Investigating the utility of EndoFLIP, a novel diagnostic modality for assessing anal canal function

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Submitted for the degree of MD(Res)

By

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Declaration

I, Lalit Kumar confirm that the work presented in this thesis is my own. Where information has been derived from other sources, I confirm that this has been indicated in the thesis.

Abstract

Evaluation of anal sphincter function is of critical importance in the investigation and management of faecal incontinence, a problem which affects 2% of the general population. Structural assessment by ultrasound is of proven value in defining aetiology and treatment of faecal incontinence, but the currently available physiological assessments are of little value. This is in part because existing physiological techniques assess static properties, whilst faecal incontinence is a dynamic event. Additionally, the heterogeneous structures of the muscle complex surrounding the anal canal require evaluation of the biomechanical properties at multiple locations along the anal canal, not the single measurement of pressure currently available to us with existing techniques.

The EndoFLIP technique is a novel methodology that has been used to assess physiology of the upper gut. It allows determination of serial cross-sectional areas (CSAs) during distension thereby providing a detailed segmental geometric and mechanical properties. Custom made probes better suited for anorectum were made as a part of this study to evaluate the biomechanical properties of the anal canal in healthy volunteers. This was followed by study of patients with anorectal dysfunction, specifically dysfunction of the internal anal sphincter and those suffering from scleroderma. The hypothesis for the study was that the dynamic mechanical properties vary at different locations in the anal canal, in response to the anal distension.

The study proved a good in-vitro repeatability of the custom-made anorectal probes. This was followed by validation of EndoFLIP in the healthy subjects by confirmation of the pre-existing knowledge of the anorectum besides demonstration of the sampling reflex in real life. A comparison of healthy cohort with morphologically intact faecal incontinence patients and scleroderma patients highlighted a number of significant differences amongst these groups during resting, squeeze and RAIR phase. The clinical utility of EndoFLIP was proven by the new knowledge that was gained during this study.

Impact Statement

Disorders of the function of the anus and rectum resulting in faecal incontinence are common. The studies have reported the prevalence of faecal incontinence in adults to be between 0.4% and 18%. The existing modalities to investigate anorectal physiology are not without limitations. This research project studied a novel investigating modality EndoFLIP which uses impedance planimetry technique to study the anorectal function.

This research study has led to the development of specialised anorectal probes for the EndoFLIP device besides testing the impedance planimetry as an investigating modality to study the anorectal function. The healthy control group in this study helped validate the use of EndoFLIP besides confirming the repeatability of EndoFLIP. The next part of this research project, studying patients with faecal incontinence despite no morphological abnormality and those with faecal incontinence secondary to scleroderma helped confirm the clinical utility of EndoFLIP by providing new knowledge about the anorectal physiology.

As a result of close working with the industry this research project has been successful in developing the EndoFLIP probes specifically suited for anorectal studies. By validating and confirming the clinical utility of EndoFLIP, this research study has paved way for its use in further anorectal studies. Future research projects can benefit from this pilot study by using the custom-made anorectal probes, the balloon inflation volumes and the normality range established during this study. Furthermore, the methodology of this study can be easily replicated in other centres for future studies.

The new knowledge obtained during this study has helped improve our understanding of pathology of faecal incontinence in specific patient groups. This can be used to improve the existing clinical practice and potentially result in better patient outcomes. Additionally, future research projects can build upon the knowledge gained during this project and study other patient groups with anorectal dysfunction, specifically those who have had pelvic radiotherapy, anterior resection surgery or neuromodulation. Commercial projects can also be undertaken to further improve and market the anorectal probes developed during this study.

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Communications and awards arising from this thesis

Publications

1. Internal anal sphincter: Clinical perspective. Kumar, L, Emmanuel, A. The Surgeon, August 2017, Volume 15, Issue 4, Pages 211–226

Presentations

1. EndoFLIP : A new diagnostic modality for demonstrating anal canal function & sampling reflex, Oral presentation, Digestive Disease Week (DDW), Chicago, USA, May 2014. Kumar. L, Zaman. F, Emmanuel. A
2. EndoFLIP : A novel modality for demonstrating anal canal function, Oral presentation, United European Gastroenterology Week (UEGW), Vienna, Austria, October 2014. Kumar. L, Zaman. F, Emmanuel. A
3. Validation of EndoFLIP : a new diagnostic modality for measuring anal canal function, Poster presentation, Digestive Disease Week (DDW), Chicago, USA, May 2014. Kumar. L, Zaman. F, Emmanuel. A
4. EndoFLIP : a new diagnostic modality for measuring anal canal function, Poster presentation, United European Gastroenterology Week (UEGW), Vienna, Austria, October 2014. Kumar. L, Zaman. F, Emmanuel. A

Awards

1. UCL Graduate School Research Projects Fund of £1500 (December 2012)
2. UCL Graduate School Conference Fund of £500 (July 2014)
3. Travel Bursary of €1000 for UEG Week (October 2014)

Acknowledgements

I would like to thank all the patients who took part in this research study. Their spirit of participating in the study despite knowing that the study is not going to have a direct and immediate impact on their own management plan exemplifies the compassion and benevolence of the human race. I would remain indebted to these patients forever.

I am equally grateful to my supervisor, Prof Emmanuel for all his support and guidance throughout this protracted project. He has been a great mentor and I have learnt from him not only at professional but also at personal level. No words will ever suffice to express my gratitude to him.

I would also like to thank the GI Physiology staff, especially Dr Raeburn who works tirelessly to ensure that the Unit delivers the best patient care. She has been a source of immense help right from the day I joined the Unit and I owe my knowledge of anorectal manometry to her.

I would also like to acknowledge my research colleagues, Mr Jonathan Gosling for his help with the MATLAB coding, Mr Abhilash Paily for helping me with the study protocol and ethics application and Dr Shamaila Butt for her help with recruiting the Scleroderma patients. They made this difficult journey enjoyable and fun.

This thesis would not have been possible without the support of my parents and my brother. No words can ever suffice my expression of thanks to them. I won't be what I am without them.

Last but not the least, I would like to thank my wife for being so supportive and tolerating me through all the ups and downs of my research.

Dedicated to my father

CHAPTER 1

INTRODUCTION & BACKGROUND

1.1 Introduction

Faecal incontinence is a debilitating condition which is often under-reported. Although it is difficult to accurately determine the incidence of faecal incontinence due to underreporting, a range of 2-24%, depending on the population studied, has been reported [2-7]. Despite a disagreement about incidence rates of FI, there is a widespread acceptance of its increased prevalence in the older population [8]. FI has a significant negative impact on quality of life, which worsens with an increase in the quantity of faecal loss [9,10]. Moreover, it is associated with a huge socioeconomic burden [11,12].

Maintenance of continence is a complex process requiring interplay between colon, rectum, the internal and external anal sphincter complex, pelvic floor and inter-related motor and sensory neural pathways, all governed by higher centres [13]. Out of many factors controlling the continence, the anal canal, being the final determining step, with its sphincter complex is undoubtedly critical.

1.2 Anal canal

J. Symmington coined the term anal canal in 1888 [14] but it was Milligan and Morgan who laid the foundation of the surgical anatomy of anal canal [15]. The anal canal is surrounded by internal (IAS) and external anal sphincters (EAS) and extends from the upper aspect of the pelvic diaphragm to the anus. Its beginning is marked by the acute angulation at the anorectal junction due to the traction applied by puborectalis muscle (PRM). Based upon these structures, the anal canal can be divided into three main parts; proximal end formed by IAS and PRM, mid part comprising of IAS and EAS and distal part composed of EAS only. Such segmental division is not only based upon anatomical but also physiological grounds and has previously been described in the literature using 3D ultrasound [16], vector manometry [17] and EndoFLIP [18,19].

Voluntary muscles of the anal canal (PRM & EAS)

PRM and EAS are the skeletal muscles which are under the voluntary control and help maintain continence by different mechanisms. PRM is one of the three components of levator ani muscle, which forms the pelvic diaphragm, the other two being iliococcygeus and the pubococcygeus. It forms a muscular sling at the anorectal junction and is responsible for maintaining about 90 degrees angulation at rest at this site thereby preserving continence [20]. During defecation, the PRM relaxes making the anorectal angle more obtuse (about 110-130 degrees) thereby facilitating evacuation. PRM has also been demonstrated to contribute to the squeeze pressure in the proximal anal canal [16,21]. EAS, on the other hand, is primarily

responsible for generating voluntary squeeze at the distal end of the anal canal. It has three parts - deep, superficial and subcutaneous parts which are considered to be a single functional and anatomical entity. The fibres of the deep part blend with those of PRM at the proximal anal canal. The superficial EAS, situated in the mid anal canal, surrounds the IAS and the subcutaneous EAS, lying below the level of IAS, constitutes the distal most part of the anal canal. The superimposition of EAS over IAS makes it difficult to study the functionality of each muscle separately. This is evident from the fact that there is no universal agreement over the contribution of each muscle towards the resting anal pressure, with estimates ranging between 15 to 50% for the EAS contribution [22].

