individual cylinders. A correction factor (E) is applied when the longitudinal image did not pass precisely through the long central axis of the gallbladder, thereby

causing the maximal diameter of the longitudinal image to be less than that of the cross-sectional image.

Dodds WJ et al then set out to compare the measurements of gallbladder volume calculated by simple ellipsoid method with those obtained by the sum-of-cylinders method (73). The ellipsoid method was calculated by the formula:

$$\text{Gallbladder Volume (GBVol)} = \text{Length (L)} \times \text{Height (H)} \times \text{Width (W)} \times 0.523$$

It was determined that a correction factor is not needed for the ellipsoid method. The authors have measured both in-vitro model and in-vivo studies and demonstrated very good correlation for both methods in both situations. The average difference between the values obtained was negligible.

Despite the wide use of ultrasonography, it remains controversial in its role for gallbladder motility assessment in gallbladder dyskinesia. The use of three-dimensional probes is becoming promising due to the more reliable gallbladder delineation and volume calculation. However, further studies need to be performed with three-dimensional ultrasound probes, in particular with fatty meal stimulus combination.

1.8 Magnetic Resonance Cholangiography

Magnetic resonance (MR) imaging is an excellent imaging modality without the need for radiation. For an MR imaging study, the subject will have to place in an MR imaging scanner which consists of a large magnet. Hydrogen protons align within the magnetic field, and due to their magnetic moment generate a spin. The application of a tuned radiofrequency leads to the absorption of energy and changes a proportion of the spin alignment to be antiparallel to the magnetic field (higher energy state). When the radiofrequency pulse is switched off this energy is released and can be measured. The strength and characteristics of the released energy can be reconstructed as an image. Although MR imaging is an excellent imaging modality, there are a few disadvantages whereby certain patients with pacemaker implants or claustrophobia are not suitable for the study. The MR imaging scan also takes longer, although this is similar to the time required for biliary scintigraphy.

MR imaging has been used widely for the assessment of gallbladder pathology and symptoms-related to gallbladder disease. This is because MR imaging has the capability to delineate well the anatomy of the gallbladder and the biliary system in relation to the adjacent liver and organs. It is also good in detecting gallbladder and biliary system disease owing to its high soft tissue contrast.

Magnetic resonance (MR) cholangiography can be used to assess gallbladder function by evaluating the volume and subsequent ejection fraction with the use of either fatty meal stimulus or the infusion of cholecystokinin. The use of MR cholangiography requires the use of specific contrast agent which

are taken up by the hepatocytes and subsequently excreted into the biliary system. The mechanism is akin to the use of Technetium-99m labelled iminoacetic acid which is used in hepatobiliary scintigraphy. The reason this can be done is because MR cholangiography can image repeatedly within a same region in a wide field of view to assess the movement of the specific organ, and in this case the gallbladder.

The initial studies were conducted on the use of MR cholangiography for assessment of acute cholecystitis. Kim et al compared conventional T2-weighted MR cholangiography with MR cholangiography with the use of biliary excreted contrast agent in the assessment of acute cholecystitis (74). The study was also conducted in conjunction with hepatobiliary scintigraphy which demonstrated excellent correlation between MR cholangiography and hepatobiliary scintigraphy as well as with surgical findings. In addition, Fayad et al has shown up to 100% positive predictive for the diagnosis of acute cholecystitis with functional MR cholangiography (75).

Lee et al has used MR cholangiography to assess the pattern of bile distribution in the biliary tree and the small bowel as well as determining the gallbladder ejection fraction in healthy volunteers (76). This study has demonstrated different distributions of gallbladder ejection fractions within healthy individuals. A further study from a different group compare hepatobiliary phase of gadoteric acid-enhanced magnetic resonance and biliary scintigraphy for evaluation of cystic duct patency and gallbladder contractility in patients suspected for having gallbladder dyskinesia (77). This was a small study that recruited only 18 patients. However, the preliminary data demonstrated

concordance on the calculated gallbladder ejection fractions for both imaging modalities. This indicates that MRI has a potential for assessment of gallbladder motility by calculating the gallbladder ejection fraction.

Fidler et al also conducted a study in comparing MR cholangiography with biliary scintigraphy (78). In addition, this group has also added CT cholangiography into the study for comparison. They have recruited both subjects with functional biliary pain and healthy subjects for comparison. The study used Sincalide as the gallbladder stimulant and showed all three modalities showed comparable calculated gallbladder ejection fractions.

MR cholangiography is a promising imaging modality for the assessment of biliary dyskinesia. It has the advantage of no radiation. However, the MR imaging scanner resources is limited as the MR imaging scanner can be used for a wide variety of imaging indications. Furthermore, no studies have been performed to assess gallbladder motility following fatty meal stimulus by MR cholangiography. Therefore, it can be concluded that the use of MR cholangiography as a first-line imaging modality for assessment of gallbladder dyskinesia is not appropriate due to the lack of evidence currently.

1.9 Computed Tomography

Computed tomography (CT) is a widely used imaging modality with ever increasing indications. However, the use of computed tomography as a first line imaging modality for assessment of gallbladder disease should be discouraged in view of the high radiation involvement. Most of the gallbladder disease requiring structural assessment can be imaged by ultrasonography.

Computed tomography is an imaging modality which utilises X-rays, similar to plain film radiography. This involves lying the subject within the CT scanner who will be irradiated with a narrow beam of X-rays rotating around the subject very quickly. The X-rays will be detected and formed an image which is three-dimensional as opposed to plain film radiography depicting only two-dimensional image. CT scanner are mainly used to assess structural or pathological changes. The newer CT scanners have developed the capability to assess certain physiological parameters such as blood perfusion.

Due to its inherent high radiation, CT scanner is rarely considered appropriate for assessment of biliary motility for functional biliary disorders. It is used more frequently to assess for any structural or pathological abnormality of the gallbladder when ultrasonography is inconclusive.

As discussed within the section in MR cholangiography, Fidler et al has performed a prospective study comparing gallbladder motility assessment and calculation of gallbladder ejection fraction for subjects with functional biliary disorders and healthy subjects using biliary scintigraphy, MR cholangiography and CT

cholangiography (78). The study demonstrated comparable gallbladder ejection fractions in all three modalities. This indicates that CT cholangiography has the potential to assess gallbladder motility. Further studies need to be conducted to assess the use of CT cholangiography, especially the radiation exposure.

1.10 Study Rationale

The initial hypothesis states that there will be good correlation for the measurement of gallbladder ejection fraction between biliary scintigraphy and two-dimensional as well as three-dimensional ultrasound following fatty meal stimulus.

Key research questions:

1. Is there a good correlation between gallbladder ejection fraction measured with biliary scintigraphy and two-dimensional and three-dimensional ultrasound method following a set fatty meal stimulus?
2. If a good and positive correlation is found for gallbladder ejection fraction between biliary scintigraphy and two-dimensional as well as three-dimensional ultrasound methods, can normal values of gallbladder ejection fractions be established using two-dimensional and three-dimensional ultrasound method in healthy volunteer subjects?
3. Can two-dimensional and three-dimensional ultrasound methods complement the use of biliary scintigraphy in the assessment for biliary dyskinesia?

CHAPTER 2

MATERIALS AND METHODS

2.1 Introduction

This chapter will describe the two phases of the study design in detail with regards to the study population, methodology and analysis strategies. Both phases of the study will be discussed below.

This is an observational prospective single centre study of which the author is the Chief Investigator. This research study is sponsored by the University of Warwick as part of higher research degree study.

2.2 Ethical Approval

This prospective study was conducted in compliance with the Research Ethics Committee (REC) of the United Kingdom Health Departments Research Ethics Service (REC reference number 16/WM/0216). As there is no additional ionising radiation involved from the nuclear medicine study component, further approval from the Administration of Radioactive Substances Advisory Committee (ARSAC) was not required.

2.3 Sample Size Calculations

2.3.1 Phase One

A previous study (79) found that the correlation coefficient between gallbladder ejection fraction measured on scintigraphy and three-dimensional ultrasound was $r=0.856$. Using the method given by Hulley et al (80), a Type I error rate, $\alpha=0.05$ (two-tailed), Type II error rate, $\beta=0.1$ and an expected correlation coefficient of $r=0.856$, the total sample size required to determine if a correlation coefficient differs from zero is given by:

$$N = \left[\frac{\left(Z_{\alpha} + Z_{\beta \frac{\pi}{2}} \right)^2}{C} \right] + 3 = 9$$

Where $C = 0.5 \times \ln \left[\frac{(1+r)}{(1-r)} \right]$ and Z refers to the standard normal deviate.

Thus, the minimum number of subjects for this phase is $n=9$.

Although the calculated minimum number of subjects for phase one were 9, the plan was to recruit up to 30 patients within phase one taken into consideration of the number of clinical referrals made for biliary scintigraphy per year within the institution. This number was felt to be an achievable target based on the referral projections over the period of research study.

The next Results chapter have documented the reasons why the initial plan of 30 patients could not be recruited within the timeline.

2.3.2 Phase Two

Minimum sample size for determining reference ranges is a difficult subject, with few papers found in the literature regarding the appropriate number of subjects (81). Various authors have suggested values between 120-200 or more for a statistically sound estimate of the 95% reference interval (82-85).

In this instance rather than a reference range with upper and lower limits we are concerned only with a lower reference limit i.e. a gallbladder ejection fraction lower than the limit can be considered statistically abnormal. Following the work undertaken by Jennen-Steimetz et al (81) for a one-tailed 95% reference interval lower limit, where the sampled limit is within two percentiles of the true population limit with a 90% confidence probability, the minimum number of subjects is $n=171$ for parametric data.

2.4 Study Population

2.4.1 Phase One

Phase one of the study involved recruitment of 13 subjects with clinical symptoms and signs suggestive of biliary dyskinesia following investigations by the clinical team to exclude other pathological biliary disorders. All phase one subjects were prospectively recruited from patients who have been referred by the clinical team for investigation of biliary dyskinesia by biliary scintigraphy. Sources of referral included gastroenterology, general surgery or other relevant clinical specialty teams. All patients referred for biliary scintigraphy from these clinical teams to assess for biliary dyskinesia will be considered for the study.

The inclusion criteria are:

- a) Subjects requiring biliary scintigraphy for the diagnostic work-up of biliary dyskinesia
- b) Subjects can consent for the study, following the guidance published by the General Medical Council as Good Practice in Research and Consent to Research (86)
- c) Subjects must be over age 18 years old.
- d) Subjects have not had previous surgical intervention for biliary symptoms such as cholecystectomy.
- e) Gallbladder can be visualised on biliary scintigraphy
- f) Other biliary pathologies or structural abnormalities have been excluded by other forms of imaging.

The exclusion criteria are:

- a) Subjects whom gallbladder cannot be visualised on ultrasonography
- b) Subjects whom ultrasonography detected other pathological or structural abnormalities of the gallbladder such as presence of gallbladder stones or gallbladder polyp.
- c) Subjects who are normally excluded from biliary scintigraphy even based on the clinical referral for diagnostic assessment of biliary dyskinesia such as pregnancy and breast feeding as well as being on certain medications which will affect the validity of the biliary scintigraphy study.

The subject information leaflet regarding the study was sent out with subject's appointment letter regarding the study. On the day

of the study, the subject will meet with the chief investigator to further discuss about the study and to obtain consent.

2.4.2 Phase Two

Phase two of the study involves recruitment of healthy volunteer subjects with no symptoms suggestive of biliary dyskinesia or functional biliary disorder as described in the introduction chapter. This meant that the healthy volunteer subjects do not have symptoms described within the Rome IV criteria for functional gallbladder disorder diagnosis (2).

The inclusion criteria are:

- a) Healthy volunteer subjects with no symptoms described by the Rome IV diagnostic criteria for functional gallbladder disorder.
- b) Healthy volunteer subjects can consent for the study, following the guidance published by the General Medical Council as Good Practice in Research and Consent to Research (86)
- c) Healthy volunteer subjects must be over age 18 years old.
- d) Healthy volunteer subjects have not had previous surgical intervention for biliary symptoms such as cholecystectomy.

The exclusion criteria are:

- a) Healthy volunteer subjects whom gallbladder cannot be visualised on ultrasonography
- b) Healthy volunteer subjects whom ultrasonography detected other pathological or structural abnormalities of the

gallbladder such as presence of gallbladder stones or gallbladder polyp.

- c) Healthy volunteers who are pregnant, breast-feeding or on medications who will be normally excluded from the diagnostic criteria for biliary scintigraphy clinically.

Health volunteer subjects for phase two of the study will be recruited from staff working within the recruitment hospital. Information for healthy volunteer subjects' recruitment will be disseminated within the recruitment hospital. The targeted cohort of healthy volunteer recruitments within hospital staffs was specifically detailed within the submission to the Research Ethics Committee and has been approved.

The healthy volunteer subjects will be recruited to match the demographics of phase one study subjects as similar as possible. Similar numbers of healthy volunteer subjects will be recruited as the number of subjects recruited for phase one.

The healthy volunteer subjects will receive the subject's information leaflet for the study together with the standard information leaflet regarding the biliary scintigraphy appointment. The subject's information leaflet contains information regarding the nature of the study, the selection criteria, the whole procedure of the study and contacts for the relevant people involved in the study. Healthy volunteer subjects will meet the chief investigator on the day of the ultrasonography procedure to discuss about the study and consent is also obtained at this stage.

2.5 Biliary Scintigraphy

All subjects attending the phase one of the study will undergo a standard biliary scintigraphy protocol based on the department's standard operating procedure. The biliary scintigraphy is performed by members of staff who are listed in the nuclear medicine department as approved Ionising Radiation (Medical Exposure) Regulations (IRMER) operator list. The biliary scintigraphy requires a set meal (see below), followed by four hours fasting before the appointment time.

To prepare the gallbladder, the subject must consume a set meal which has a set amount of caloric content. The set meal contains two medium slices of toast with thinly spread margarine or butter. Subject will then fast for four hours before the biliary scintigraphy study. They may drink water if needed. No narcotics (including smoking) or chewing gum are allowed after the set meal has been consumed until the end of the test as this will affect the biliary scintigraphy test. If the subject is on morphine-related medication, this will be discontinued for twelve hours before the test.

Following four-hour fast after the set meal, subjects will be administered 100 MBq ^{99m}Tc -mebrofenin intravenously 30 minutes before the imaging commences. The amount of injected activity is based on the recommended diagnostic reference level from the ARSAC guidance (87). Subjects will be remained fasted the duration between the injection and the test.

The biliary scintigraphy lasts for 45 minutes. The test is acquired as a continuous dynamic phase imaging. The dynamic phase imaging camera set-up is as below:

Camera Orientation	H-mode, Anterior/Posterior
Detectors	1 & 2
Collimator	Low Energy General Purpose (LEGP)
Matrix	128
Zoom	1.0
Contouring	On
Acquisition Mode	Dynamic
Frame Time/Acquisition Length	45 x 60s

All subjects are imaged supine with the feet towards the gamma camera to obtain a diagnostic view of the hepatobiliary system. All subjects are imaged with the Discovery NM/CT 670 or Optima NM/CT 640 gamma cameras (General Electric Healthcare, Milwaukee, United States of America).

At 30 minutes post-injection, the subjects are placed on the gamma camera. The dynamic imaging is commenced at this point. After 5 minutes of imaging, the subject is given a fatty meal stimulus which consists of a Mix 1 x 125 ml Fortisip Compact (Nutricia Ltd, Wiltshire, UK) with 75 ml Calogen (Nutricia Ltd, Wiltshire, UK). The total fat content is approximately 50 g. The subject will be scanned for additional 40 minutes following the fatty meal stimulus.

The biliary scintigraphy raw images will be sent for processing following protocol as any standard biliary scintigraphy performed. The Xeleris 3 Workstation (General Electric Healthcare,

Milwaukee, United States of America) is used to perform the analysis. The raw data is initially checked for motion and corrected if necessary. The dynamic phase is processed by drawing region of interest (ROI) around the gallbladder which will subsequently generate a time-activity curve. The gallbladder ejection fraction is calculated by taking the difference in counts between the maximum and minimum point of the time-activity curve after the time-point of fatty meal stimulus.

All the biliary scintigraphy analysis were performed by qualified nuclear medicine technologists and clinical scientists working within the Nuclear Medicine department who have been routinely involved in biliary scintigraphy analysis.

2.6 Ultrasonography

The ultrasound was performed on similar LOGIQ E9 ultrasound machines (General Electric Healthcare, Milwaukee, United States of America). A curvilinear ultrasound probe and a three-dimensional ultrasound probe will be used for the ultrasound study.

For the first phase of the study which involves subjects referred for biliary scintigraphy to assess for biliary dyskinesia, ultrasound will be performed just before the commencement of the biliary scintigraphy (20 minutes after administration of radiopharmaceutical agent) and immediately upon completion of the biliary scintigraphy. Subjects will be scanned with the ultrasound machine while lying on the gamma camera. During the ultrasound procedure, the gallbladder will be identified and ensure no pathology is identified. Three orthogonal plane measurements

of the gallbladder will be acquired with the curvilinear ultrasound probe. This is performed by placing the probe over the gallbladder area, and the long axis was aligned with the longest gallbladder dimension. The maximal gallbladder length (L) and the anteroposterior dimension (height [H]) were measured (Figure 2.1a). The probe was then rotated in a right-angle plane for viewing of the cross-section of the gallbladder, and the maximal transverse gallbladder diameter found. The greatest transverse gallbladder diameter (width [W]) was measured (Figure 2.1b). All measurements were obtained from inner wall to inner wall. Three measurements will be obtained for each orthogonal plane. This is followed for three-dimensional acquisition of the gallbladder with the three-dimensional ultrasound probe. Similarly, the three-dimensional acquisition will also be acquired three times. The ultrasound procedure will be repeated immediately after completion of the biliary scintigraphy study. The similar images for both 3 orthogonal plane measurements and three-dimensional images will again be acquired.

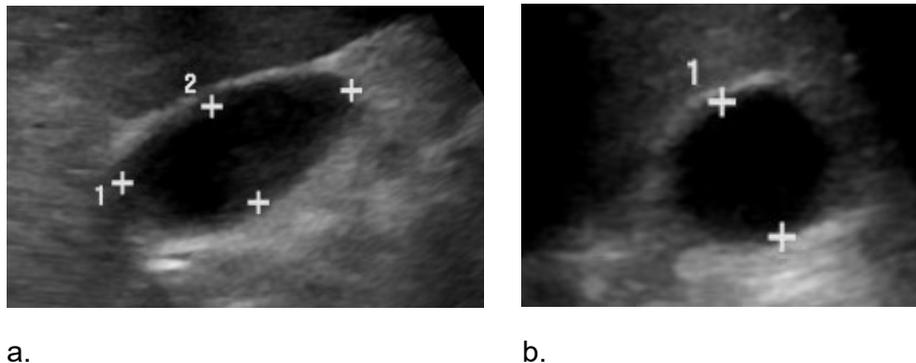


Figure 2.1 Ultrasound images of the gallbladder in perpendicular planes.

a, Ultrasound image shows ultrasound probe placed along long axis of the gallbladder for measurement of maximum length and anteroposterior dimension (height) (*callipers* 1 and 2).

b, Ultrasound image obtained at a perpendicular plane to image of (a) (transverse image of gallbladder) shows maximum transverse gallbladder diameter (width) (*callipers* 1).

For the second phase of the study which involves recruitment of healthy volunteer subjects, subjects will not undergo biliary scintigraphy. Only ultrasound procedure will be performed on the healthy volunteer subjects. Similar to the subjects recruited for phase one of the study, healthy volunteer subjects will be required to consume the same set meal specified for the phase one subjects and fast for four hours following the set meal. Once fasted for four hours, healthy subjects will undergo the ultrasound procedure which will be the same as the phase one subjects. Ultrasound will be performed at every 15 minutes interval for four times. Following the first ultrasound study, healthy volunteer subjects will consume the fatty meal stimulus as described above for the phase one study. Three further ultrasound studies were acquired following consumption of the fatty meal challenge at 15 minutes, 30 minutes and 45 minutes. This is to follow the timeline similar to biliary scintigraphy for phase one subjects. There will again be measurements of the gallbladder with the curvilinear ultrasound probe in three orthogonal planes as well as three-dimensional acquisition with the three-dimensional ultrasound probe. All measurements and images will be acquired on three consecutive times

All ultrasound procedures are performed by the chief investigator who is a certified clinical radiologist based on the Royal College of Radiologist training scheme (88).

2.7 Image Analysis

All image analysis will be completed on vendor native platforms by one of the local investigation team, supervised by the Chief Investigator. Each set of subject results were reviewed by the Chief Investigator to ensure consistency in analysis as well as discussed and approved by the Chief Investigator. The consistency of the result was maintained by repeated analysis of the biliary scintigraphy study by the Chief Investigator. The Chief Investigator will have executive decision if the local investigation team cannot agree a data set analysis.

On the scintigraphy images, gallbladder ^{99m}Tc -HIDA activity will be represented by the acquired counts from a region of interest (ROI) drawn around the margins of the gallbladder and corrected for overlying/underlying uptake in the liver parenchyma (avoiding the nearby hepatic tree) with a similarly sized region of interest placed over the upper right lobe of the liver. Gallbladder ejection fraction will be computed as the lower of two measurements of GB contents measurements, divided by the higher and reported as a percentage (Figure 2.2).

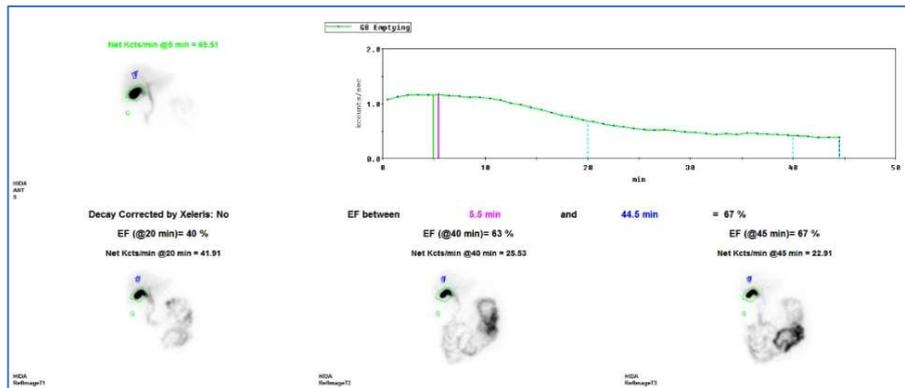


Figure 2.2 An example of biliary scintigraphy demonstrating a region of interest (ROI) drawn around the gallbladder with a time-activity graph generated and calculated gallbladder ejection fraction (GBEF).

For gallbladder measurements, the average measurements for the gallbladder length, height and width were calculated. Gallbladder volume is calculated as a prolate ellipsoid shape with the formula as depicted above.

As described in the ultrasound section of the introduction chapter, the ellipsoid method of calculation is a validated method of calculating the gallbladder volume (73).

Measurements were taken at 15 minutes interval for the healthy volunteer subjects was to assess the pattern of gallbladder contraction and drainage as well as refilling. As each ultrasound scan can take between 5-10 minutes, it is not feasible to shorten the duration of the interval to scan more frequently. The final assessment point was taken at 45 minutes to coincide with the end time for the phase one subjects. Frequent ultrasound measurements could not be carrying out for phase one subjects due to difficulty in getting good sonography windows as patient is undergoing biliary scintigraphy at the same time.

Unfortunately, gallbladder volume from the three-dimensional images could not be analysed due to data transfer error whereby volumetric information for the three-dimensional sweep were not available. Therefore, gallbladder ejection fraction calculation could not be obtained from the three-dimensional acquisition.

2.8 Statistical Analysis

Statistical advice was obtained from two statisticians based at the Warwick Medical School, Dr Peter Kimani and Dr Nick Parsons.

Microsoft Excel[®] (Microsoft Office 365 ProPlus) spreadsheet is utilised for data input and compilation. Statistical analysis has been performed using IBM[®] SPSS[®] Statistic program (IBM[®] SPSS[®] Statistics, Version 27) and Microsoft Excel[®] (Microsoft Office 365 ProPlus) spreadsheet. The demographics of the subjects and healthy volunteer subjects are presented as categorical information. Tables, linear and bar graphs were presented to demonstrate the difference in calculated gallbladder ejection fraction for both biliary scintigraphy and ultrasonography study. Normality of the data were investigated by frequency histogram charts and normality tests such as Kolmogorov-Smirnov and Shapiro-Wilk. A scatterplot of the gallbladder ejection fractions from both modality in phase one subjects have been performed with calculation of both Pearson's and Spearman's correlation coefficient to determine the correlation between both biliary scintigraphy and ultrasonography calculated gallbladder ejection fraction. In addition, a Bland-Altman plot has also been generated to compare the two measurements of the same variables. A non-parametric Mann-Whitney-Wilcoxon test has also been applied on the phase one study to determine any

significance between the biliary scintigraphy and ultrasonography methods.

For phase two of the study, a line plot was performed to assess the gallbladder volume change over the 45 minutes interval. A bar graph is also produced to assess the overall gallbladder ejection fraction for each healthy volunteer subjects. As gallbladder volume can be calculated from the orthogonal measurements over three time points following fatty meal stimulus, the changes of gallbladder volume will be assessed to investigate the mean time of refilling following contraction. This will be presented as a line graph demonstrating the change in volume of all the healthy volunteer subjects with interposition of the mean results.

2.8.1 Phase one and phase two results comparison.

A Mann-Whitney-Wilcoxon test will be performed between the mean gallbladder ejection fraction of phase one subjects demonstrating abnormal gallbladder ejection fraction on biliary scintigraphy (gallbladder ejection fraction <35%) and healthy volunteer subjects in phase two.

A Mann-Whitney-Wilcoxon test will be performed between the mean gallbladder ejection fraction of phase one subjects demonstrating normal gallbladder ejection fraction on biliary scintigraphy (gallbladder ejection fraction >35%) and healthy volunteer subjects in phase two.

CHAPTER 3

RESULTS

3.1 Introduction

The results are presented in two parts. The results from the phase one of the study involving subjects requiring biliary scintigraphy with ultrasound will be presented first. This is followed by results from healthy volunteer subjects in phase two of the study.

Demographics for both phases of the study is illustrated as below:

	Phase One Subjects	Phase Two Healthy Volunteer Subjects
Number	13	13
Mean age (range)	36 (23-68)	30 (23-50)
Gender Male:Female	5:8	6:7

Table 3.1 demonstrates demographic differences between phase one and phase two subjects.

3.2 Phase One

During the period of recruitment, there were 40 patients who were referred for biliary scintigraphy for assessment of biliary dyskinesia following exclusion of anatomical or pathology cause of patient's symptoms. Only 13 patients were recruited during this period due to difficulty in coordinating and assessing ultrasound equipment as only two ultrasound machines are compatible with the three-dimensional ultrasound probe. As the biliary scintigraphy can only be performed during specific time of the day

due to the requirements for the dietary and fasting preparation, this further limits the availability of the ultrasound machines which are normally utilised for clinical work. In addition, as all the ultrasound are performed by the chief investigator, there is also restrictions in availability of the chief investigator, as ultrasonography can normally only performed during dedicated research time.

The phase one subjects yielded three groups of results based on biliary scintigraphy results:

- Group A: Gallbladder ejection fraction could not be calculated from the biliary scintigraphy
- Group B: Abnormal gallbladder ejection fraction which is defined as a calculated ejection fraction of <35%.
- Group C: Normal gallbladder ejection fraction which is defined as a calculated ejection fraction of >35%.

3.2.1 Group A: Gallbladder ejection fraction could not be calculated from the biliary scintigraphy

There were four subjects which gallbladder ejection fraction could not be calculated from the biliary scintigraphy. Gallbladder ejection fraction can only be calculated when there is a reduction in the tracer activity over time for biliary scintigraphy as demonstrated on Figure 2.2 in the Chapter for Methodology.

All four subjects have tracer activity seen within the gallbladder at the beginning of the study. Following ingestion of the fatty

stimulus, there is either further increased or plateau of tracer activity within the gallbladder with no evidence of tracer excretion. An example of such a patient is depicted in Figure 3.1 and 3.2.