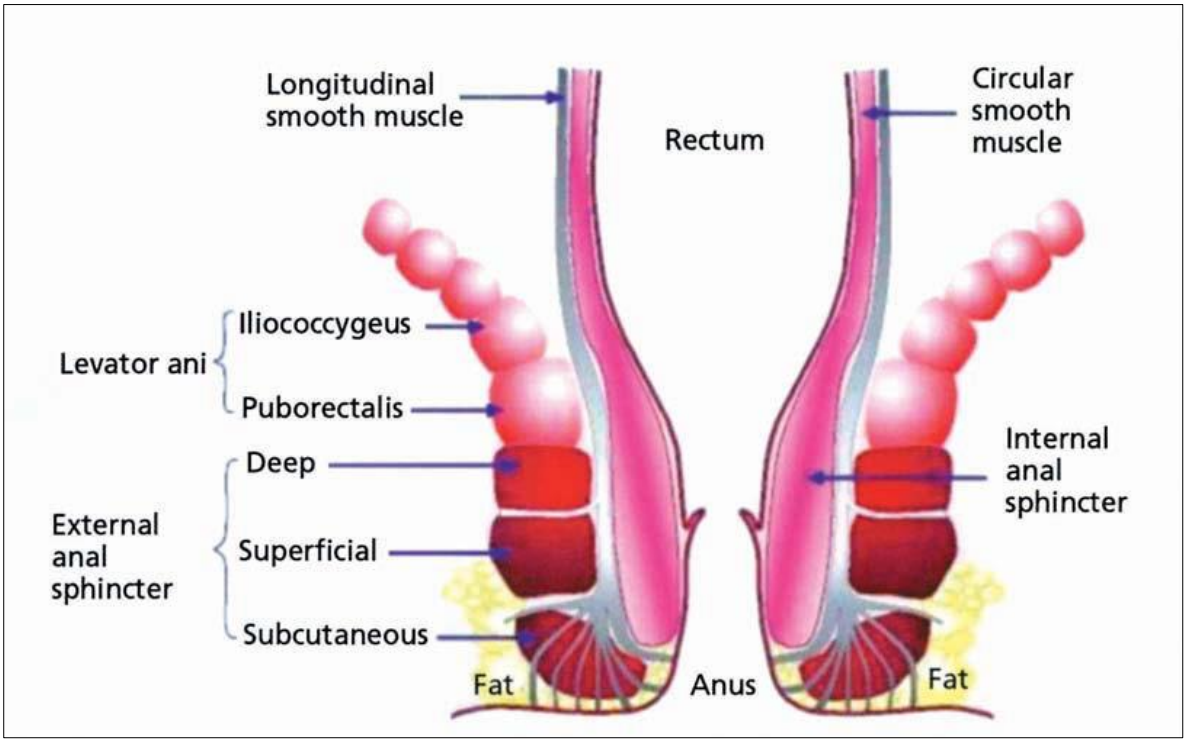


Figure 1.1. Anatomy of anal canal

Involuntary muscle of the anal canal (IAS)

The internal anal sphincter is the involuntary ring of smooth muscle in the anal canal. It is the major contributor towards resting pressure in the anal canal with its dysfunction (due to structural or functional causes) leading to passive incontinence of faeces. Despite advancement in diagnostic techniques, an accurate assessment of IAS still remains a challenge as the presenting symptoms do not always correlate well with the manometric findings. In one study, faecal leakage was found to have a sensitivity of 98.9%, a specificity of 11% and a positive predictive value of 51% for detecting low resting anal pressure [23].

Moreover, there is a significant overlap in anal canal pressures when comparing healthy individuals with those suffering from faecal incontinence [24-26]. This research project aimed to study the IAS function in detail and as such it was deemed imperative to compile a summary of our current knowledge of IAS, which is presented in rest of this chapter.

1.2.1 Anatomy of internal anal sphincter

The internal anal sphincter is a ring of smooth muscle formed by the continuation of the involuntary circular muscle of rectum into the distal anal canal (Fig. 1). As such it is not under voluntary control. Caudally the IAS extends just proximal to the lowest part of the external anal sphincter (EAS) [27]. It is about 2.5 cm long and 2-5 mm thick in size. The IAS has been found to be thicker in females [28,29] but does not have any correlation to body weight or parity [30,31]. It increases in thickness with advancing age [32], a phenomenon more pronounced in females after the age of forty to fifty [28]. Histological studies have shown this rise in bulk to be due to an increase in the amount of connective tissue [33-35], a finding which correlates well with the manometry findings of decreased resting pressure with ageing [36,37].

1.2.1.1 Laplace law

The Laplace's law describes the pressure-volume relationship in a sphere or cylinder and has been used to understand physiological features of tubular structures in the body. It states that

$$\text{Pressure} = (2 \times \text{Thickness} \times \text{Tension}) / \text{Radius}$$

Where,

Pressure = The pressure inside the cylinder

Thickness = Thickness of the cylinder's wall

Tension = Tension within the cylinder's wall

Applying this to the anal canal, the inward pressure of anal canal depends upon the radius and thickness as well as the wall tension (or tone) of the anal canal. This would suggest that an increase in thickness with age would also lead to an increase in the resting pressure of the anal canal. However, the opposite (i.e. a decrease in resting pressure with advancing age) has been found to be true in real life [38]. This has been suggested to be due to decrease in tone of the IAS with advancing age and has been proven by histologic studies confirming increased collagen deposition and decreased smooth muscle with increasing age [34]. In other

words, to understand why a thicker IAS is weaker all four variables of Laplace's law need to be taken into consideration. Whereas thickening of the IAS leads to an increased pressure the increasing diameter and decreasing wall tension (tone) of IAS leads to lower anal pressures. The net result is a low resting pressure seen in the elderly population.

1.2.2 Physiology of internal anal sphincter

1.2.2.1 Innervation of internal sphincter

The IAS is dually innervated by the autonomic and the enteric nervous system [39]. The parasympathetic and sympathetic nervous systems are responsible for exerting both excitatory and inhibitory influences on the smooth muscle whereas the enteric system influences the tone [40].

In general, IAS is believed to be parasympathetically innervated by first, second and third sacral nerves via pelvic plexus and sympathetically from both the thoracolumbar outflow and hypogastric nerves [41,42]. Moszkowicz *et al*. in their topographic study of IAS in human foetuses demonstrated the autonomic innervation to be through inferior rectal plexus which originated from the posteroinferior fibres of the inferior hypogastric plexus [43].

The enteric nervous system is organised into an interconnected network of neurons and glial cells that are grouped into ganglia located in two major plexuses: the myenteric (Auerbach's) plexus and the submucosal (Meissner's) plexus. Its components form entire reflex pathways that control peristaltic contractions independent of extrinsic innervation from the autonomic nervous system [44].

1.2.2.2 Effects of parasympathetic innervation on IAS

The parasympathetic fibres have been shown to have an inhibitory effect on the tone of IAS thereby causing its relaxation. *In vitro* studies such as those by Burleigh *et al.* and O'Kelly *et al.* helped explain the effects of muscarinic receptors stimulation. The former study revealed that stimulation of muscarinic receptor by acetylcholine leads to relaxation of IAS [45] and the latter demonstrated that muscarinic receptor stimulation is mediated indirectly via nitric oxide (NO) release.

Sustained hypertonicity of the IAS leading to ischaemia of the anal lining has long been hypothesised to be the cause of anal fissure [46] and although lateral internal sphincterotomy results in high healing rates, the associated complication of incontinence led to an increasing

interest in the pharmacological agents that can relax IAS and promote healing. The property of IAS relaxation, on stimulation of muscarinic receptors via acetylcholine, led to a trial of bethanechol for treating anal fissures. Bethanechol was found to reduce the resting anal pressure by about 20% in patients with anal fissure [47]. However, due to many superior products available for medical management of anal fissure bethanechol is not prescribed generally.

Another therapeutic agent for managing anal fissure, which acts on cholinergic neurones, is botulinum toxin. In the initial study by Jost et al. [48], it was injected in the EAS causing it to be temporarily paralysed due to the prevention of the presynaptic release of acetylcholine. This led to a reduction of anal pressure thereby leading to healing of anal fissure. However, in the subsequent studies, it was injected in the IAS or the intersphincteric groove [49-51] and although one would have expected an increase in the resting anal pressure (due to its inhibitory effect on the acetylcholine release) it was found to still cause a reduction in the resting anal pressure. The possible explanation of this was provided by Jones et al. in their study on porcine IAS, which established that botulinum has the ability to block sympathetic nerves by reducing noradrenaline release, thereby leading to IAS relaxation [52]. Results from clinical studies using Botox have been discussed in detail in the treatment section of this chapter.

1.2.2.3 Effects of sympathetic innervation on IAS

The effects of sympathetic system are known to be mediated via the alpha and beta-adrenergic receptors both of which are present on the IAS. Depending upon which adrenoceptor subtype is stimulated, the sympathetic innervation exerts excitatory or inhibitory influence on the IAS.