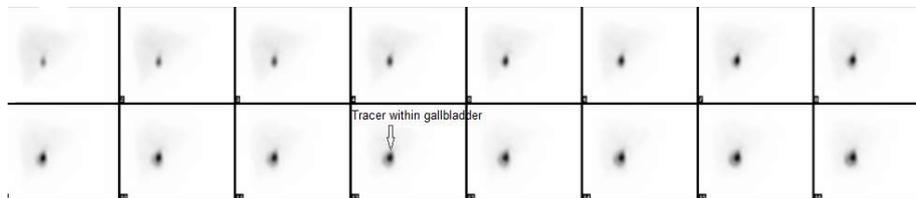


Figure 3.1 demonstrate dynamic images of biliary scintigraphy after fatty meal stimulus with increasing tracer activity seen within the gallbladder but no evidence of excretion into the small bowel

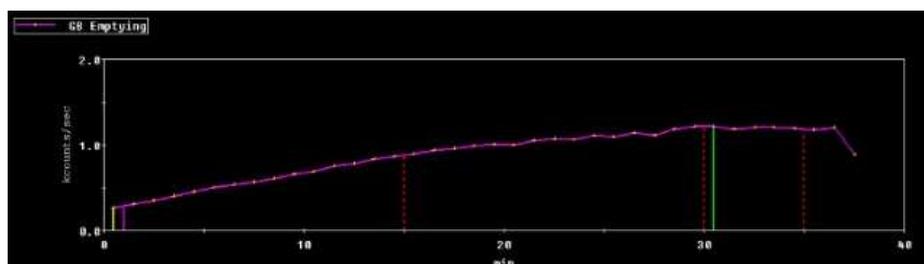


Figure 3.2 demonstrate the time-activity curve for the biliary scintigraphy of Figure 3.1 which shows increasing tracer activity within the gallbladder with no evidence of excretion.

In two of the four subjects who did not show any tracer excretion from the biliary scintigraphy, there was a correlation with the gallbladder ultrasound findings which demonstrated an increased in gallbladder volume. However, in the other two subjects, there was a reduction of gallbladder volume with a calculated gallbladder ejection fraction of 21.5% and 28.8% which are inconsistent with the findings on biliary scintigraphy.

3.2.2 Group B: Abnormal gallbladder ejection fraction which is defined as a calculated ejection fraction of <35%

There were three subjects which demonstrated poor gallbladder ejection fraction (<35%) based on biliary scintigraphy analysis. The calculated gallbladder ejection fraction for the three subjects were 9%, 23% and 31% respectively. Two of the subjects demonstrated better calculated ejection fraction based on ultrasonography while one of the subjects demonstrated an increased in gallbladder volume on ultrasound measurements. This is better depicted on Table 3.2 demonstrating the gallbladder ejection fractions for both ultrasonography and biliary scintigraphy methods for the three subjects and Figure 3.3 which shows how the calculated gallbladder ejection fraction compared between the two modalities on the bar chart.

Patients	Ultrasound Gallbladder Ejection Fraction (%)	Biliary Scintigraphy Gallbladder ejection fraction (%)
1	51	31
2	0	23
3	45	9

Table 3.2 demonstrates the gallbladder ejection fractions for both ultrasonography and biliary scintigraphy methods for the three subjects.

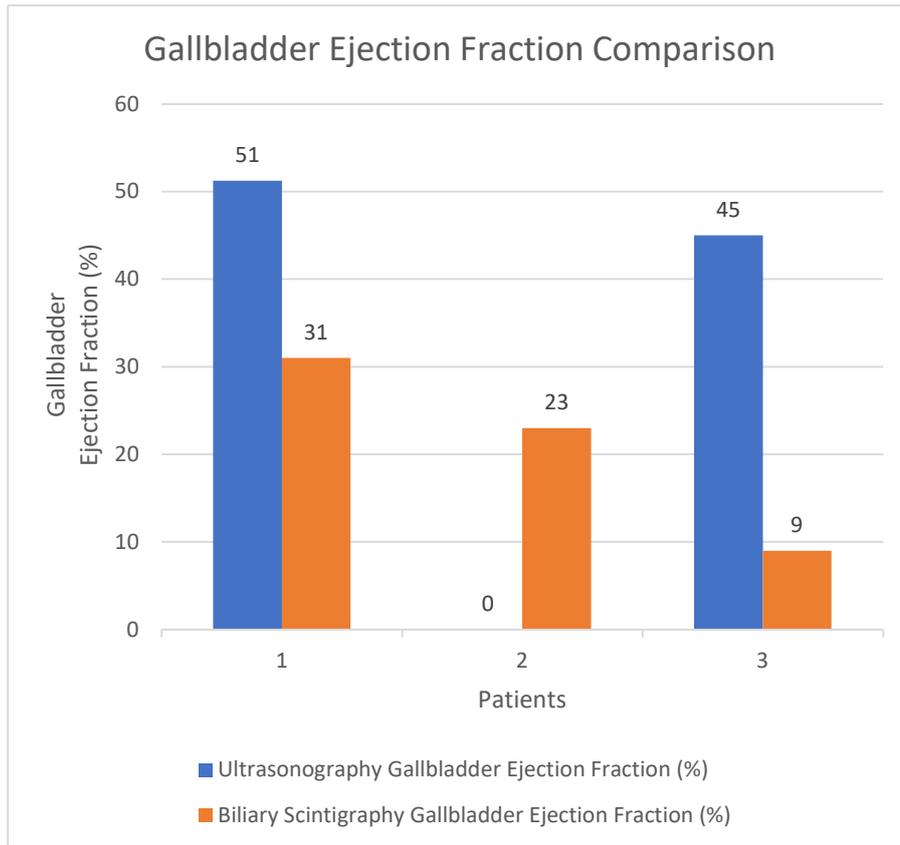


Figure 3.3 demonstrate the difference in gallbladder ejection fractions between ultrasound measurements and biliary scintigraphy for subjects with abnormal gallbladder ejection fractions based on biliary scintigraphy.

3.2.3 Group C: Normal gallbladder ejection fraction which is defined as a calculated ejection fraction of > 35%

There were six subjects who demonstrated normal gallbladder ejection fraction (>35%) based on biliary scintigraphy analysis. None of the subjects in Group C demonstrated an increased in gallbladder volume based on ultrasonography assessment. Four of the six subjects showed a higher calculated gallbladder ejection fraction from the biliary scintigraphy when compared to ultrasound volume assessment. One of the subjects demonstrated a calculated gallbladder ejection fraction of 29.1% based on ultrasound assessment which is much lower than gallbladder

ejection fraction calculated from biliary scintigraphy. The results are better depicted on Table 3.3 demonstrating the gallbladder ejection fractions for both ultrasonography and biliary scintigraphy methods for the six subjects and Figure 3.4 shows how the calculated gallbladder ejection fraction compared between the two modalities on the bar chart.

Patients	Ultrasound Gallbladder Ejection Fraction (%)	Biliary Scintigraphy Gallbladder ejection fraction (%)
1	81	95
2	29	51
3	62	67
4	93	87
5	70	40
6	86	99

Table 3.3 demonstrates the gallbladder ejection fractions for both ultrasonography and biliary scintigraphy methods for the six subjects.

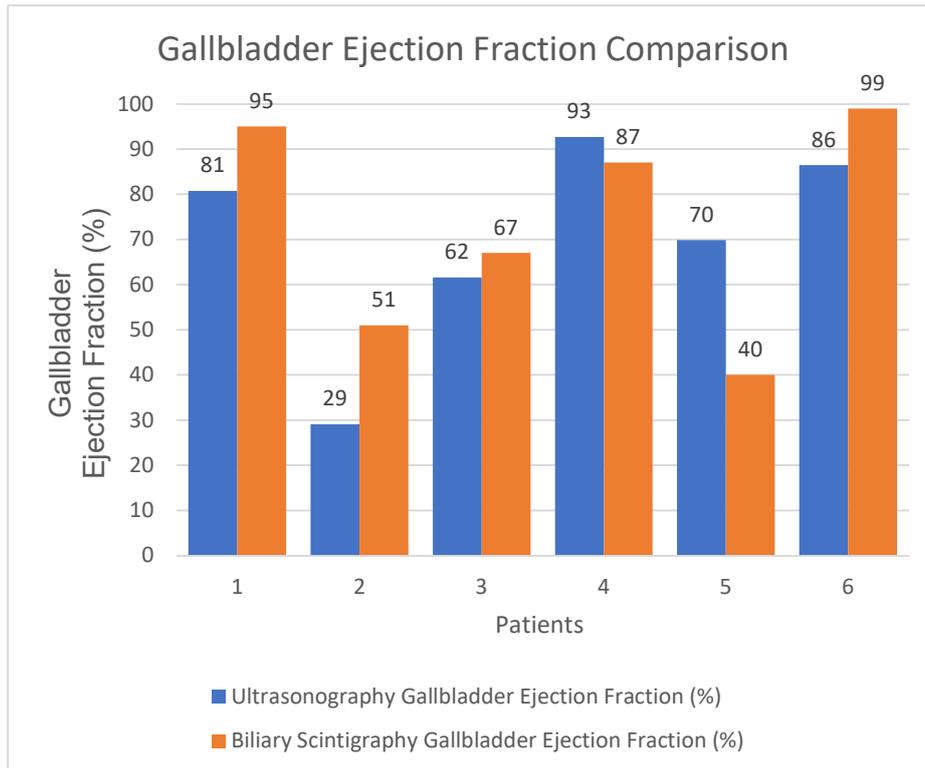


Figure 3.4 demonstrate the difference in gallbladder ejection fractions between ultrasound measurements and biliary scintigraphy for subjects with normal gallbladder ejection fractions based on biliary scintigraphy.

As described above, there were four subjects in phase one of the study who demonstrated abnormal biliary scintigraphy whereby gallbladder ejection fraction could not be calculated due to non-excretion of ^{99m}Tc -HIDA tracer into the small bowel from the gallbladder. Therefore, these four subjects were excluded from the calculation of correlation between biliary scintigraphy and ultrasonography assessment of gallbladder ejection fraction.

The remaining nine subjects demonstrated variation in the calculated gallbladder ejection fractions from biliary scintigraphy and ultrasonography assessments with 4 subjects demonstrating a higher value from biliary scintigraphy assessments and 5 subjects demonstrated higher calculated ejection fractions from

ultrasonography assessments. This is better depicted in Figure 3.5 which show the correlation for each subject between the two modalities in line graph. This demonstrates the fact that neither biliary scintigraphy or ultrasonography assessment methods consistently over- or under-estimates the calculated gallbladder ejection fraction between the two modalities.

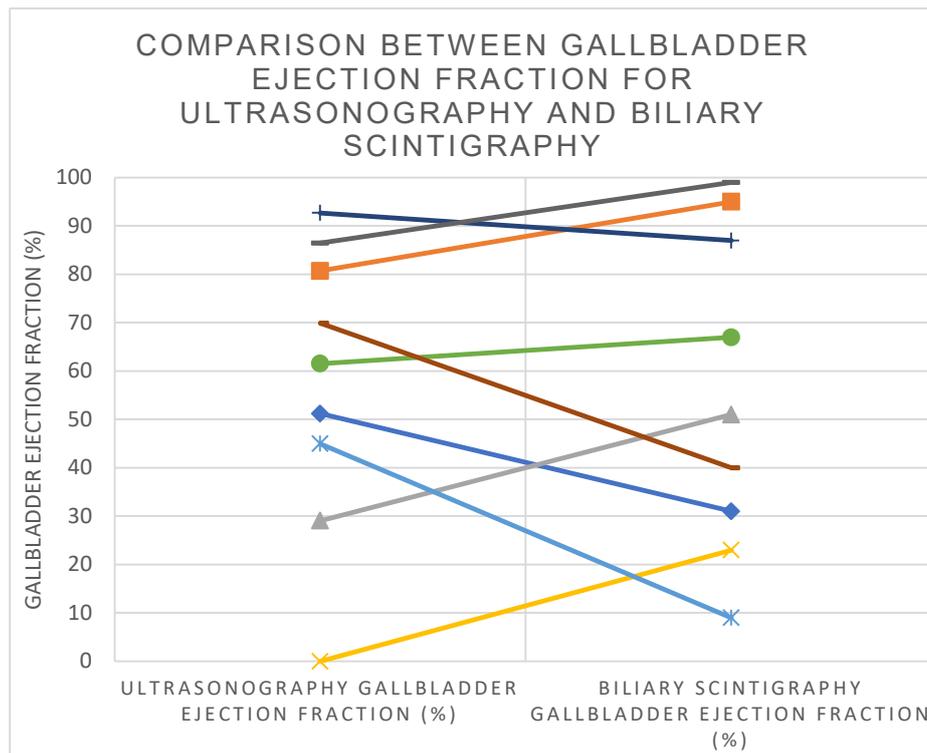


Figure 3.5 demonstrate the differences in gallbladder ejection fraction calculated from both modalities for each subject.

Both ultrasonography and biliary scintigraphy gallbladder ejection fractions have similar mean and standard deviations 56.1 (SD \pm 32.7) and 56 (SD \pm 32.9). There is some difference seen on the box and whisker plot as shown on Figure 3.6.

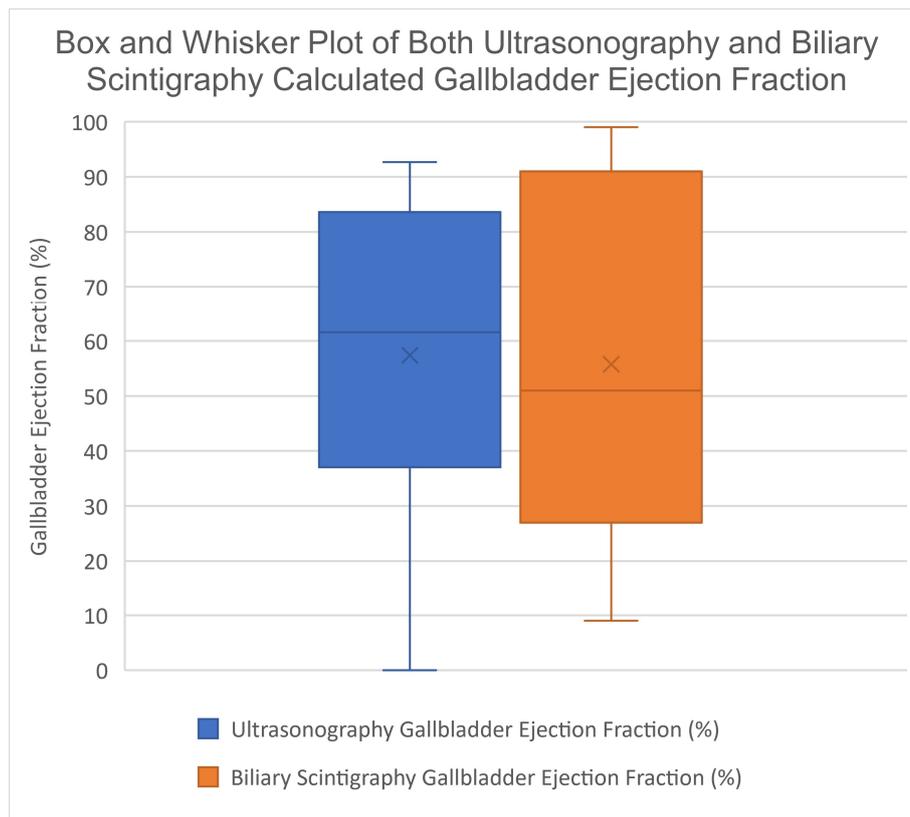


Figure 3.6 demonstrate the comparison of gallbladder ejection fractions from both modalities on a box and whisker plot.

The results for both gallbladder ejection fractions obtained from ultrasonography and biliary scintigraphy techniques were checked for normal distribution using the Kolmogorov-Smirnov and Shapiro-Wilk tests.

The Kolmogorov-Smirnov test demonstrated a p-value of 0.200 for both ultrasonography and biliary scintigraphy gallbladder ejection fractions which is above 0.05 indicating normal distribution. The Shapiro-Wilk test for normality demonstrated a p-value of 0.537 for biliary scintigraphy method and p-value of 0.674 for ultrasonography method in gallbladder ejection fraction calculation which is above 0.05, indicating normal distribution.

Frequency histogram for both ultrasonography and biliary scintigraphy methods have also been plotted to assess for normal distribution. Figure 3.7 below demonstrating frequency histogram of the gallbladder ejection fraction based on ultrasound method and shows a non-normal distribution with negative skewness.

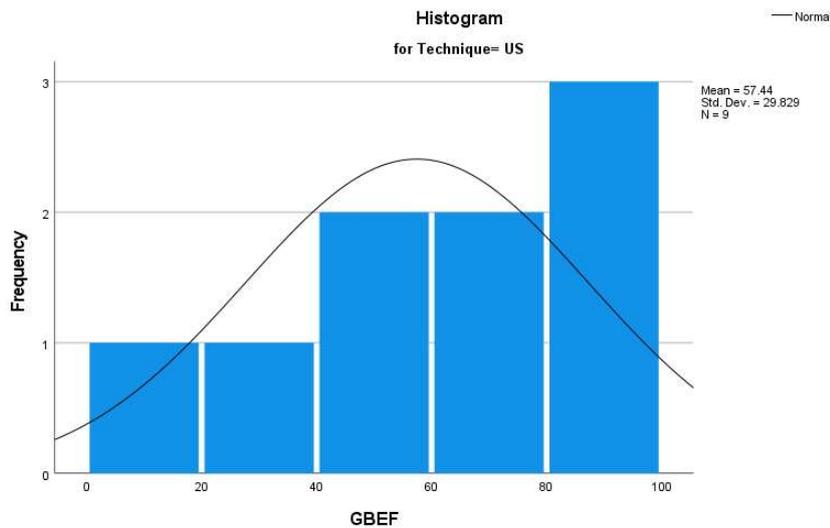


Figure 3.7 demonstrates frequency histogram of the gallbladder ejection fraction based on ultrasound method.

Figure 3.8 below demonstrates frequency histogram of the gallbladder ejection fraction based on biliary scintigraphy method and shows a non-normal distribution with negative skewness.

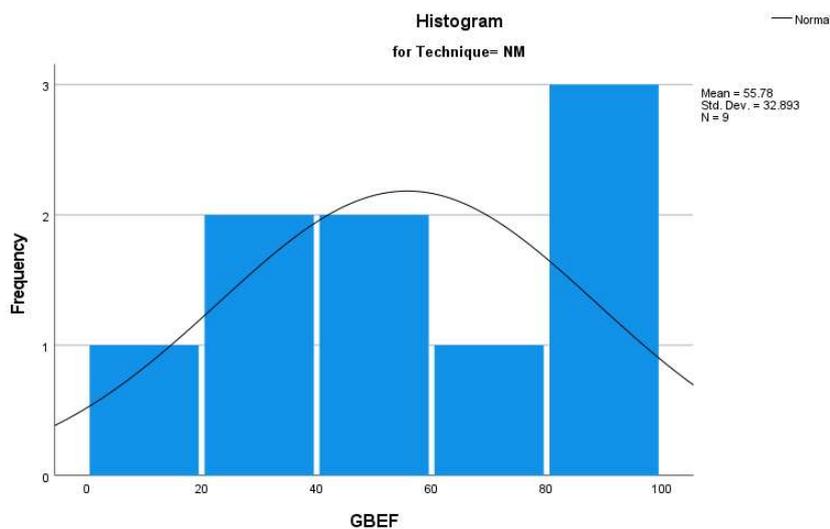


Figure 3.8 below demonstrates frequency histogram of the gallbladder ejection fraction based on biliary scintigraphy method.

Therefore, there is contradiction between the results from the frequency histograms and both Kolmogorov-Smirnov and Shapiro-Wilk tests. The normality tests demonstrate that both data have a normal distribution, but the frequency histograms demonstrate non-normal distribution with negative skewness. This is very likely due to the small size sample. Although the gallbladder ejection fraction results for both imaging modalities may have a normal distribution if a larger sample was obtained, this cannot be demonstrated on the frequency histogram due to a small sample size within this study.

In view of the possibility of a non-normal distribution, the correlation between both modalities for the assessment of gallbladder ejection fraction is assessed with Spearman's correlation coefficient as the data is considered as non-parametric. The Spearman's ρ correlation was calculated with a value of 0.783 and a p-value of 0.013 (significant if $p < 0.05$). This

indicates a moderate positive correlation between both modalities in the measurement of gallbladder ejection fraction. This is best demonstrated on the scatter plot on Figure 3.9 showing the correlation.

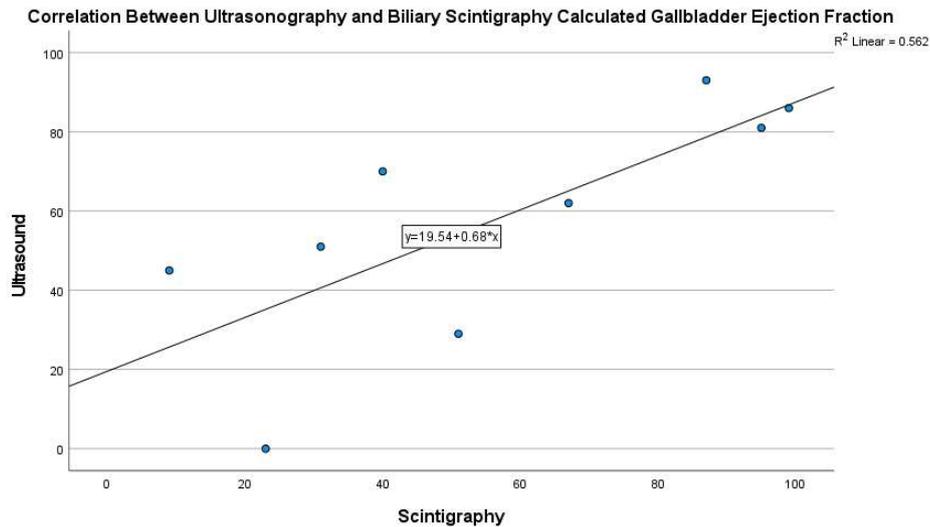


Figure 3.9 is a scatter plot graph demonstrating the correlation between both modalities.

A Bland-Altman plot (Figure 3.10) has also been performed which demonstrated that the differences between the calculated gallbladder ejection fractions are within the 2 standard deviations limit although there is still quite high range of differences observed.



Figure 3.10 demonstrates a Bland-Altman plot of the differences between the calculated gallbladder ejection fractions from both modalities.

Therefore, there is moderate correlation between both modalities based on the Pearson's correlation coefficient. The differences between the calculated gallbladder ejection fractions are within 2 standard deviations based on the Bland Altman plot.

In addition, a Mann-Whitney-Wilcoxon test has also been employed to test the difference in the mean of both gallbladder ejection fraction values from ultrasonography and biliary scintigraphy. The calculated p-value is 1.0 indicating no significant difference between the 2 mean values which would be consistent with the known moderate correlation.

3.3 Phase Two

For phase two of the study, a total of 14 healthy volunteer subjects consisted of 6 males and 8 females between the age range of 23-50 (mean 30) were recruited within the local radiology and nuclear medicine department. Unfortunately, one of the healthy volunteer subjects was excluded as the initial ultrasound study before fatty meal stimulus demonstrated a contracted gallbladder which is difficult to define the luminal measurements. Therefore, only 13 healthy volunteer subjects were analysed for the study. All healthy volunteer subjects fulfil the criteria stipulated in methodology chapter. None of the healthy volunteer subjects experience any symptoms during the study following ingestion of the fatty meal stimulus.

As described in the methodology, phase two healthy volunteer subjects had 4 ultrasonography measurements of the gallbladder over a period of 45 minutes to chart the gallbladder contraction throughout the period by calculating the change in gallbladder volume.

The volume of each individual healthy volunteer patients is charted over the period of 45 minutes in 15 minutes interval following fatty meal stimulus. This demonstrated majority of the healthy volunteer subjects have the smallest gallbladder volume after fatty meal stimulus at 45 minutes. However, five of the healthy volunteer subjects had the smallest gallbladder volume before 45 minutes; two healthy volunteer subjects had the smallest gallbladder volume at 15 minutes and three other healthy volunteer subjects had the small gallbladder volume at 30 minutes.

The data for healthy volunteers will be shown within Table 3.4 which demonstrates the gallbladder volume at 0 min, 15 min, 30 min and 45 min as well as demonstrating calculated gallbladder ejection fraction at 15 min, 30 min and 45 min. This allows an overview of how the volume changes over time with the corresponding gallbladder ejection fraction.

Patients	Gallbladder volume in mL (Gallbladder ejection fraction in %)			
	0 min	15 min	30 min	45 min
1	20.2	10.1 (50)	5.0 (75)	4.9 (76)*
2	5.2	0.1 (98)*	0.8 (84)	0.8 (84)
3	14.4	2.5 (83)	0.8 (95)	0.5 (96)*
4	17.7	16.1 (9)	14.2 (20)	13.8 (22)*
5	6.9	2.7 (60)	2.7 (61)	0.9 (87)*
6	21.7	21.7 (0)	17.0 (22)*	18.3 (16)
7	23.0	9.6 (58)	7.8 (66)	7.3 (68)*
8	22.9	11.7 (49)	7.2 (68)	6.2 (73)*
9	12.4	6.9 (45)	2.7 (78)	1.3 (90)*
10	11.8	1.4 (88)	1.3 (89)	1.1 (91)*
11	7.7	7.6 (2)*	11.5 (0)#	14.2 (0)#
12	11.9	1.3 (89)	1.3 (89)*	3.6 (70)
13	6.4	0.4 (94)	0.1 (99)*	0.4 (94)

Table 3.4 which demonstrates the gallbladder volume at 0 min, 15 min, 30 min and 45 min as well as demonstrating calculated gallbladder ejection fraction at 15 min, 30 min and 45 min.

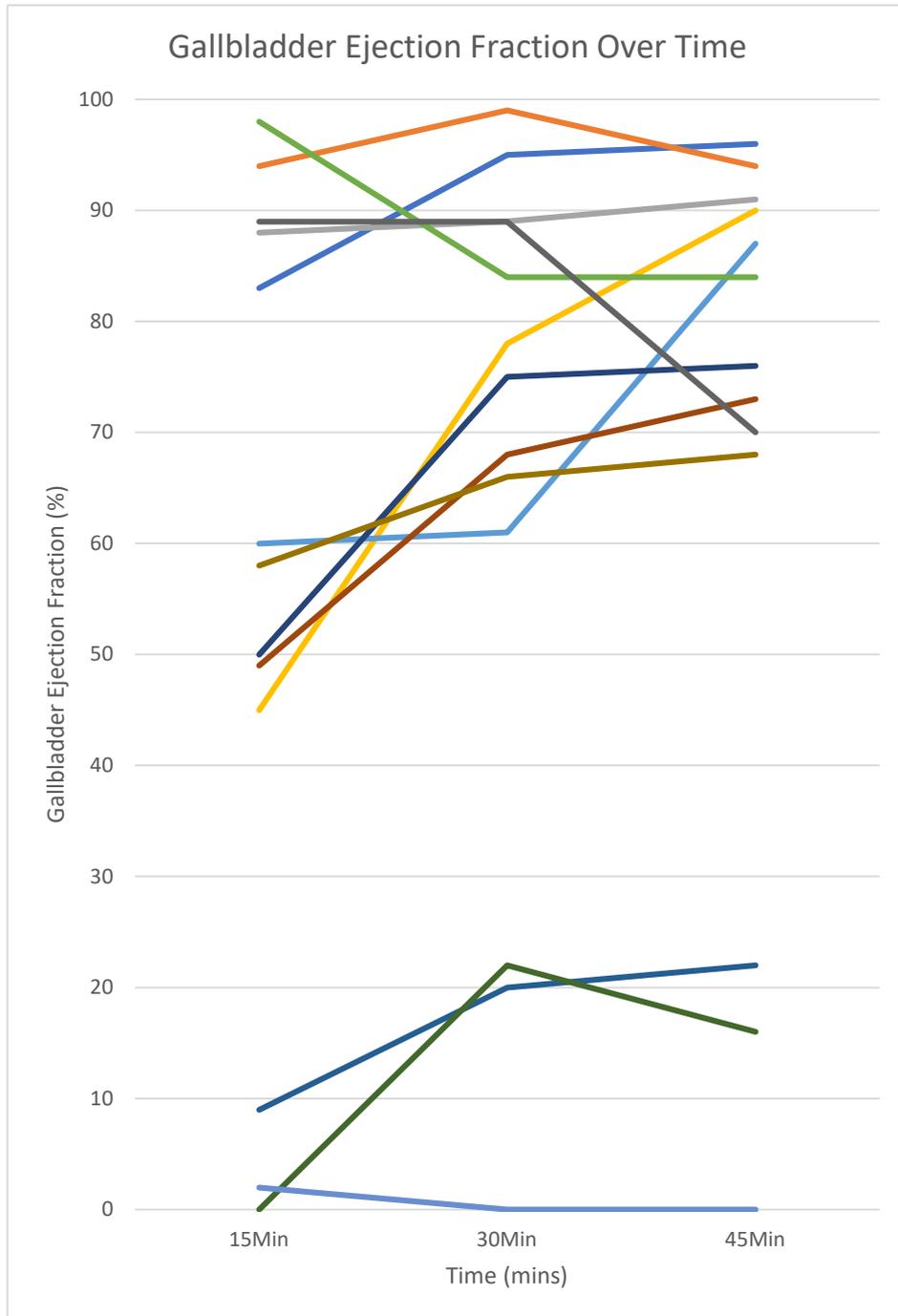


Figure 3.12 shows the changes in gallbladder ejection fraction for each individual healthy volunteer subjects over the 45 minutes period with 15 minutes interval.