Stimulation of alpha-adrenoceptors is known to have an excitatory effect on the smooth muscles of IAS. GI tract with its abundant smooth muscles has been confirmed to be influenced by alpha-adrenoceptors [53,54]. The effects of alpha-adrenoceptors have also been established on the smooth muscle of IAS. Of the two known subtypes, α1 and α2, the former has been shown to cause IAS contraction without affecting the RAIR induced IAS relaxation whereas the latter inhibits RAIR-induced IAS relaxation but does not increase the IAS contractility [55,56]. The effect of α1 adrenoceptors on the IAS contractility has been suggested to be mediated via noradrenaline.

This IAS contraction inducing property of α1 adrenoceptors has been clinically used in the form of phenylephrine, a potent α1 adrenoceptor agonist, to provide symptomatic

improvement in FI patients. However, to avoid the undesirable effect of an elevated blood pressure, due to stimulation of α1 adrenoceptors present on the blood vessels, only the topical form of phenylephrine has been trialled. Another α1 adrenoceptor agonist, methoxamine, has recently been studied for its clinical value in FI patients.

Although a better understanding of the α1 adrenoceptors raised hopes of developing newer and effective therapeutic agents to treat FI, this has not been the case in reality. Phenylephrine was only found beneficial in a limited number of patients with FI and methoxamine despite showing promising results in phase I trial [57] failed to demonstrate any significant improvement in phase II trial. The possible explanation of their limited beneficial effect is that the diseased IAS is less responsive to adrenergic neurotransmitters than the healthy muscle. This has certainly been the case in an in-vitro study carried out on IAS of patients suffering from FI [58]. Moreover, the fibrosed part of the IAS is unlikely to be responsive to the pharmacological therapy, thereby varying the effectiveness of treatment in different patient groups. The results of clinical studies using these topical agents have been discussed further in the treatment section of this chapter.

Beta-adrenoceptors, the other type of adrenergic receptors, have been shown to mediate the inhibitory effects of sympathetic nerve stimulation in the IAS [59] and other parts of the GI tract [60]. Several clinical studies have already established the influence of β adrenoceptors on the GI tract as shown in table 1.1. In IAS, the presence of all the three subtypes of β adrenoceptors (β1, β2 and β3) was established by a recent study, which found a varying level of inhibitory effect was exerted on the IAS by each of the three receptor subtypes [59]. β1-agonists were found to be the most superior inhibitors of IAS as compared to β2 or β3- agonists, producing about 70% IAS relaxation vs 40% and 30% by β2 and β3- agonists respectively [59]. However, achieving selective gastrointestinal β1 or β2 adrenoceptor modulation alone without the associated cardiovascular effects is not possible thereby limiting the role of pharmacological agents acting on these receptor subtypes in gastrointestinal disorders. β3 adrenoceptor subtype, which received recognition only in the last two decades [61] has led to a new interest in studying its use as potential adrenergic modulation site that can be used in the patients with anal fissure. Although it showed promising results in animal studies, producing up to 90% relaxation in rat IAS, it has been found to have only a limited capacity to relax human IAS (30%) [59]. Further studies are warranted before any conclusions can be made on the use of β3 adrenoceptor.

Pharmacological agent	Organ	Effect on motility
Non-selective β agonist [62]	Stomach	
β1 agonist [63]	Oesophagus	
β1 agonist [64]	Rectosigmoid	
β1 agonist [59]	IAS	
β2 agonist [63]	Oesophagus	
β2 agonist [62]	Stomach	
β2 agonist [64]	Rectosigmoid	
β2 agonist [59]	IAS	
β3 agonist [59]	IAS	
β1 antagonist [62]	Stomach	
Non-selective β antagonist [65]	Intestine	

Table 1.1. Effects of different β adrenoceptors on gastrointestinal motility

1.2.2.4 Effects of enteric system on IAS

The enteric nervous system (ENS) exerts its influence upon the IAS through the interplay of several neurotransmitters. These neurotransmitters cause relaxation of the IAS during defecation [66]. Relaxation of IAS has been shown to occur in response to rectal distension (RAIR) and to electrical field stimulation in organ bath studies. Studies confirmed that the relaxation response remained unaffected by muscarinic receptor and β-adrenoceptor antagonists but was blocked by tetrodotoxin, thereby confirming its non-adrenergic and non-cholinergic (NANC) origin [40].

A number of neurotransmitters have been identified over the years exerting an inhibitory (Nitric Oxide (NO), Adenosine triphosphate (ATP), Vasoactive Intestinal Peptide (VIP), Carbon Monoxide (CO), ANF and Calcitonin gene-related peptide (CGRP)) and excitatory (Dopamine, Angiotensin II, Substance P, bombesin and Neuropeptide Y (NPY)) influence on the IAS [67].

Of these, nitric oxide has been found to be the principal neurotransmitter mediating relaxation in the IAS [68]. This generated an interest in researching exogenous sources of nitrates which when degraded by cellular mechanism release nitric oxide. One such Nitric oxide donor is nitroglycerin ointment which has become a popular first choice in managing anal fissures. It has been shown to cause a rapid reduction in the resting anal pressure of healthy subjects [69] and patients with anal fissure [70] and more importantly have a significant effect on healing of anal fissures [71]. Results from studies using GTN have been discussed further in treatment section of this chapter.

1.2.2.5 Inherent myogenic tone

The myogenic nature of IAS was first reported by an in vivo study done by Frenckner *et al.*in 1976. They found that anal canal had a residual pressure despite maximal relaxation of IAS induced by rectal distension in patients who underwent either bilateral pudendal nerve block (thereby paralysing the striated sphincter muscles), low spinal anaesthesia (depriving IAS of its parasympathetic supply) or high spinal anaesthesia (depriving IAS of its sympathetic supply) [72].

Culver et al found no change in the peak anal pressure in their study on opossum despite abolishing external sphincter activity with pancuronium bromide and using phentolamine (alpha adrenergic antagonist) to abolish the tonic adrenergic activity of IAS. Furthermore, the use of tetrodotoxin, obliterating the tonic neural activity, did not modify the anal canal pressure [73]. These results led them to conclude that the basal tone is IAS is primarily myogenic.

In vitro study of porcine IAS by Cook et al. revealed that this myogenic tone and spontaneous activity depends on extracellular calcium and flux across the cell membrane. The IAS strips prepared for organ bath study lost the tone and spontaneous activity when placed in calcium-free solution. Moreover, the addition of nifedipine which is an antagonist of L-type calcium channel (the main subgroup of Ca channel in smooth muscle cells), also leads to abolished tone in IAS [74].

The calcium channel blockers (CCB) have been used in an attempt to modulate IAS tone based upon the above characteristics in the patients with anal fissure. Oral diltiazem was found to reduce resting anal pressure but did not gain widespread popularity due to associated

effect of reducing the systemic blood pressure. Topical diltiazem has become common in treating anal fissure and has been reported to have similar efficacy as GTN in treating anal fissure while bearing a lower adverse event profile [75]. Another CCB, nifedipine (used orally or topically) has also been trialled for treating anal fissure but failed to gain popularity due to a high rate of side effects associated with it [75].

There has been an upsurge in research to understand the molecular mechanisms underlying the basal IAS tone. Several studies have shown activation of Rho kinase (ROCK) by GTP·RhoA to play a significant role in maintaining this basal myogenic IAS tone [76,77]. IAS relaxation demonstrated in studies as a result of ROCK inhibition [78] makes it an exciting new therapeutic agent to treat hypertensive IAS disorders such as Hirschsprung's disease, haemorrhoids and anal fissures.

1.2.3 Pathology

The IAS is reported to contribute between 50-85% of the resting anal tone, the remainder being from the vascular anal cushions and the EAS [79,80], illustrating IAS's crucial role in maintaining continence. The IAS disease spectrum comprises of symptoms due to alteration in the pressure and as such can be divided into two subgroups, i.e. low-pressure group & high-pressure group.

1.2.3.1 Low pressure

The low pressure most often results in varying degree of incontinence to different rectal components, i.e. solid stool, liquid/semi-formed stool, gas. The common causes of low pressure are mentioned in Table 1.2

With structural defect	Without structural defect
Anorectal surgery	IAS degeneration
Obstetric injury	Scleroderma
Rectal Intussusception & Prolapse	Radiation toxicity
Trauma due to anal penetration	Diabetes Mellitus

Table 1.2. Causes of low resting anal pressure

1.2.3.1.1 Obstetric injury

Obstetric anal sphincter injuries during childbirth have long been recognised as a leading cause of faecal incontinence [81]. About 13 - 25% of women report some form of anal incontinence three to six month after vaginal or caesarean delivery [82,83] with the prevalence falling to 1 - 6% by 12 months [84,85]. Patients can suffer a range of symptoms including flatus incontinence, passive soiling, or frank incontinence of stools. Many women also experience faecal urgency which can be quite distressing. The advent of endoanal ultrasound (EAUS) in the late 1980s proved to be a key factor in recognising the obstetric injuries. Studies have reported an increase in detection rates of sphincteric injuries with the use of post-partum EAUS [86,87].