This indicates that the gallbladder has a different refilling rate following gallbladder contraction. Although most of the gallbladder still demonstrate contraction at 45 minutes following fatty meal stimulus, there are some which starts refilling as early as 30 minutes.

Gallbladder ejection fraction for the healthy volunteer subjects were calculated by taking the smallest volume obtained during the 45-minute ultrasound measurements of the volume. Of the 13 healthy volunteer subjects, 10 of the subjects demonstrated a calculated gallbladder ejection fraction of >35%. However, there were 3 subjects who demonstrated low gallbladder ejection fractions of 22%, 21.7% and 1.3% respectively. This is demonstrated on Figure 3.13 which shows the individual calculated gallbladder ejection fractions for the healthy volunteer subjects.

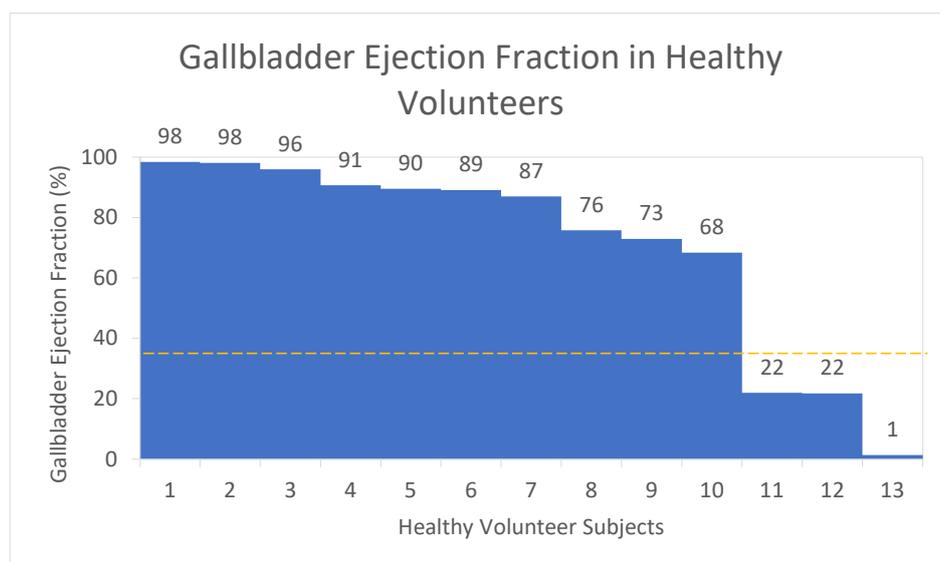


Figure 3.13 shows the individual gallbladder ejection fractions for all 13 healthy volunteer subjects as a waterfall plot with the normal gallbladder ejection fraction shown at 35% (dotted line).

Although some of the healthy volunteer subjects had earlier refilling of the gallbladder with increasing gallbladder volume at 30 and 45 minutes, if gallbladder ejection fractions were calculated by taking the volume at 45 minutes, the eventual calculated gallbladder ejection fraction remains similar, apart from one of the healthy volunteer subjects which show a change from 1.3% to - 84.4%.

The calculated mean \pm standard deviation (SD) gallbladder ejection fraction from ultrasonography for phase two healthy volunteer subjects is 70.0% \pm 33.3%.

Normality of the healthy volunteer data were tested with Kolmogorov-Smirnov and Shapiro-Wilk tests. The Kolmogorov-Smirnov test demonstrated a p-value of 0.033 and Shapiro-Wilk tests demonstrated a p-value of 0.004, both indicating a non-normal distribution spread.

Similarly, a frequency histogram of the calculated gallbladder ejection fractions calculated from ultrasonography has also been plotted to determine normal distribution which is shown as Figure 3.14 below.

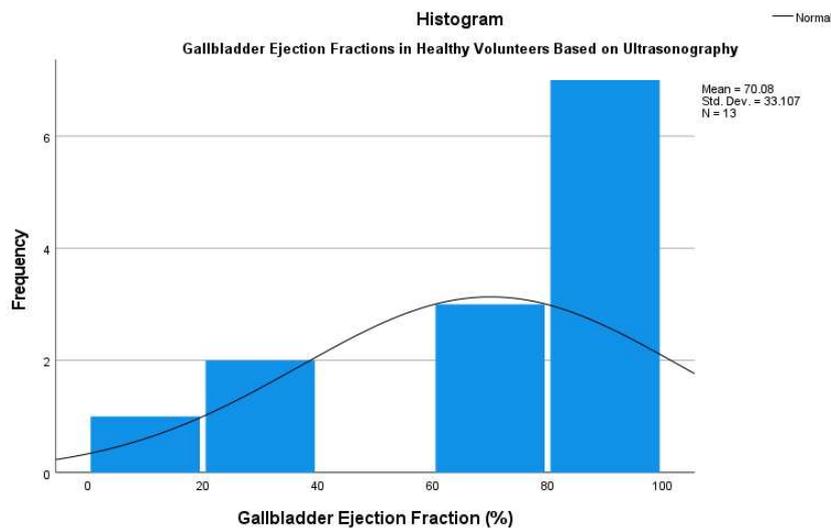


Figure 3.14 demonstrates frequency histogram of the gallbladder ejection fraction based on ultrasonography method in health volunteers.

Based on Figure 3.14 above, the histogram is not normally distributed and there is negative skewness. Therefore, the data for the healthy volunteers are not normally distributed.

3.4 Phase One and Phase Two Comparison

Phase two study is also conducted to allow direct comparison of gallbladder ejection fractions calculated from ultrasound measurements between healthy volunteer subjects with phase one subjects who have normal and abnormal biliary scintigraphy results.

There are 7 subjects within the phase one study who demonstrated abnormal biliary scintigraphy results (gallbladder ejection fraction <35%). These subjects were compared with the healthy volunteer subjects by a non-parametric Mann-Whitney-Wilcoxon test due to the non-normal distribution.

The calculated mean \pm standard deviation (SD) gallbladder ejection fraction from ultrasonography for phase one subjects with abnormal biliary scintigraphy results is $21.0\% \pm 21.8\%$ and the calculated mean \pm standard deviation (SD) gallbladder ejection fraction from ultrasonography for phase two healthy volunteer is $70.1\% \pm 33.1\%$. The calculated Mann-Whitney-Wilcoxon test demonstrates a p-value of 0.005 (significance is taken as <0.05). Therefore, the differences between healthy volunteer subjects and patient subjects with abnormal biliary scintigraphy is significant. This difference can also be appreciated on Figure 3.15 which demonstrated both data groups in a box and whisker plot.

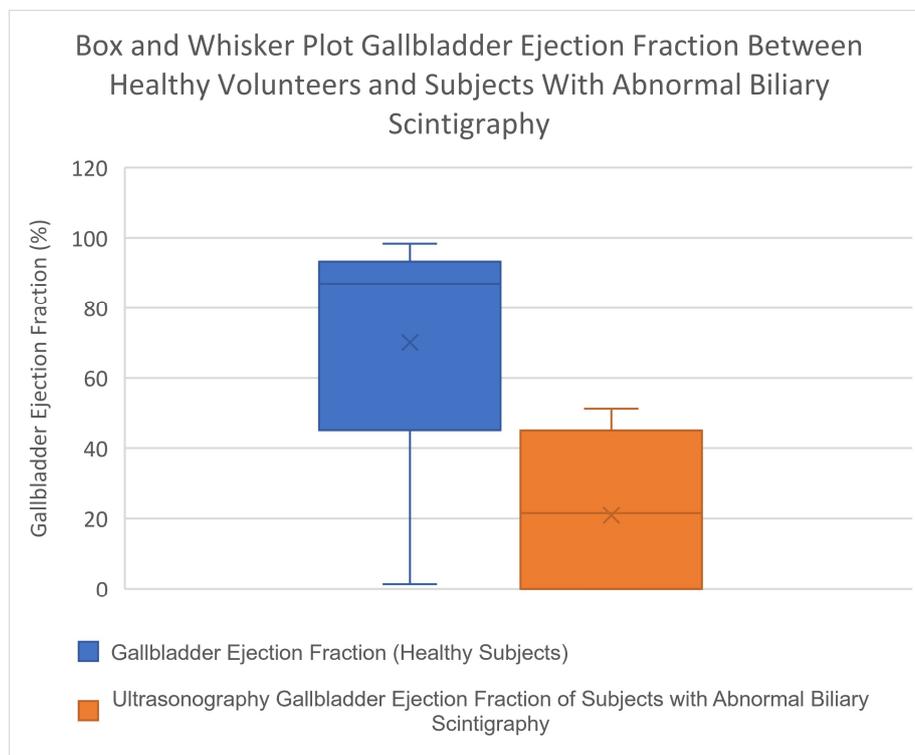


Figure 3.15 demonstrate a box and whisker plot between gallbladder ejection fraction from healthy volunteer subjects and gallbladder ejection fraction from ultrasound of patient subjects with abnormal biliary scintigraphy.

Comparison was also made of the gallbladder ejection fraction calculated by ultrasonography between healthy volunteer subjects and patient subjects who have normal biliary scintigraphy results (gallbladder ejection fraction > 35%). Similarly, a non-parametric Mann-Whitney-Wilcoxon test was used due to the non-normal distribution. The calculated mean \pm standard deviation (SD) gallbladder ejection fraction from ultrasonography for phase one subjects with normal biliary scintigraphy results is 70.3% \pm 23.2%. As noted above the calculated mean \pm standard deviation (SD) gallbladder ejection fraction from ultrasonography for phase two healthy volunteer is 70.1% \pm 33.1%. The calculated Mann-Whitney-Wilcoxon test demonstrates a p-value of 0.58 (significance is taken as <0.05). indicating there is no significant difference between the gallbladder ejection fractions from both groups. Figure 3.16 is a Box and Whisker plot that demonstrates this comparison.

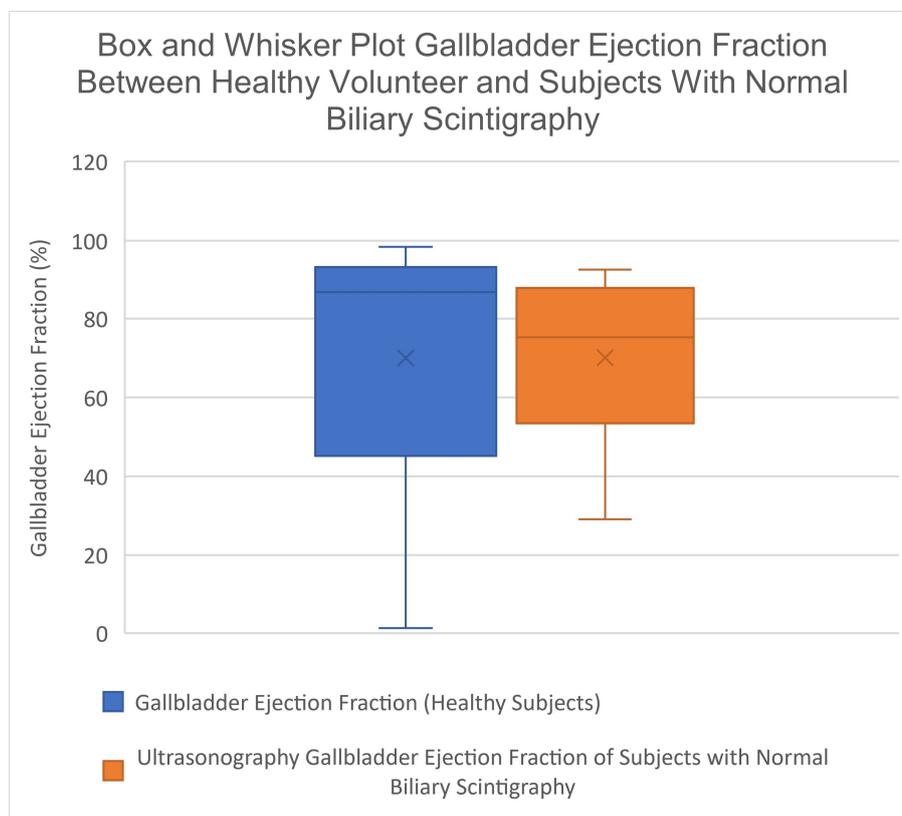


Figure 3.7 demonstrate a Box and Whisker plot between gallbladder ejection fraction from healthy volunteer subjects and gallbladder ejection fraction from ultrasound of patient subjects with normal biliary scintigraphy.

Therefore, there is significant difference in the calculated gallbladder ejection fraction by ultrasonography for patients with abnormal results on biliary scintigraphy when compared to healthy volunteer subjects or even subjects which have a normal biliary scintigraphy result.

3.5 Clinical Follow-Up

The study has not been designed for clinical follow-up in subjects within phase one of the study. However, an attempt was made to obtaining clinical follow-up details for the subjects in phase one study through hospital electronic clinical record system. There were five subjects who were referred from an external hospital which electronic clinical record were not available and clinical follow-up information could not be obtained.

For the four patients which there is increased in ^{99m}Tc -HIDA tracer activity within the gallbladder with no excretion seen or calculated on the biliary scintigraphy, two of the subjects were external hospital referral and therefore, clinical follow-up information was not available. One of the subjects had resolution of symptoms and was there reassured and discharge from clinic with a view of re-referral from the general practitioner if the symptoms recur. The remaining subject was felt to have type 2 Sphincter of Oddi dysfunction with further assessment on follow-up demonstrating abnormal liver function blood test results. The subject was offered

sphincterotomy as a treatment regime for the Sphincter of Oddi dysfunction.

There were three subjects who demonstrated a reduced gallbladder ejection fraction $< 35\%$ and classified as consistent with the diagnosis of gallbladder dyskinesia. One of the subjects had cholecystectomy. Follow-up after cholecystectomy was not available yet. One of the subjects had developed further symptoms due to a separate gastrointestinal disorder and was therefore managed appropriately for the separate disease. The remaining subject was an external hospital referral and therefore no clinical follow-up information available.

There were six subjects who demonstrated normal gallbladder ejection fraction defined as $>35\%$ as shown above. Two of the subjects within this category were external hospital referral and therefore clinical follow-up information were not available. Three of the subjects were reassured on clinical follow-up. One of the subjects however had persistent severe symptoms and underwent cholecystectomy. There is no clinical follow-up yet following the cholecystectomy. Interestingly, the subject who underwent cholecystectomy due to persistent severe symptoms have a calculated gallbladder ejection fraction of 29.1% based on ultrasonography.

CHAPTER 4

DISCUSSION

4.1 Introduction

As the study has been conducted in two phases, discussion for the results will be focussed initially in the respective phases of study. This will be followed by discussion of the entire study results.

As discussed in the introductory and result chapters, the initial study design for phase one of the study was developed to assess the correlation between gallbladder contraction as measured by gallbladder ejection fraction between biliary scintigraphy method and ultrasonography method. To date, there has not been any published studies for adult patients with symptoms suggestive of biliary dyskinesia following standardised fatty meal stimulus, with a comparison of biliary scintigraphy and ultrasonography.

4.2 Sample Size

4.2.1 Phase One

Sample size calculation have been discussed in the methodology chapter. However, further discussion has been included within this chapter to explain the justification for the sample size calculation in more detail. A concern would be that a minimum cohort of 9 subjects is very low. The literature cited in this study design relating to the investigation of the relationship between

gallbladder ejection fraction measured on both modalities (54, 55, 75 and 85) had cohort sizes ranging from 10-22; no statistical consideration was given for the cohort sizes. A range of statistical findings were given, for example one paper found a significant correlation ($P=0.04$) but poor degree of association (adjusted $r^2=0.21$) and another a significant correlation ($P<0.01$) and moderate degree of association ($r^2=0.53$).

The method given in this study design (80) represents the minimum number of data points required to prove the inter-variable correlation is non-zero, given an expected (or in this design a value from previous evidence) correlation coefficient. It is however acknowledged that this represents one part of the analysis. Consideration should be given also to the significance of the result as well as the confidence interval associated with the correlation coefficient. One possible method to investigate clustering of results is the 95% prediction interval which will vary in width depending on the clustering of results.

4.2.2 Phase Two

As discussed in the methodology chapter, the suggested minimum number of healthy volunteer subjects needed to be recruited to allow statistically sound results is 171 healthy volunteer subjects. It has been felt that for a single centre, this number may be difficult to achieve. Further analysis with the same method given in Jennen-Steinmetz (81) for a one-tailed 95% reference interval lower limit, where the sampled limit is within three (rather than two as in the original study design) percentiles of the true population limit with a 90% confidence probability, the minimum number of subjects is $n=77$ for parametric data.

Nonetheless, as comparison is required between phase one and phase two subjects, it was decided that comparable number healthy volunteer subjects to phase one study will be recruited with similar age range and gender to match phase one subjects. As demonstrated on results demographics, the healthy volunteer subjects are younger compared to the phase one study for both calculated mean age and upper limit of the age range but remains similar. There is also comparable gender distribution for both cohorts.

4.3 Normal Value for Biliary Scintigraphy Following Fatty Meal Stimulant

As discussed within the introduction chapter, there is no consensus for a normal value for gallbladder ejection fraction on biliary scintigraphy following fatty meal stimulus. This is due to the variable fatty meal stimulus which has been investigated in different situations producing variable results.

For biliary scintigraphy calculated gallbladder ejection fraction following biliary scintigraphy assessment with synthetic cholecystokinin infusion, there is consensus recommendations from an interdisciplinary panel to accept a normal gallbladder ejection fraction defined as $\geq 38\%$ based on a protocol involving synthetic cholecystokinin infusion of $0.02 \mu\text{g}/\text{kg}$ over 60 minutes for subjects undergoing biliary scintigraphy (33).

Ziessman et al conducted a study on healthy volunteers using a lactose-free fatty meal food supplement as gallbladder stimulant for biliary scintigraphy to establish normal values (34). They have

established a range of 33% to 95% (mean \pm SD, 62.6% \pm 21.3%) from the study. The statistical analysis determined the lower range of normal to be 32.6% on this study. Therefore, it was concluded that a lower limit of normal range is taken as 33%.

A systematic review with meta-analysis was performed to assess cholecystectomy for biliary dyskinesia (90). Within this paper, the authors have reviewed 29 studies, most of which have used synthetic cholecystokinin as gallbladder stimulant but there were also a few studies which used fatty meal stimulus. Most of the studies have set an accepted normal gallbladder ejection fraction of $\geq 35\%$.

Based on the literature review as described above, we have also decided on a normal gallbladder ejection fraction of $\geq 35\%$ for fatty meal simulated biliary scintigraphy. This has also considered of the original Rome III criteria for functional biliary disorder which states that abnormal gallbladder ejection fraction of $<40\%$ is considered abnormal (91). We feel that taking 35% as the lower limits of normal value is a good compromise between all the different studies and between the use of synthetic cholecystokinin and fatty meal stimulus.

4.4 Phase One

As highlighted on the study rationale section within the introductory chapter, the first clinical question was to establish if a good correlation between gallbladder ejection fraction measured and calculated by ultrasonography is consistent and correlated with biliary scintigraphy method. Therefore, phase one of the study was performed with clinical patients first instead of healthy

volunteers. It is only after subsequent analysis that demonstrated a moderate positive correlation between gallbladder ejection fraction assessment with ultrasonography and biliary scintigraphy which allows the commencement of phase two of the research to establish normal gallbladder ejection values on ultrasonography assessment in healthy subjects.

The use biliary scintigraphy is more widely established when compared to ultrasonography in the assessment of biliary contraction is mainly due to the reliability in gallbladder ejection fraction assessment. The use of radionuclide tracer allows the assessment of a three-dimensional process such as contraction of the gallbladder through a two-dimensional imaging. This is because the changes in the radioactivity of the tracer within the gallbladder allows an accurate quantitation of the gallbladder ejection fraction. The measurement of the gallbladder dimensions can be inconsistent as discussed above due to the different shapes of the gallbladder and therefore makes reliable assessment of the gallbladder volume difficult.

There have been a few studies which compared gallbladder contraction assessment with biliary scintigraphy and ultrasonography in patients diagnosed with biliary dyskinesia using synthetic cholecystokinin as gallbladder stimulant. Siegel A et al have conducted a study on adult patients with histories of recurrent abdominal pain but no evidence of gallstones by ultrasonography using biliary scintigraphy and ultrasonography following Sincalide infusion (58). The authors have demonstrated only a weak correlation between the gallbladder ejection fraction calculation from biliary scintigraphy when compared to ultrasonography. They concluded that the gallbladder volume

used to determine the gallbladder ejection fraction from ultrasonography were less reliable. This is because calculation for gallbladder volume is from the assumption that gallbladder has a ellipsoid shape which is not always the case. Irshad et al has performed a prospective study in evaluating patients with suspected biliary dyskinesia comparing the standard two-dimensional ultrasonography and three-dimensional ultrasonography with biliary scintigraphy using Sincalide as gallbladder stimulant (79). The study has demonstrated good agreement between all three methods of assessments for gallbladder ejection fraction, although the ultrasonography methods calculated higher values when compared to biliary scintigraphy. In addition, they have also assessed the accuracy of volume measurements for both two-dimensional and three-dimensional ultrasonography method with exact balloon volume. The study has shown a much higher accuracy for the three-dimensional ultrasonography method.

Cay A et al have performed a study of comparing subjects presented with symptoms suggestive of biliary dyskinesia in the paediatric population (age 6 – 16 years old) (39). The study however did not make direct comparison between ultrasonography and biliary scintigraphy as biliary scintigraphy was not performed. Ultrasonography assessment of the gallbladder volume was performed before and after gallbladder simulation with fatty meal in the symptomatic paediatric population which was then compared to healthy paediatric volunteers. The study demonstrated a significantly reduced gallbladder ejection fraction in patient cohorts when compared to the healthy volunteer cohorts. Therefore, the authors have concluded that the use of ultrasonography with fatty meal stimulus

is a safe, cheap and reliable option to investigate for biliary dyskinesia in children in view of the lack of radiation.

A separate study by Rådberg et al have observed a phenomenon whereby following liquid test meal, the gallbladder volume when measured by real-time sonography reduces for 80 minutes but subsequently increased again indicating refilling (92). This contrasts with the concomitant biliary scintigraphy which demonstrated further tracer excretion from the gallbladder beyond 80 minutes. They have also shown within the study that when a separate series of repeated intravenous injections of ^{99m}Tc -HIDA given after the test meal, there is still tracer accumulation within the gallbladder even when the gallbladder is contracted. The author hypothesize that the gallbladder acts more like a 'bellow' than a reservoir with intermittent filling and emptying. This phenomenon can also be observed within this study whereby two subjects which demonstrated a reduction in gallbladder volume following fatty meal stimulus but on biliary scintigraphy showed further increased of tracer activity within the gallbladder. This suggests that the mechanism for gallbladder dyskinesia is complex and probably involves a combination of gallbladder contraction and gallbladder concentration. Nonetheless, the mechanism of gallbladder dyskinesia is not fully understood.

Within our phase one study, there are four subjects whereby the gallbladder ejection fraction could not be calculated. Three out of four of the subjects demonstrated further increased of ^{99m}Tc -HIDA tracer within the gallbladder throughout the study duration after fatty meal stimulus with no evidence of excretion. One out of four of the subjects demonstrated a static ^{99m}Tc -HIDA tracer activity within the gallbladder throughout the study duration after fatty

meal stimulus. As described above, two of the subjects which demonstrated further increased in ^{99m}Tc -HIDA tracer activity within the gallbladder have a reduction in gallbladder volume following fatty meal stimulus indicating a degree of gallbladder contraction, although the calculated gallbladder ejection fraction remains low. For the other two subjects, the gallbladder volume further increases by end of the study which has a negative correlation with the biliary scintigraphy findings. This may imply a different pathophysiological process for patient's symptoms such as Sphincter of Oddi dysfunction as opposed to biliary dyskinesia.

Sphincter of Oddi dysfunction is a separate entity from gallbladder dyskinesia under Rome IV classifications more broadly categorised as functional biliary sphincter disorder. The diagnosis is mainly considered in patients with biliary-type pains after cholecystectomy, when stones and other pathologies have been excluded (91, 93). The functional biliary sphincter disorder is divided into three clinical types. Type 1 demonstrates a dilated bile duct with elevated liver enzymes. Type 2 demonstrates either a dilated bile duct or elevated liver enzymes. Type 3 on the other hand do not demonstrate any abnormalities based on anatomical or biochemical assessments. This diagnosis is mainly considered in patients following cholecystectomy as it is believed that the sphincter dynamics are altered following cholecystectomy (94). This is because normal gallbladder contraction will stimulate the relaxation of the sphincter of Oddi to facilitate bile drainage and excretion into the small bowel. However, there will be a disruption of this connection or reflex following cholecystectomy which can alter the Sphincter of Oddi behaviour to contractile effects of CCK on the Sphincter of Oddi (95). Although this is classified mainly as a diagnosis following cholecystectomy, there can be altered Sphincter of Oddi mechanism even in the presence of gallbladder,

thus causing biliary-type symptoms similar to biliary dyskinesia (96). Manometry is the gold standard for diagnosis of Sphincter of Oddi dysfunction which is shown to be reproducible with good interobserver variability (97, 98). Biliary scintigraphy has been used for the diagnosis of Sphincter of Oddi dysfunction although it is more limited due to variability in study protocol and different outcome measurements. There is a variable reported sensitivities and specificities (97-101). The use of biliary scintigraphy is even more limited in the assessment of Sphincter of Oddi dysfunction in patients with intact gallbladder. However, the significant increase in ^{99m}Tc -HIDA tracer activity within the gallbladder following fatty meal stimulus may suggest a degree of Sphincter of Oddi dysfunction. The expected pattern of excretion based in biliary scintigraphy for patients suspected for sphincter of Oddi dysfunction is ^{99m}Tc -HIDA tracer activity is seen accumulating within the gallbladder at the start of the study with activity subsequently being excreted into the common bile duct. As there is resistance at the sphincter of Oddi, ^{99m}Tc -HIDA tracer activity will be seen to backflow into either the gallbladder or the intrahepatic biliary ducts visually. Quantitatively, this can be determined by measuring either the counts within the gallbladder or the liver hilum.

For the two subjects who demonstrated both increased in gallbladder volume on ultrasonography and increased in ^{99m}Tc -HIDA tracer activity within the gallbladder may fulfil the criteria for suggesting an underlying diagnosis of sphincter of Oddi dysfunction. The biliary scintigraphy dynamic images for both patients however do not show ^{99m}Tc -HIDA tracer activity within the common bile duct. We may postulate that under such a scenario, the cystic duct which connects the gallbladder to the common bile duct may be the cause of functional obstruction to the excretion of biliary content and ^{99m}Tc -HIDA tracer into the

common bile duct. However, there have not been documented functional cystic duct disorders for individuals with intact gallbladders. Cystic duct can produce symptoms similar to symptoms for biliary dyskinesia in individuals who had cholecystectomy. Interestingly, one of the subjects had clinical follow-up as documented on the results chapter suggestive of possible Sphincter of Oddi dysfunction. The remaining three subjects with biliary scintigraphy results which could not calculate an ejection fraction, the assumption of underlying gallbladder dyskinesia is made in the absence of any other abnormal findings.

All four subjects with abnormal biliary scintigraphy results whereby a gallbladder ejection fraction could not be calculated also have abnormal or reduced gallbladder contraction based on ultrasonography findings as indicated by the calculated gallbladder ejection fractions. This indicates both imaging modalities correlates well with biliary scintigraphy findings, although there are also very low calculated gallbladder ejection fractions within the phase two healthy volunteer subjects which will be discussed later.

For the three subjects who demonstrated abnormal biliary scintigraphy (GBEF <35%), One of the subjects demonstrated a conflicting result whereby the biliary scintigraphy demonstrated further reduction of ^{99m}Tc -HIDA tracer activity within the gallbladder and being excreted into the small bowel while the calculated gallbladder volume from the ultrasound study has increased at the end of the study. This suggests possibility of an early refilling of the gallbladder following poor gallbladder contraction. Despite the refilling of the gallbladder, there is still excretion of biliary content into the common bile duct

simultaneously, which explains the continuous reduction in ^{99m}Tc -HIDA tracer activity within the gallbladder towards the end of the study by 45 minutes post fatty meal ingestion. This hypothesis is supported by a study by Jazrawi et al which demonstrated simultaneous refilling of the gallbladder with continuous excretion of ^{99m}Tc -HIDA tracer activity following ingestion of meal (102). Howard et al has also demonstrated phases of emptying and refilling of the gallbladder in response to solid meal by continuous measurements by ultrasonography (103) further supporting this mechanism as an explanation for the phenomenon.