The rate of anal sphincter disruption in primiparous women has been reported to be as high as 27 to 35 % and between 4 - 8.5% in multiparous women [88]. The risk factors include the use of forceps, prolonged second stage of labour, high birth weight and occipito-posterior presentation. The Royal College of Obstetricians and Gynaecologists classify the obstetric sphincter injury from first to the fourth degree with an increasing severity upon moving up (Fig. 1.2 – 1.3). Involvement of IAS occurs in grade 3c (both EAS and IAS torn) and 4 (injury involving EAS, IAS and the anal epithelium).

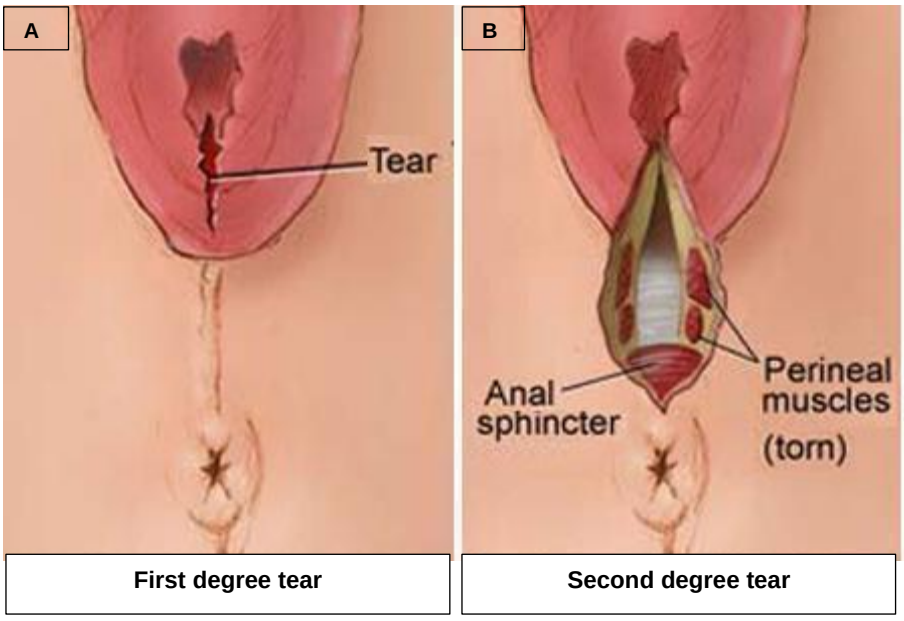


Figure 1.2. Obstetric anal sphincter injury grades 1 & 2

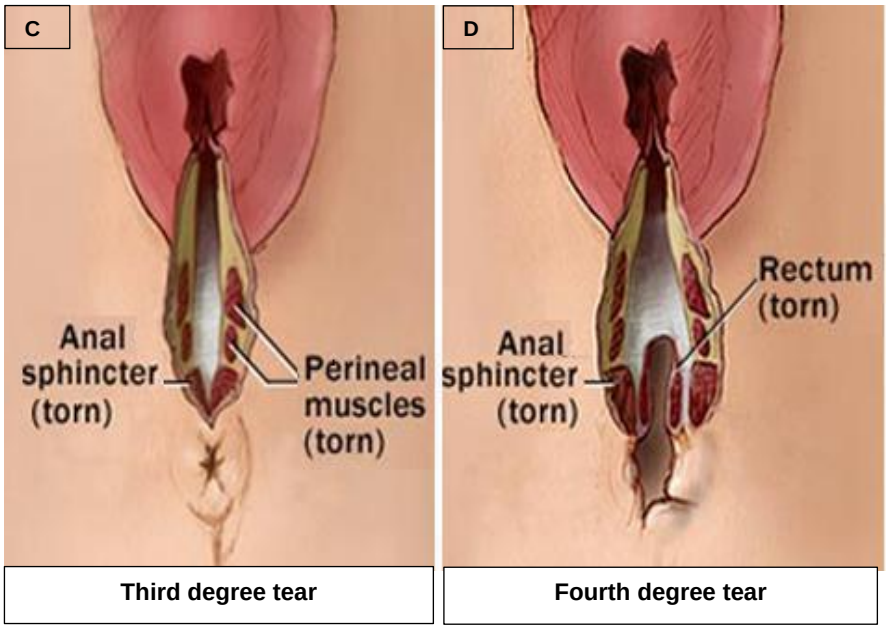


Figure 1.3. Obstetric anal sphincter injury grades 3 & 4

The association of anal sphincter injury and FI has been demonstrated in several studies. In a large study of 500 patients, Mahony et. al. found a significant association between IAS injury during childbirth and faecal incontinence and recommended that the presence of an IAS defect should be sought carefully in cases of obstetric anal sphincter injury [89]. Another study showed the fourth-degree tears and an episiotomy to be associated with persistent IAS defect on an ultrasound done 6-12 months postpartum despite repair of the anal sphincter lacerations at the time of delivery. Furthermore, the IAS defect was more strongly associated with incontinence symptoms than EAS defect as demonstrated by the Faecal Incontinence Severity Index [90].

With regard to treatment, it is recommended that IAS should be repaired if its torn and can be identified separately [88,91]. The prognosis for future continence problems is worse in women with injuries to the internal anal sphincter or rectal mucosa [88] which in part relates to the poor maintenance of IAS integrity following repair compared to the EAS. There is little doubt that obstetric sphincter injuries can have a disastrous effect on patient's quality of life. A combination of early recognition of these injuries, provision of supportive treatment and a decision about mode of future pregnancies tends to reduce the disease burden. With increasing awareness of how common the sphincter injuries are and their long-term consequences on patient's life, this combination approach is becoming a common practice.

1.2.3.1.2 Anorectal surgery

Anorectal surgical procedures such as haemorrhoidectomy, anal dilatation, fistula surgery, low anterior resection can cause structural and functional changes in the sphincter complex eventually leading to symptoms of faecal incontinence [92-97]. Better awareness of sphincter damage has meant that certain procedures are no longer done (such as Lord's dilatation) and others are done more cautiously (limited lateral sphincterotomy, haemorrhoidectomy). Evolution of surgical practice has also led to a reduction in iatrogenic injuries.

a. Haemorrhoidectomy

Haemorrhoidectomy has been well known to be associated with complication of faecal incontinence. A large multicentre study of patients who underwent haemorrhoidectomy reported that 33% of patients (139 of 507) had impaired anal continence post procedure and of these 29% (40 of 139) claimed this to be a direct result of the haemorrhoidectomy [98]. The incontinence is believed be from factors such as iatrogenic injury to the sphincters, loss of haemorrhoidal cushion leading to a reduced resting pressure and inadvertent anal dilatation from the retractors during haemorrhoidectomy [99].

Different surgical techniques used for haemorrhoidectomy have been shown to cause varying degree of damage to the IAS [95,100-102]. A study comparing EAUS findings in symptomatic vs asymptomatic patients post haemorrhoidectomy revealed damage to sphincter complex in 63% of the patients (10 out of16). Peters et al. observed that IAS was significantly thinner in the patients who underwent conventional haemorrhoidectomy with a diathermy as compared to haemorrhoidectomy with ligasure, a bipolar electrosurgical device, (1.8 mm vs 2.5 mm, p=0.005). Moreover, this finding was not explained by a difference in age or sex distribution between the two groups and led the authors to conclude that the IAS damage was due to the use of diathermy. A histological study revealed presence of sphincter muscles in 16% of the patients undergoing haemorrhoidectomy [103]. The most recent technique of transanal haemorrhoidal dearterialization (THD) has shown some promising results without affecting the continence, but further clinical trials are needed to evaluate the long-term results [104-106].

b. Anal dilatation

Anal dilatation has previously been a mainstay for treating colorectal diseases such as haemorrhoids and anal fissure. Lord popularised the procedure [107] for which different techniques have been described from using four fingers in the anal canal to provide lateral distraction [108] to using a Parks' retractor opened to a set distance in an attempt to

standardise the technique [109]. However anal dilatation fell out of favour due to the reports of faecal incontinence post procedure.

A study from St Marks by Speakman et al. reported damaged internal sphincter on EAUS in 11 of 12 men, presenting with FI post anal dilatation, with extensive fragmentation noted in 10 of these subjects [110]. Similar results were reported by Snooks *et al.* who found IAS damage (in 8 of 10 patients) through a combination of manometric and EMG tests in patients who underwent anal dilatation [111]. A study of 32 consecutive patients undergoing manual dilatation reported incontinence in 12.5% of the patients. Of 20 patients who agreed for EAUS, 13 were found to have sphincteric defects [112]. With these widespread findings of sphincteric damage and faecal incontinence post anal dilatation this procedure is rarely used these days.