For the remaining six subjects in phase one study who demonstrated normal gallbladder ejection fraction on biliary scintigraphy (gallbladder ejection fraction $>35\%$) have corresponding gallbladder ejection fraction calculated from ultrasonography similarly $>35\%$ apart from one subject of which the calculated gallbladder ejection fraction from ultrasonography is 29.1%. Although other studies comparing biliary scintigraphy and ultrasonography assessment of gallbladder ejection fractions have demonstrated a higher gallbladder ejection fraction calculated from ultrasonography (79), this is not the case for this study. As mentioned in results chapter, there is similar number of subjects whereby gallbladder ejection fraction calculated from biliary scintigraphy is higher than ultrasonography calculation and vice versa. Therefore, it is not consistent that either biliary scintigraphy or ultrasonography assessment will underestimate or overestimate the gallbladder ejection fraction.

The one subject which demonstrated normal biliary scintigraphy assessment but a calculated gallbladder ejection fraction of $<35\%$ from ultrasonography assessment interestingly had subsequent

cholecystectomy for persistent of symptoms. This may suggest a combined role of assessing for gallbladder dyskinesia with biliary scintigraphy and ultrasonography to increase the sensitivity of disease detection. Nonetheless, the normal values for gallbladder ejection fraction from healthy volunteers following fatty meal stimulus can be variable and demonstrate very low gallbladder ejection fraction which will be discussed below. Hence, a low gallbladder ejection fraction calculated from ultrasonography may not necessarily reflect gallbladder dyskinesia.

Overall, from phase one of the study, there is good correlation between the nine subjects which comparison could be made demonstrating a Spearman's ρ correlation calculated value of 0.783 and all the differences of the calculated gallbladder ejection fractions from both modalities fall within two standard deviation limits and the Bland-Altman chart.

4.5 Phase Two

There have been a few studies in healthy volunteer subjects with fatty meal stimulus to established normal values of gallbladder ejection fraction with ultrasonography method. One of the reasons is there is a huge variation in results through different studies and therefore it is difficult to establish a normal range.

Donald et al has conducted a study on normal subjects with a set standard meal that have been established within their institution (104). The authors have demonstrated a wide range of gallbladder ejection fractions from -10% to 99%. They have also repeated the study on different days for the same normal subjects. In this situation, they have shown in addition a gallbladder ejection fraction variation within the same normal from 6% to 87%. This shows that a single study for gallbladder contraction with ultrasonography can be misleading.

The study to assess reproducibility of gallbladder ejection fraction from biliary scintigraphy with fatty meal stimulus has also been performed (105). They have shown no significant difference of the mean \pm SD of gallbladder ejection fractions calculated on both occasions in healthy volunteers with a correlation coefficient of 0.473. The authors have concluded that gallbladder ejection fraction measured by fatty meal biliary scintigraphy is reproducible.

Another study was performed to investigate the correlation between biliary scintigraphy and ultrasonography with standard fatty meal in healthy volunteers (89). The authors have demonstrated good correlation between both modalities, with a

calculated higher gallbladder ejection fraction on biliary scintigraphy compared to ultrasonography. They have also shown good reproducibility of both modalities when both studies were repeated on the same healthy volunteers on a separate day. In addition, as the authors have also tracked the gallbladder ejection fraction at 15 minutes, 30 minutes, 45 minutes and 60 minutes; they have further concluded that biliary scintigraphy demonstrated continuous excretion even after 45 minutes which is less evident on ultrasonography.

As demonstrated on our study, there is a huge range of gallbladder ejection fraction calculated from the healthy volunteer subjects from 1.3% to 98.4%. This is not dissimilar to some of the studies discussed above. Nonetheless, most of the healthy volunteer subjects (10 out of 13) have calculated gallbladder ejection fractions > 35%. Also similar to other studies, this study has demonstrated early refilling in some patients before 45 minutes. The maximal gallbladder ejection fraction was calculated by taking the smallest volume of the gallbladder calculated after fatty meal stimulus before refilling has occurred. Further analyses to look at the difference in calculated gallbladder ejection fractions at 45 minutes and the maximal gallbladder ejection fraction for healthy volunteer subjects who had early refilling have been performed. This shows that even if gallbladder ejection fraction at the end of the study at 45 minutes were calculated, it would have probably provided the same interpretation outcome as the difference is not significantly different, apart from the healthy volunteer subject with a calculated gallbladder ejection fraction of 1.3%.

The gallbladder ejection fractions calculated from phase two healthy volunteer patients do not have normal distribution based on the normality tests and frequency histograms. A lower limit normal value is difficult to established due to the small number of healthy volunteer subjects and the extremely wide variation in gallbladder ejection fractions calculated.

4.6 Phase One and Phase Two Comparison

As phase one was performed as a standard investigation pathway for patients with symptoms suggestive of biliary dyskinesia, it is recognised that not all phase one subjects will be diagnosed with biliary dyskinesia. Therefore, comparison of ultrasonography calculated gallbladder ejection fraction between phase one subjects, and phase two healthy volunteer subjects have been performed in two groups.

Based on the standard interpretation of biliary scintigraphy within our institution with fatty meal stimulus, subjects with gallbladder ejection fraction <35% will be considered abnormal and indicates biliary dyskinesia in the correct clinical context based on Rome IV criteria. Within this group, the four phase one subjects which gallbladder ejection fraction could not be obtained from biliary scintigraphy who have been discussed above are also included as these subjects are also considered to have abnormal biliary scintigraphy. Therefore, comparison of gallbladder ejection fraction calculation from ultrasonography was made between phase one subjects with abnormal biliary scintigraphy and phase two healthy volunteer subjects to assess if a significant difference can be shown. The results demonstrated marked difference in the

mean gallbladder ejection fraction between the two groups; which is also statistically significant.

The second group for comparison was made between phase one subjects with normal biliary scintigraphy (GBEF >35%) and phase two healthy volunteer subjects. Both groups have shown very similar mean calculated gallbladder ejection fractions from ultrasonography method with no statistically significant difference.

This indicates that the use of ultrasonography alone with fatty meal stimulus to assess gallbladder contraction by measuring changes in gallbladder volume and calculate the gallbladder ejection fraction can potentially discriminate between patients with biliary dyskinesia from patients without biliary dyskinesia based on Rome IV criteria in assessment of gallbladder ejection fractions.

4.7 Three-Dimensional Ultrasonography Volumetric Gallbladder Assessment

As describe in methodology chapter, although the original study was designed to include gallbladder ultrasound assessment with a three-dimensional probe to obtain volumetric data, unfortunately, due to data acquisition error, the volumetric data available was not suitable for volumetric analysis. However, it is worth discussing the value of three-dimensional volumetric gallbladder assessment in gallbladder dyskinesia with reference to literature review.

Three-dimensional ultrasonography is increasingly utilised for different indications and becoming a useful diagnostic tool for evaluation of different organs. This is because the three-dimensional ultrasonography allows a three-dimensional image reconstruction with a single sweep of the ultrasonic beam. This then allows a more accurate and repeatable evaluation of anatomic structures with variable appearances. The advances in three-dimensional ultrasound probes have also improved the image quality. In conjunction, newer software also allows a more rapid and easy method for evaluating gallbladder structure and volume in real time.

There have been very limited studies comparing three-dimensional ultrasound assessment of the gallbladder with either two-dimensional ultrasound method or biliary scintigraphy. We will discuss the published studies on the usage of three-dimensional ultrasonography for gallbladder disease assessment.

An initial study on the use of three-dimensional ultrasonography was performed by Hashimoto et al which comprises of comparing with two-dimensional ultrasound assessment for both in vitro balloons of various shapes and healthy male volunteers following standard meal as gallbladder stimulus (106). The gallbladder volume from two-dimensional ultrasonography is calculated using the sum-of-cylinders method. The authors have demonstrated no significant difference in the measured gallbladder volumes between both ultrasound methods for both in-vitro and healthy male volunteer studies.

Similarly, another group has also conducted very similar study comparing three-dimensional ultrasonography with two-dimensional ultrasonography assessment of in-vitro balloon and healthy volunteers (107). In addition, they have also recruited patients with gallstones into the study. The study shows good correlation between both three-dimensional and two-dimensional assessment of gallbladder volumes.

Serra et al has also conducted a very similar study comparing both three-dimensional and two-dimensional assessment of gallbladder volume in healthy volunteers with fatty meal stimulus (108). They have also demonstrated no significant difference between a skilled and unskilled operator for the three-dimensional ultrasonography assessment as opposed to the two-dimensional ultrasound assessment.

Yoon et al has conducted a study to compare three-dimensional ultrasonography with biliary scintigraphy for assessing gallbladder contractility with fatty meal stimulus in patients suspected of gallbladder disease (60). The study has demonstrated good correlation in gallbladder ejection fractions measured on both modalities. The calculated Pearson's correlation coefficient in this study is 0.92.

Irshad et al has conducted a very comprehensive assessment of the three-dimensional ultrasound with comparison to two-dimensional ultrasound assessment of in-vitro balloon as well as patients with suspected gallbladder dyskinesia (79). A further comparison was also made between three-dimensional ultrasonography with biliary scintigraphy using Sincalide as

gallbladder stimulant. The study demonstrated good correlation and concordance across all three modalities for three-dimensional ultrasound, two-dimensional ultrasound and biliary scintigraphy. In addition, they have also shown no significant inter-operator variability for three-dimensional ultrasound assessment.

Although there has only been a handful of studies involving three-dimensional ultrasound assessment of gallbladder structure and volume to determine gallbladder contractility and ejection fraction, all studies have demonstrated consistent results with good correlation to both two-dimensional ultrasound assessment and biliary scintigraphy with either synthetic cholecystokinin or fatty meal stimulant. This indicates that the use of three-dimensional ultrasound gallbladder assessment is a promising tool for a more accurate diagnosis of gallbladder disease. This can also be appreciated that gallbladder shape can be variable, and the assumption of an ellipsoid shape does not always hold. Three-dimensional ultrasound of the gallbladder can also assess the change in shape of the gallbladder which will allow of better understanding of gallbladder contractility mechanism.

4.8 Pitfalls and Limitations of the Study

There are several technical pitfalls and limitations involved in this study with regards to image acquisition from both modalities.

As discussed in the introduction chapter, the biliary scintigraphy is performed as a two-dimensional acquisition. The calculation of the activity of ^{99m}Tc -HIDA tracer is by drawing a region of interest around the gallbladder to track the change in activity throughout the duration of the acquisition. As with any two-dimensional acquisition, there is an inherent problem with overlying structures. In this case, the small and large bowel can overlap the gallbladder either anterior or posteriorly. This means that when ^{99m}Tc -HIDA tracer is excreted into the small bowel, there is potential of overlapping ^{99m}Tc -HIDA tracer activity within the gallbladder and excreted ^{99m}Tc -HIDA tracer activity transiting through the bowel. This will artificially increase the ^{99m}Tc -HIDA tracer within the region of interest. Unfortunately, such a pitfall cannot be easily corrected. Therefore, the careful assessment of ^{99m}Tc -HIDA tracer transit through the bowel need to be taken. Careful review of the biliary scintigraphy studies in phase one did not demonstrate any obvious overlap of bowel and gallbladder but subtle overlap remains a possibility.

There is also technical problem with movement of gallbladder during the image acquisition for biliary scintigraphy. Subtle movements from respiration are normally accepted and can be corrected. However, large movements are difficult to correct which makes drawing of the region of interest for assessment difficult. This means that a larger region of interest will have to be drawn and throughout the duration of the acquisition, some parts of the

region of interest may fall out of the gallbladder or include the biliary ducts or bowel. This can artefactually increase the ^{99m}Tc -HIDA tracer activity within the gallbladder. There were a few biliary scintigraphy studies within phase one with some motions which most of them are corrected for motion with the analysis program.

For this study protocol, the standard protocol for biliary scintigraphy is part of the departmental biliary scintigraphy protocol for assessment of patients with symptoms suggestive of biliary dyskinesia. The protocol for biliary scintigraphy has included a specific meal with a set caloric content for patients to ingest before fasting for four hours prior to the biliary scintigraphy study. This protocol was introduced as patients fast for different durations previously despite being advised to fast for only six hours prior to the study. The specific meal prior to fasting would provide a more constant biliary contraction to allow a good relaxation during fasting period. Nonetheless, this specific protocol has not been studied to assess how a specific meal prior to fasting will affect the subsequent gallbladder contraction during biliary scintigraphy.

From an ultrasonography perspective, the measurements of gallbladder by two-dimensional ultrasound can be variable. This can be variable due to position of the gallbladder and motion. Frequently, the subject will have to breath in to allow better delineation of the gallbladder on ultrasound assessment. The gallbladder can have a different shape during inspiration and expiration. Therefore, the degree of inspiration may render a change in measurement due to the change in shape rather than true contraction. Therefore, within this study, all gallbladder

ultrasound measurements were performed three times and an average taken to reduce this effect. There are also occasions when after the gallbladder is contracted, the initial position to view the gallbladder on ultrasound is no longer optimum and a different position needs to be obtained. This happens rarely within this study. Most of the ultrasound measurements of the gallbladder were taken at a similar position for each patient.

As discussed above, the gallbladder can have different shape and following gallbladder contraction, the shape may change. Therefore, the assumption of the gallbladder in an ellipsoid shape does not always hold. Fiddler et al has demonstrated on CT cholangiography the different shapes of gallbladder which do not conform to the standard ellipsoid shape (78). They have also found that gallbladder contraction occurs more at the neck and as the neck of the gallbladder are not easily assess on two-dimensional ultrasound, there is potential in underestimating the degree of gallbladder contraction. Consideration need to be taken with regards to the accuracy of gallbladder volume calculation by the ellipsoid method for all patients.

The main limitation of this study is the data error in three-dimensional ultrasound acquisition which would have allowed a better assessment of gallbladder shape and contraction. As discussed above, three-dimensional ultrasound appears to offer a more robust and detailed assessment of the gallbladder with reduced variability.