1.2.3.1.3 Rectal prolapse

Aetiopathogenesis of faecal incontinence in rectal prolapse is multifactorial and poorly understood. However, IAS dysfunction is known to be one of the definitive causal factors of incontinence in this group. In cases of external prolapse, a reduction in both resting and squeeze pressures [13] leading to incontinence has been postulated due to several factors including internal sphincter dilatation [113], rectal prolapse and intermittent activation of the recto-anal inhibitory reflex (RAIR) [114], nerve damage from stretching due to perineal descent [115], and reduction of the rectal reservoir [116]. In contrast, incontinence in the internal prolapse (or rectal intussusception) has been speculated to be due to attenuation of the RAIR mechanism [117,118]. Another possible cause is a reduction in IAS tone as shown in a recent study of 515 patients which found an inverse relationship between resting pressure and the grade of internal prolapse without an effect on squeeze pressure [13].

1.2.3.1.4 Anal penetration

Anal penetration is another factor reported to cause damage to the anal sphincters. A study comparing 40 anoreceptive to 18 non-anoreceptive males by Miles *et al.* concluded that anal intercourse (AI) was associated with reduced anal canal resting pressure and an increased risk of minor anal incontinence [119]. Chun *et al.* demonstrated the same results in their study on 28 males engaging in AI. On further examination with EAUS, they reported thinner anal sphincters in anoreceptive group, albeit not statistically significant, but no disruption of the IAS or EAS was seen. There were no complaints of faecal incontinence by the study subjects [120].

In contrast to passive AI, unwanted anal penetration was found to be associated with structural internal anal sphincter damage in all the 7 patients who were studied by Engel *et al.* [121] though clearly this study was compromised by ascertainment bias.

1.2.3.1.5 IAS degeneration.

This was first described by a study conducted at St Marks Hospital in which Vaizey et al identified a manometric and sonographic abnormality in EAUS of 38 patients who had a history of isolated passive soiling. All of the 38 patients had a functionally and structurally normal external anal sphincter along with normal pudendal nerve terminal motor latencies. They were found to have low maximum anal pressure at rest, and an abnormally thin, poorly defined, and echogenically altered internal sphincter on ultrasound. They concluded IAS degeneration was responsible for FI in 3.5% of the patients [122]. Another recent study reported similar rate of 4.3% for IAS degenration, noted on EAUS in absence of any other cause for FI [123].

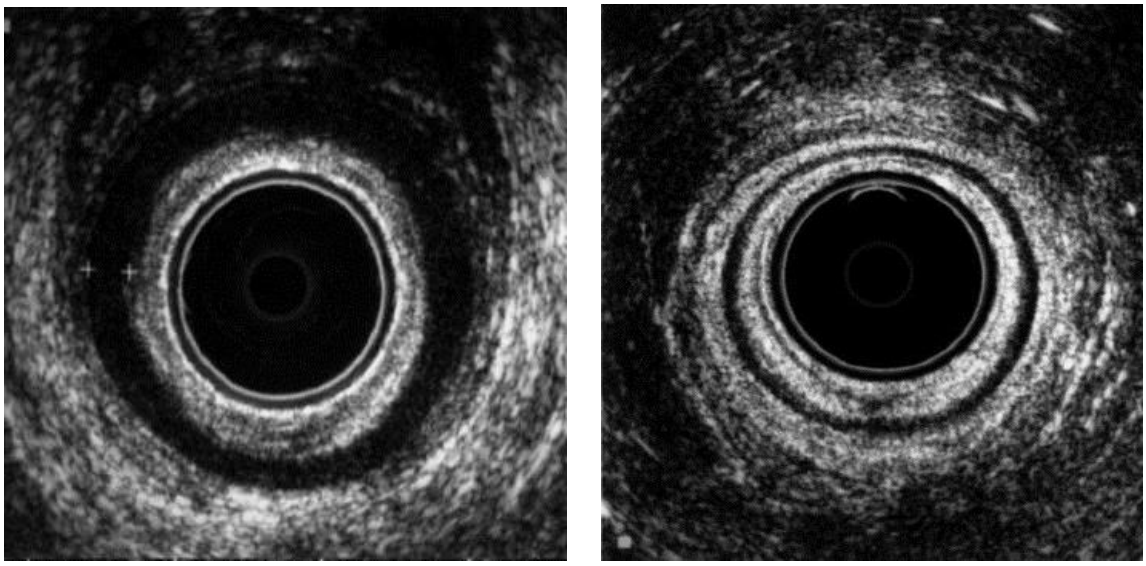


Figure 1.4. EAUS of asymptomatic patient demonstrating a normal IAS (left) and a patient with IAS degeneration (right)

Secondary IAS degeneration and FI is already a well-recognized complication of progressive systemic sclerosis. In these patients the IAS atrophy is attributed to collagen deposition leading to fibrosis; vasculopathy leading to ischaemic atrophy; and neuropathy. However, the patients in the two studies reporting IAS degeneration did not have any recognised cause for the FI. The only common feature in both these studies was the older age group in which IAS degeneration was noted (mean age of 63 in study by Vaizey et al. and 75 in study by Albuquerque et al.). A possible explanation for this finding is that collagen deposition takes place with advancing age besides a decrease in smooth muscles. This has been previously

confirmed by a histological study which found a significant correlation ($p = 0.001$) between the collagen content and patient's age (Neurogenic FI patients). Furthermore, they also reported an excess collagen content in FI patients as compared to controls (55% vs 33% respectively, $p = 0.013$) [124]. An increase in connective tissue [125] and fibrotic changes [126] in IAS with advancing age have also been reported in separate studies.

Vaizey et al. also postulated likewise that IAS degeneration seen in their study was due to muscle atrophy [122]. Although an increase in thickness of IAS with age has been well documented [30,127,128], this has been shown to be due to an increase in the proportion of fibrous tissue rather than the smooth muscle of IAS [124]. Vaizey et al, confirmed this finding in their study and reported an increase in echogenic apperance suggesting an increased fibrous tissue.

1.2.3.1.6 Scleroderma

Scleroderma, an autoimmune connective tissue disorder is characterised by excess collagen production, chronic inflammation and vascular changes, involving multiple organs. GI tract is affected in up to 90% of these patients with oesophageal involvement being the most common. The anorectum is second commonest site of involvement and affects 50–70% of patients [129].

There have been a number of studies which have investigated anorectal physiology in this patient group (with anorectal manometry) and found low resting anal pressure, attributing it to the involvement of IAS [130-133] whereas the squeeze pressure, generated by EAS, has been reported to be within normal limits [134-136].

With the development of better anorectal imaging modalities, such as EAUS and Endoanal MRI, the research interest moved from measuring physiological parameters towards detecting the morphological involvement of IAS in these patients. Engel et al. were the first one to report the IAS atrophy in scleroderma patients [137]. Since then two other studies by DeSouza et al. and Koh et al. have confirmed the finding of IAS atrophy in this patient group using MRI and EAUS respectively [138,139].

A study from our centre compared scleroderma patients with and without anorectal symptoms. Although one would have expected to see morphological differences in IAS in these two patient subgroups, the study demonstrated that all these patients had thinned and atrophic IAS (on EAUS) without any significant difference in the atrophy scores or IAS thickness between them [140]. The result from this study points towards other possible factors (apart

from structural) at play in the IAS which lead to faecal incontinence in these patients. It can be that functional changes in the sphincter are responsible for FI in these patients, given that this has already been shown to be the case in the oesophageal involvement. The effects of scleroderma on IAS biomechanics remain unexplored and the new technology using impedance planimetry, EndoFLIP, might be able to provide some new information in these patients as discussed later in chapter 5.

1.2.3.1.7 Radiation toxicity

Pathophysiology of radiation damage

Ionising radiation cause direct and indirect damage to the DNA. The nuclear chromatin is directly targeted leading to DNA damage. The intracellular membrane is also damaged due to the disruption of rigidity pf phospholipid bilayer and electric gradient plasma thereby affecting the cell integrity. Formation of free radicals from ionisation of water molecules leads to indirect damage to the DNA. Small DNA disruptions may get repaired thereby maintaining cell function and ability to divide. However, severe damage causes immediate arrest of cell division, leading to cell death.

The radiation is more damaging to the cells with short mitotic cycle, such as intestinal mucosal cells as opposed to endothelial and smooth muscle cells which divide slowly [141]. Within a few days of irradiation, intestinal mucosa becomes denuded as the high proliferating epithelial cells suffer a significant damage and are not replaced owing to the damage to the progenitor cells. The radiation damage further leads to nuclear atypia, bacterial invasion, vascular congestion and oedema. The electron microscopy of epithelium reveals decreased microvilli, disruption of tight junctions, thickened basement membrane and damaged mitochondria. These changes tend to result in diarrhoea, electrolyte imbalance and malabsorption in the acute setting. The late effects are the result of vascular damage leading to ischaemia and fibrosis. Ischemic necrosis and ulceration, as a result of chronic ischaemia, are often visible on colonoscopy and usually lead to extensive fibrotic changes [142].