As with any study, there can be a number of potential bias. Selection bias may occur for phase one subjects referred for

biliary scintigraphy as the referral is based on clinicians. There will be bias with regards to patient investigation and management for biliary dyskinesia amongst different clinicians. The selection bias can be reduced by having a larger pool of referrals.

Operator bias can be a limitation or advantage within this study. The ultrasound is only performed by a single operator within this study which can contribute inconsistencies in measurement techniques. However, it can also produce a constant systematic error if the operator performs an error constantly in all the ultrasound assessment.

Finally, the biggest limitation is the small number of subjects recruited into the study, in particular, within phase one of the study. The small number of subjects limits the statistical analysis which as demonstrated within the Results Chapter, a normal distribution cannot be assumed and therefore a non-parametric statistical method had to be used. This also affects the strength in the calculated correlation and significance of the observed data.

CHAPTER 5

CONCLUSION AND FUTURE WORK

5.1 CONCLUSION

The results from this study has demonstrated a good correlation between biliary scintigraphy assessment and two-dimensional ultrasound assessment of the gallbladder ejection fractions with fatty meal stimulus. The study also demonstrated significant difference between the subjects considered to have gallbladder dyskinesia by biliary scintigraphy when compared to healthy volunteer subjects using ultrasound assessment.

This suggests that ultrasound assessment of gallbladder ejection fraction by volume calculation can be considered for assessment of gallbladder dyskinesia. As described in the section on limitations, the number of subjects within this study makes interpretation of the results limited with regards of statistical significance.

The results from phase two of the study has demonstrated a wide range of gallbladder ejection fractions within healthy volunteer subjects. This makes it difficult to adopt it reliably for general usage of ultrasound in the evaluation of biliary dyskinesia.

The results from this study however remains very similar to results presented in published literature which have similar methodology and patient population. This indicates that patient and healthy subjects are heterogenous in gallbladder contraction and response to fatty meal stimulus. The underlying mechanism for gallbladder dyskinesia may be far more complex and an assessment based on gallbladder ejection fraction alone is not sufficient to establish the diagnosis confidently.

5.2 FUTURE DIRECTIONS

Although there has been extensive work done within the subject of biliary dyskinesia assessment and management, there remains a significant void of good data on multicentre trials. There is also no consensus on the type of fatty meal required to produce a more consistent gallbladder response even in healthy subjects.

5.2.1 Fatty Meal

Further work can be done in assessing how the body response to fatty meal in the production of endogenous cholecystokinin to stimulate gallbladder contraction. It is unlikely that a single set fatty meal and be established to be utilised universally. If the fatty meal response is based on caloric content or fat content, perhaps a few similar fatty meal choices can be made available for a multicentre trial in both assessing healthy and patient subjects.

5.2.2 Ultrasonography

Three-dimensional ultrasonography appears to have very promising role based on the few limited publications. All the publications are single centre studies. Therefore, to establish the use of three-dimensional ultrasound for the assessment of gallbladder contractility and disease, a multicentre prospective trial comparing between healthy volunteers and patients' needs to be performed. This will also need to be performed for both synthetic cholecystokinin and fatty meal stimulus. In three-dimensional ultrasound we had problems with data transfer. To make it successful in a multicentre study it would be necessary for there to be improvements in the reliability of data transfer. The software for three-dimensional analysis will also need to be reliable across all vendors and sites.

There are advances in three-dimensional reconstruction from two-dimensional images with computer software which can help in determining the shape, contractility and other parameters of bile excretion from the gallbladder such as gallbladder wall strain (109). Such software will need further evaluation with comparison to other different modalities, including CT and MR cholangiography.

5.2.3 Biliary Scintigraphy

The practice of nuclear medicine has also changed dramatically with introduction of new imaging and reconstruction methods. One of the newer introductions is the capability of performing fast acquisition of three-dimensional scintigraphy with new imaging equipment. This indicates there is a potential of also assessing gallbladder contraction with biliary scintigraphy in three-dimensional real time acquisition. The continuous three-dimensional acquisition of the hepatobiliary scintigraphy will allow simultaneous radioactivity counts and volume change throughout the entire scintigraphic acquisition. In addition, all Single Photon Emission Computed Tomography – Computed Tomography (SPECT-CT) imaging machines have Computed Tomography (CT) capability. This enables the CT acquisition at the beginning and end of the study to allow direct visualisation of the gallbladder morphology and volume calculation. The changes in the volume based on hepatobiliary scintigraphy and CT can determine the change in concentration and resolve any difference between radioactivity counts of the hepatobiliary radiopharmaceutical and the gallbladder morphological volume. This will allow a better understanding of different gallbladder disease state, including biliary dyskinesia.

5.2.4 Functional Magnetic Resonance Cholangiography

As discussed in the Introduction Chapter, there has been a few small studies utilising functional magnetic resonance cholangiography for the assessment of gallbladder function. This involves the administration of hepatobiliary contrast agent which similar to the hepatobiliary radiopharmaceuticals, demonstrate a predominant route of excretion through the biliary pathway.

Although the literature is very limited for the use of functional magnetic resonance cholangiography in assessing gallbladder function, this is a very promising way to detect gallbladder dyskinesia. In addition, it can also assess the concentration of the hepatobiliary contrast agent as well as the morphology of the gallbladder in a single study. The dynamic magnetic resonance cholangiography will obtained imaging sequences which demonstrates real time changes in the gallbladder contraction to allow exact visualisation of the gallbladder contraction. This can help to resolve the query on whether gallbladder contraction occurs globally or regionally.

The concentration of the hepatobiliary contrast within the gallbladder can be determine by the signal intensity of the study and hence provides the information if contraction and drainage can occur simultaneously with gallbladder concentration. Furthermore, magnetic resonance imaging will also provide anatomical information regarding the biliary tracts and detect anatomical variation or obstruction within the biliary tract.

Finally, the standard magnetic resonance imaging contrast can also be utilised to study the gallbladder wall enhancement which

can provide information regarding chronic cholecystitis. In chronic cholecystitis, there is general slower enhancement of the gallbladder wall.

5.2.5 Clinical Outcomes

Last of all, although clinical outcomes following a diagnosis made or confirmed by an imaging modality is not specifically addressed within the study, the main role of an imaging method should always be considered in the context of clinical outcome of patients. Therefore, future multicentre trials regarding either biliary scintigraphy or ultrasound assessments will need to be evaluated also based on patient's management and clinical outcome.

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APPENDICES

Appendix A

Phase 1 subjects: gallbladder measurements in 3 planes for 3 times at the start of the study before fatty meal stimulus.

Pre-L1	Pre-L2	Pre-L3	Pre-A1	Pre-A2	Pre-A3	Pre-H1	Pre-H2	Pre-H3
4.9	5.2	5.0	1.9	2.0	2.0	2.7	2.5	2.6
2.8	2.7	4.1	2.1	2.1	2.0	2.1	1.9	1.9
6.7	5.9	6.0	3.1	2.9	2.8	3.0	3.4	3.5
5.2	5.2	5.2	1.7	1.6	1.7	2.1	2.1	2.4
4.4	4.4	4.8	1.2	1.8	1.4	3.5	3.1	3.2
6.5	6.5	5.8	3.9	4.0	4.2	6.1	6.1	6.0
7.8	7.5	6.4	2.2	2.1	2.1	3.9	2.8	2.6
2.9	3.2	3.6	1.6	1.7	1.6	1.6	1.5	1.7
3.6	3.7	4.2	1.8	2.2	2.3	2.3	2.2	2.5
6.5	6.3	6.1	2.4	2.4	2.1	2.1	2.7	2.3
4.9	5.4	4.8	1.9	2.0	2.1	2.3	2.3	2.5
2.1	2.1	2.5	0.9	0.9	1.1	3.4	1.7	1.6
5.6	4.4	4.3	3.3	3.2	2.9	2.8	4.0	3.9

Appendix B

Phase 1 subjects: gallbladder measurements in 3 planes for 3 times at the end of the study.

Post-L1	Post-L2	Post-L3	Post-A1	Post-A2	Post-A3	Post-H1	Post-H2	Post-H3
4.6	4.2	4.4	1.5	1.3	1.4	2.1	2.0	2.1
4.5	4.0	4.3	1.8	1.7	1.9	1.9	1.9	2.0
6.4	6.4	5.9	2.7	2.6	2.9	2.8	2.8	2.7
4.3	3.2	3.1	0.9	1.0	0.7	1.4	1.2	1.0
4.9	4.5	4.6	1.6	1.6	1.6	4.2	3.7	4.0
5.0	5.1	5.0	3.1	3.6	3.7	6.3	6.2	6.2
6.3	5.6	5.8	1.8	1.9	1.7	4.9	5.0	5.2
1.9	2.1	2.4	1.3	1.2	1.2	1.6	1.8	1.9
2.8	2.6	2.7	2.1	1.8	1.9	1.2	1.1	1.3
3.9	2.7	2.8	0.8	1.0	0.6	0.9	1.2	0.9
4.6	4.4	4.5	1.2	1.1	1.2	1.5	1.4	1.2
2.1	1.5	1.4	0.8	0.7	0.7	0.5	0.6	0.5
4.6	4.5	4.6	2.6	3.1	3.2	2.9	2.8	2.7

Appendix C

Phase 2 volunteers: gallbladder measurements in 3 planes for 3 times at the start of the study.

OL1	OL2	OL3	OH1	OH2	OH3	OA1	OA2	OA3
5.5	4.8	4.9	2.4	2.7	2.8	2.4	3.2	3.1
3.4	3.6	3.7	1.4	1.5	1.4	1.9	2.0	1.9
4.8	3.7	3.9	1.9	1.8	2.3	3.1	3.5	3.4
4.6	4.3	5.8	3.4	2.8	2.8	2.0	2.3	2.6
5.9	6.3	5.9	1.3	1.6	1.9	1.6	1.4	1.1
5.7	5.8	5.6	2.9	2.3	2.7	2.1	3.1	3.1
4.2	4.0	3.9	4.0	3.9	3.9	2.5	2.8	3.0
5.1	4.8	4.5	3.9	3.6	3.3	2.9	2.5	2.2
4.4	3.8	4.3	3.5	2.3	3.7	1.5	2.1	1.8
4.9	5.0	5.2	2.9	2.6	2.9	1.4	1.3	2.1
5.3	5.1	4.6	2.2	2.0	2.0	1.4	1.3	1.6
7.0	6.7	5.8	2.3	2.0	2.4	1.9	1.3	1.5
4.6	4.3	5.0	2.2	2.5	1.9	1.0	1.5	1.1

Appendix D

Phase 2 volunteers: gallbladder measurements in 3 planes for 3 times at 15 minutes of the study.

15L1	15L2	15L3	15H1	15H2	15H3	15A1	15A2	15A3
3.4	4.3	4.0	2.7	2.5	2.5	2.1	1.8	1.9
1.4	1.4	1.0	0.5	0.5	0.4	0.4	0.3	0.3
2.7	3.1	2.8	1.5	1.7	1.4	1.1	1.2	0.9
5.9	6.2	5.9	2.6	2.6	2.5	1.9	2.0	2.1
6.1	5.4	5.5	1.2	1.3	1.1	0.8	0.8	0.7
5.6	4.8	5.3	3.7	3.5	4.5	2.0	1.8	2.3
4.1	3.3	2.9	2.8	2.5	2.3	2.0	2.2	2.1
4.4	4.1	4.0	2.5	2.4	2.2	2.0	2.5	2.3
3.1	3.5	3.7	2.9	2.4	2.7	1.7	1.1	1.5
4.1	3.8	3.7	1.0	1.3	1.2	0.7	0.6	0.5
4.0	4.0	4.6	2.0	2.1	2.4	1.7	1.7	1.4
3.1	2.7	3.2	0.9	1.0	0.9	0.7	1.0	1.0
3.4	2.4	3.3	0.8	0.6	0.7	0.8	0.6	0.7

Appendix E

Phase 2 volunteers: gallbladder measurements in 3 planes for 3 times at 30 minutes of the study.

30L1	30L2	30L3	30H1	30H2	30H3	30A1	30A2	30A3
3.4	3.4	3.8	1.9	1.5	1.5	1.5	1.6	1.9
2.7	1.8	2.3	0.9	0.8	1.4	0.6	0.6	0.8
3.1	2.6	2.8	1.0	0.7	0.7	0.8	0.6	0.5
6.2	6.4	6.0	2.8	2.4	2.7	1.7	1.6	1.7
3.3	3.2	2.9	1.5	1.6	1.5	1.0	1.0	1.2
4.3	4.2	4.8	3.4	3.0	2.9	2.6	2.2	2.3
2.9	3.0	2.9	2.6	2.4	2.5	2.0	2.2	1.9
3.8	4.4	3.9	2.1	2.2	2.4	1.6	1.3	1.7
1.7	2.2	2.5	2.3	2.4	1.6	1.2	1.1	1.2
4.2	3.9	3.6	1.1	0.9	1.2	0.7	0.5	0.6
4.6	4.4	4.6	1.9	1.9	2.2	2.5	2.2	2.6
2.3	3.0	2.7	0.9	0.8	1.0	1.2	1.1	0.8
1.0	1.4	1.5	0.4	0.5	0.5	0.4	0.4	0.5

Appendix F

Phase 2 volunteers: gallbladder measurements in 3 planes for 3 times at 45 minutes of the study.

45L1	45L2	45L3	45H1	45H2	45H3	45A1	45A2	45A3
3.7	3.0	3.6	1.7	1.7	1.9	1.5	1.6	1.5
2.4	2.4	3.6	0.9	0.9	0.8	0.7	0.6	0.7
2.0	1.7	2.3	0.7	0.7	0.8	0.7	0.7	0.7
5.7	5.5	5.0	2.7	2.6	2.3	1.8	2.0	2.0
3.8	4.2	4.0	0.7	0.7	0.6	0.9	0.6	0.5
5.6	4.9	4.9	3.5	3.3	2.8	2.4	1.9	2.1
3.1	3.1	2.7	2.4	2.4	2.3	1.9	1.8	2.3
3.0	3.4	3.5	2.4	3.1	2.0	1.5	1.5	1.3
1.5	1.7	1.7	1.4	1.6	1.5	0.9	1.1	1.0
3.5	3.1	3.1	0.9	1.0	1.3	0.7	0.5	0.6
4.5	4.4	4.5	3.0	2.8	3.0	2.1	2.2	1.9
4.9	4.3	4.4	1.6	1.6	1.8	0.8	0.9	1.0
2.1	2.2	2.3	1.0	1.0	0.8	0.7	0.6	0.7