Radiation induced changes in rectum and anal sphincters

Ionising radiation has long been recognised as causing bowel dysfunction. Most of the early studies attributed this to decreased reservoir capacity and compliance of rectum in addition to nervous degeneration [143-145]. Varma et al. were the first to show a significant reduction in resting pressure and physiological sphincter length in patients following radiotherapy, thereby suggesting IAS dysfunction [146]. Several other studies also reported an increase in

symptoms of incontinence and a reduced RP in irradiated patients as compared to patients treated with surgery alone [147-149]. Kusunoki et al established that anorectal dysfunction in the irradiated patients is dose dependent, with patients receiving a high-dose radiation (80Gy) having lower RP as compared to those receiving low-dose (30Gy) or no radiation. The sphincter dysfunction post radiotherapy is not limited to rectal/anal carcinomas but is also found in other pelvic malignancies such as prostate, bladder and cervix [150-152].

Numerous studies have reported indirect evidence of IAS dysfunction (due to radiation), based on manometric findings and clinical symptoms, but De Silva et al. are credited with confirming this histologically. They reported an increased collagen deposition and hypertrophy of the myenteric plexus in irradiated IAS and proposed that as the cause for IAS dysfunction [153]. Lorenzi et al. recently published the first study providing a physiological basis for chemo-radiotherapy (CRT) induced impairment of IAS function in patients treated for rectal cancer. In their organ bath studies, using human IAS, they established that pelvic irradiation not only damaged the intrinsic nerves but also affected the muscular component of the IAS [154].

Despite several histological and physiological studies, the pathophysiology of IAS dysfunction due to irradiation remains poorly understood. The data from these studies has been inconsistent with some studies suggesting anal sphincter weakness to be common post radiation [145,152,155,156] while the others claim that sphincter function remains unaffected [157-159]. Moreover, imaging of the IAS has not found any morphological aberrations in post pelvic radiation patients [155,160] thereby leaving functional sphincter problem a likely possibility. Interestingly, there is no study that has looked at the biomechanical properties of the IAS in this patient group. Such studies can possibly provide some fresh perspective and help clarify the confusion in the literature on this subject.

1.2.3.2 High pressure

Low pressure in the anal canal due to the above-mentioned pathological disorders usually leads to FI. At the other end of the spectrum are anorectal disorders such as anal fissure, hereditary myopathy and internal sphincter achalasia which are characterised by an abnormally high pressure generated by IAS. The pathophysiology for this increase in resting pressure is not completely understood but has been discussed below for each condition.

1.2.3.2.1 Anal fissure

Anal fissure is a tear in the squamous epithelium of the distal anal canal usually extending from below the dentate line to the anal verge. It has long been postulated to be a result of elevated resting pressure (RP) in the anal canal which eventually leads to ischaemia of the anal lining [46,161]. Several studies have demonstrated an increase in maximal RP above 90mmHg to be associated with anal fissure [162-164]. Moreover, the gold standard treatment [165] of lateral internal sphincterotomy has been proven to reduce the RP, on manometry, to allow for the healing [162], thereby providing further evidence for high RP to be the causal factor for anal fissure. The primary cause of high resting pressure in the first place still remains an enigma, although several theories exist about its increase being secondary to trauma from passing hard stools resulting in spasm [166], psychological stress [167] and being idiopathic in nature [168].

1.2.3.2.2 Hereditary myopathy

This rare autosomal-dominant inherited myopathy of IAS was first described in a series of three patients from the same family having severe proctalgia fugax and constipation [169]. Similar cases were subsequently reported by Konig *et al*., Portilla *et al*. and Zbar *et al.* in their studies [170-172]. All the patients in these studies were found to have an abnormally high resting pressure with massive ultraslow waves. The anal pain was noted to coincide with these ultraslow wave peaks. The ultrasound usually reveals a grossly thickened IAS in these patients. The treatment is in the form of IAS strip myectomy which on histology reveal characteristic finding of vacuolation and presence of inclusion bodies [173].

1.2.3.2.3 Internal sphincter achalasia

Internal sphincter achalasia is a rarely diagnosed disease of IAS in which it does not relax fully to rectal distension. It presents in a similar way as Hirschsprung's disease with symptoms of constipation and gradual bloating. However, unlike in Hirshsprung's the rectal biopsy shows a normal pattern of the ganglion cells and a normal acetylcholinesterase (AchE) activity. The pathogenesis is thought to be multifactorial, including the absence of nitrergic innervations, defective innervation of the neuromuscular junction and altered distribution of interstitial cells of Cajal [174]. The management options range from conservative to surgical treatment including botox injection and IAS myotomy.

1.2.4 Assessment

1.2.4.1 Structural

The detection of a structural lesion has the highest impact in terms of identifying aetiology and potentially suggesting management options. Several modalities exist, to detect morphological defects, having their own advantages and limitations as outlined below.

1.2.4.1.1 Ultrasound

a. Endoanal ultrasound (EAUS)

Anal Endosonography was developed from urology, where the technique was used for transrectal imaging and puncturing of the prostate [175]. It was the first technique to directly visualize the separate components of the sphincter [176]. The sphincters being circular structures are ideally examined axially. An angled axial image of sphincters is possible in women by placing a standard end-firing transducer on the perineum. However, in men a standard transducer will only achieve imaging in longitudinal axis of the sphincter. As such endoprobes were developed for obtaining axial imaging of the sphincters. The initial endoanal probes used a 7-MHz receiver crystal. Increasing the frequency of the transducer probe helps to discriminate between two structures more accurately and as such a 10-MHz probe was designed specifically for use in anal canal to improve resolution. However, with an increase in frequency, the waves become more attenuated and thus are more suitable for imaging superficial rather than deeper structures [177].

The accuracy of EAUS for detecting anal sphincter defects has been demonstrated in several prospective studies that have compared US findings to the results of surgery (sphincteroplasty) [178-188]. In the largest study reported to date, US findings were prospectively compared with operative findings in 44 patients who underwent pelvic floor repair [189] and the endorectal US was found to be 100% sensitive in detecting either IAS or EAS defects in this study population. Furthermore, it has been shown to have a good reproducibility [180,182,183,190].

Although useful in the detection of internal sphincteric defects, it is limited by inherent poor contrast and does not demonstrate external sphincter accurately due to the similarity in its echogenicity to that of the ischioanal fat. A study of intraobserver agreement found a complete agreement for internal sphincter integrity in 51 consecutive patients, but disagreement related to isolated EAS injury in nine patients (18%) [182].

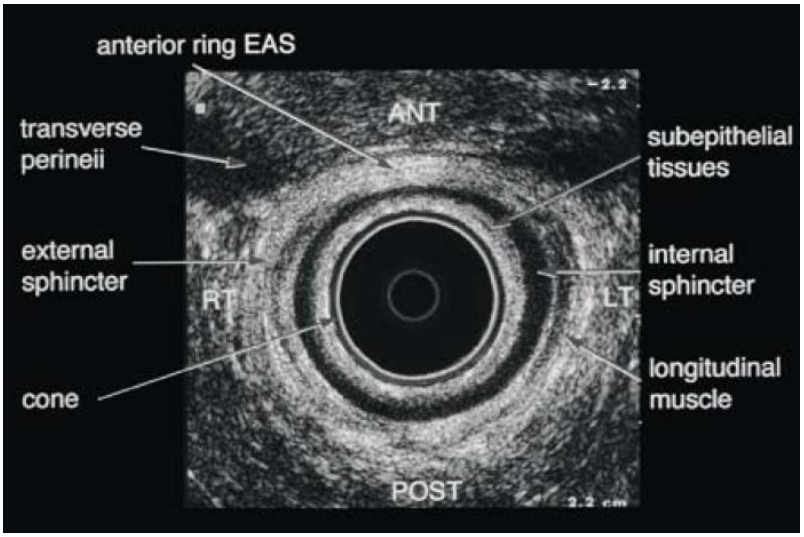


Figure 1.5. EAUS demonstrating the normal structures in the anal canal

b. Transvaginal ultrasound (TVUS)

TVUS was developed by Sultan *et al.* in 1994 in order to obtain more accurate sphincter muscle measurements by avoiding distortion of epithelial structures and compression of sphincters due to the anal probe. They clearly visualized puborectalis, EAS, IAS, anal submucosa and anal cushions and concluded that TVUS allows for accurate imaging of anal sphincters and their defects [191]. Further uses of TVUS for identifying pathologies such as rectal fistulae and perianal sepsis were established by studies from Alexander *et al*. [192] and Poen *et al.* [193].Sultan *et al.*, Alexander *et al.* and Stewart *et al.* established that accuracy of TVUS in evaluating the anal sphincters was equivalent to endoanal ultrasound [173]. However Frudinger *et al.* did not report the same level of accuracy and reported a sensitivity of 44% and specificity of 96% for the detection of IAS defects [194].

Although the interpretation of TVUS requires more expertise it has a clear advantage by being cheaper than the endoanal probe and of eliminating distortion of anal epithelium [195]. Its use has increased due to the clarity of the sphincter it offers, its ability to demonstrate important landmarks such as anorectal junction and puborectalis sling and the ease of converting it to transperineal approach for evaluating perianal sepsis [173].

c. Transperineal ultrasound (TPUS)

TPUS was developed in an attempt to improve patient acceptability by being less invasive. Hall *et al*. and Lee *et al.* reported it to be a good modality to measure IAS but not EAS by [196,197] whereas Peschers *et al.* and Huang *et al.* were able to visualize all the layers as described by EAUS [198,199]. IAS was reported to be thicker on measurements by TPUS as

compared to on EAUS in two studies, presumably due to avoidance of compression of sphincters from the endoanal probe [200,201].

Although having an advantage of being less painful and more acceptable, TPUS has been found to be inferior in comparison to TVUS and EAUS at detecting the incidence of occult anal sphincter damage. This figure is comparable for TVUS and EAUS at 29% whereas for TPUS it is just 7.9% [195].

1.2.4.1.2 Elastography

Elastography is a new imaging modality based on differences in radio frequency signals following endogenous/exogenous compression due to different elastic properties of the targeted tissues or organs [202]. Simply put, it is an ultrasound technique which can produce images based on tissue stiffness. The elastography image usually comprises a red, green and blue colour corresponding to distensible, intermediate and rigid areas respectively in the targeted organ [202,203].

In the anorectal region, it identifies the IAS and EAS based on their different elastogram colour distributions. The IAS is seen as mostly red whereas EAS appears to be mostly blue. This is assumed to be due to the different type of musculature, the IAS being a smooth-muscle type possessing more elastic properties than the EAS [202].

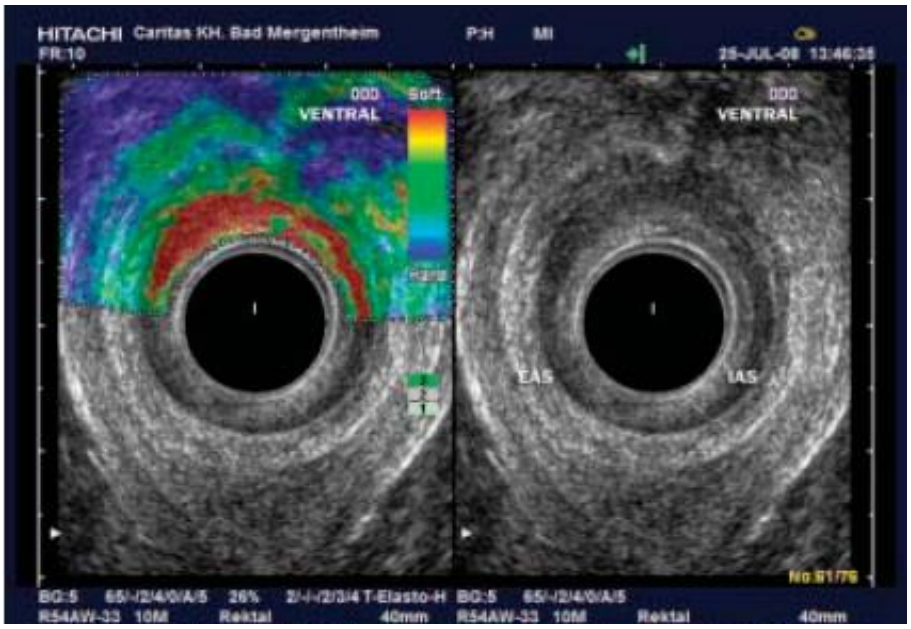


Figure 1.6 Elastogram (left) and gray-scale B-mode(right) from a single patient with normal IAS & EAS. Reproduced with permission from Taylor & Francis [204]

Although, current studies have failed to demonstrate any advantage of elastography as compared to conventional EAUS in majority of the FI patients, one of its potential uses may lie in studying the anal sphincters post pelvic radiation to detect fibrotic changes not seen with the conventional EAUS. However, due to paucity of its use in endoanal studies, it's quite early to say if elastography has potential use in studying anal sphincters and controlled trials are awaited to approve or disapprove its routine use in FI patients.

1.2.4.1.3 Endoanal MRI

Endoanal MRI was developed in the early 90s and allows detailed visualization of normal anatomy and pathologic conditions of anal sphincter [205]. It has been described as the most accurate technique for identifying sphincteric lesions and to plan optimal treatment and is superior to endoanal u/s because of its multiplanar capability and higher inherent contrast resolution [206]. IAS defects are identified as either IAS discontinuity or as the replacement of normal smooth muscle by fibrous tissue with concomitant muscle thinning [207]. It also allows for detection of thinning and atrophy of sphincters and correlates well to findings at the time of surgery and histology of biopsy specimens [206]. The drawback is that it is more expensive although in the long run it could prevent unnecessary operations and prove to be cost-effective.

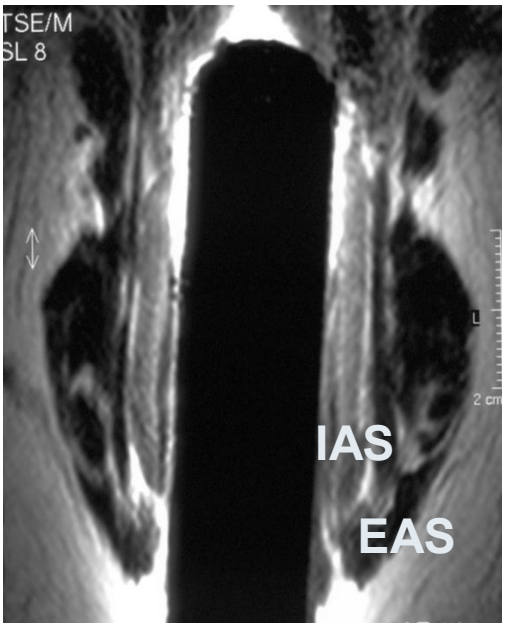


Figure 1.7 Mid coronal image showing anal sphincters

1.2.4.2 Physiological

The sphincters are not always found to be damaged structurally and can have problem with the functional aspect which has led to development of modalities which can give more information on the functional aspect of the sphincter.

1.2.4.2.1 Anorectal manometry

Anorectal manometry (ARM) is considered to be the gold standard for defining sphincter function [8] and is the most well established and commonly used technique for investigating anorectal function [208]. To a lesser extent, it has also been used for biofeedback training for

patients with constipation and faecal incontinence [209,210]. A complete assessment of anorectal function involves evaluating the following parameters: (1) anal sphincter resting and squeeze pressures, (2) rectoanal inhibitory reflex, (3) anorectal sensation, (4) changes in anal and rectal pressures during attempted defecation, (5) rectal compliance and (6) balloon expulsion test [208,211]. Of these, resting pressure and rectoanal inhibitory reflex are the direct evaluators of IAS function and the squeeze pressure helps evaluate the EAS.

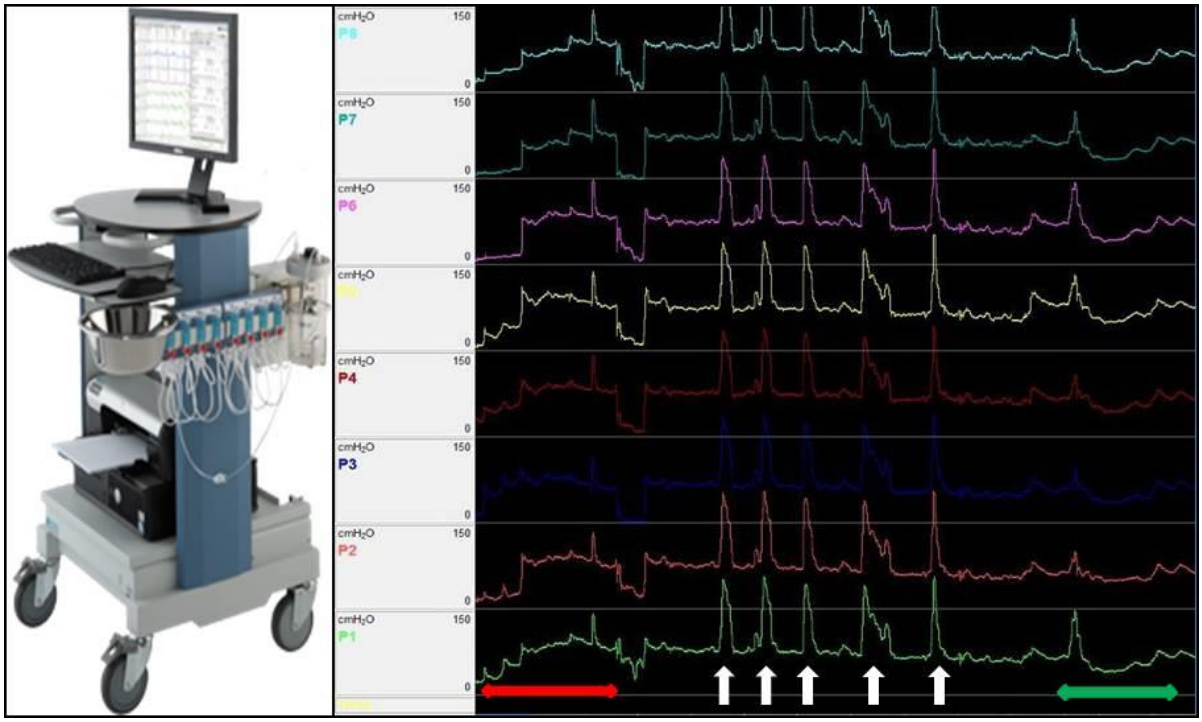


Figure 1.8 ARM setup on the left and a graph produced from ARM on the right; red horizontal marker represents resting pressure and green horizontal marker represents rectoanal inhibitory reflex. White arrows represent squeeze.

Maximum resting anal canal tone is widely acknowledged to be a key reflection of IAS function [208,212]. This resting tone prevents passive leakage of flatus and stool, thereby maintaining continence. The patients with a morphological or functional deficit of IAS have a lower resting tone explaining the incontinence in this group. However, there is no clear understanding of causes of incontinence in patients having normal pressures on anorectal manometry. This overlap in anal canal pressures when comparing healthy individuals with those suffering from faecal incontinence [24-26] indicates that conventional manometric techniques do not accurately reflect the function of the anal sphincters. Further studies are warranted to improve our understanding of underlying causes of incontinence in this group and new technologies such as EndoFLIP may be able to provide some useful information.

Another evaluator of IAS function is the recto-anal inhibitory reflex (RAIR) described by Gowers in 1877. It is an enteric phenomenon in response to rectal distension leading to a transient relaxation of the IAS. Its existence depends on intramural autonomic ganglions and its modulation on the integrity of the myenteric plexus [213]. This transient relaxation of IAS is well accepted to play a crucial part in maintaining continence [27]. A study by Kaur *et al.* reported that incontinent patients had a significantly greater relaxation of IAS in comparison to the constipated patients (P=0.001). They also noted a progressive increase in IAS relaxation with progressive rectoanal inhibitory reflex in the incontinent group but not in the constipated patients or in healthy control subjects [214]. Similar results were also seen in another study by Farouk *et al.* [215]. The greater IAS relaxation in the FI patients possibly explains the incontinence episodes in these patients. Furthermore, progressively increasing IAS relaxation (in incontinent patients) as a result of proximal anal canal filling, likely leads to a quicker delivery of faeces to the distal anal canal, where the EAS excitatory response may not be able to prevent leakage. The inability of EAS to increase its excitatory response in incontinent patients to prevent leakage has been confirmed in a study of 42 subjects (16 neuropathic FI, 16 chronic constipation and 10 healthy controls) by Zbar *et al* who looked at the different phases of RAIR, in proximal, intermediate and distal anal canal. They did not find any significant difference in the distal anal canal excitation between different groups [216]. Several other studies have also confirmed the importance of RAIR in maintaining continence. Our group while looking at incontinence in scleroderma (SSc) patients found RAIR to be absent in 46% of SSc patients with incontinence as compared to 10% in asymptomatic SSc patients and only 5% of incontinent controls [217].

Although several studies have proven that EAS contraction during RAIR undoubtedly plays an important role in maintaining continence, the fact that RAIR is absent in constipated patients (not suffering with incontinence) and present in many incontinent patients, suggests that EAS contraction is not the only mechanism responsible for maintaining continence. This possibly highlights the role of IAS as a vital defence mechanism against incontinence, especially in patients with EAS dysfunction.

Anal sensitivity is another parameter tested during ARM and is believed to play an important role in maintaining continence. The anorectal mucosa has been shown to have several different nerve endings such as Meissner's corpuscles for touch, Krause end-bulbs for acute sensation of cold, genital corpuscles for friction, and Golgi-Mazzoni bodies and Pacinian corpuscles for tension and pressure [218]. There is a heavy concentration of these receptors in the mid zone of the anal canal, also known as the transitional zone, which enables this specialised sensory epithelium to discriminate between flatus, fluid and faeces thereby forming the basis of anorectal sampling. The sampling reflex, initially proposed by Duthie and

Bennett [219], comprises of a differential relaxation of anal canal in response to a balloon inflation in the rectum. In their study, inflation of the rectal balloon led to a reduction of pressure in the upper anal canal (indicating relaxation or opening) while the pressure in the lower part remained high enough to maintain continence. Sampling essentially allows the rectal contents to be evaluated for consistency and enables passage of flatus whilst retaining faecal material. Further evidence to this characteristic property of sampling by anal mucosa is provided by the fact that anorectal surgery such as proctectomy (where the anal transitional zone is excised) leads to an increase in incontinence symptoms. Therefore, it is generally advisable to avoid any form of mucosal proctectomy to maintain the sampling feature of the anal canal.

Appreciation of this specialised function of anal mucosa led to an evolution of techniques to measure the anal sensitivity. The older methods, including needle prick test, water infusion at different temperatures and registering the awareness of presence of catheter in anus have largely been abandoned due to either lack of reliability or being cumbersome. The currently used technique for measuring anal mucosal electrosensitivity (MES) was introduced by Roe et al [220] and gained widespread acceptance due to ease of use and being a reproducible and reliable tool to measure anal sensitivity [221]. They used a catheter with two electrodes connected to a constant square wave current with variable intensity to objectify anal sensitivity. A high MES threshold reflects a decreased anal sensitivity and is invariably seen in incontinent patients. Ageing, an increase in IAS or submucosal thickness have all been shown to decrease anal sensitivity. IAS defects and combined sphincter defects also lead to decreased anal sensitivity.

Despite its widespread use, ARM has well-established limitations. One of the main caveats has been that despite decades of use there is still no standardized protocol for performing the test or interpreting its results [222-227]. Also, there is no consensus regarding normative data presumably due to differences in the manometry probes and study population in addition to the aforementioned factors [224,225]. Moreover, several authors have reported a lack of reproducibility and its inefficiency to provide precise measurement of anal canal pressures. Ryhammer et al. used a continuous pull-through technique to calculate resting pressure and a single station technique for measuring squeeze pressures. The repeatability coefficient (2 standard deviations of the difference between the two tests) was 31cmH_2O for resting pressure and 35cmH_2O for squeeze pressures with the coefficient of variation (standard deviation divided by the mean) as 22% and 33% respectively [26]. Rogers et al. used a micro balloon with a station pull-through technique and found a repeatability coefficient as 42cmH_2O for resting pressure and 65cmH_2O for squeeze pressures [228]. Bharucha et al. used a water perfused catheter with 4 radially arranged side holes using the station pull-through technique and calculated an intraobserver coefficient of variation as 26% for resting pressure and 27%

for squeeze pressure and an interobserver coefficient of variation for resting as 39% for resting and 41% for squeeze [229]. It, therefore, could be concluded that conventional manometric techniques do not provide a precise measurement of anal canal pressures.

1.2.4.2.2 Vector volume anorectal manometry

Another approach to ARM is vector volume manometry which gives a 3-dimensional view of anal sphincter pressure, allowing the pressure to be viewed at any point along the anal canal thus allowing for an objective visualization of the defect present [230]. Its ability to identify patients with incontinence symptoms and sphincter injury has been well established [82,231,232]. However, with the development of EAUS to provide accurate information about sphincter defects, the utility of vector volume manometry has become debatable. It has been suggested that it may be well suited as a predictive tool to identify patients, undergoing surgery for rectal or anal pathology, who will develop postoperative functional changes. This proposition is based on the fact that vector manometry correlates well with symptoms post rectal or anal surgery [233-235].

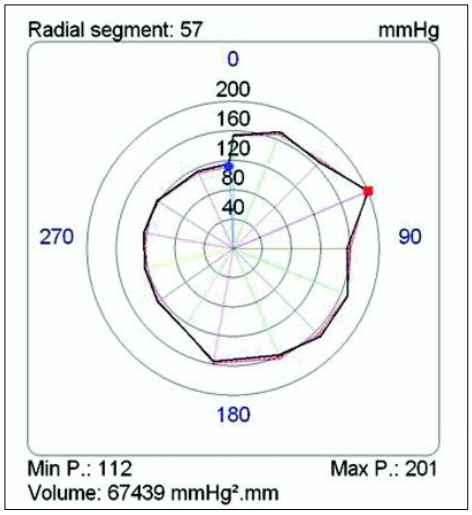


Figure 1.9. 3D view of sphincter pressure with vector volume

1.2.4.2.3 High-resolution anorectal manometry (HRAM)

High-resolution manometry is a relatively new technique primarily used for physiological studies in the oesophagus where it was found to convey a diagnostic advantage over standard manometric techniques [236]. Subsequently, this technique was adopted to study the physiology of anorectum where it provided an added advantage of simultaneously measuring circumferential pressures throughout the anal canal and rectum [237]. Jones *et al.* reported a good correlation between ARM and HRAM measurements in their prospective study of 29 patients. They also made topographic plots with the help of special software to provide a dynamic representation of pressures within the anorectum both at rest and in response to dynamic changes [236].

Further advances in the HRAM technique have resulted in 3D HRAM probe which combines the two functionalities of evaluating the physiology and anatomy at the same time. Although

this technique has been shown to be reproducible it has failed to demonstrate the sensitivity and specificity equivalent to EAUS. It was found to have a false negative rate of 14% and false positive rate of 41% in detecting the IAS defects [238].

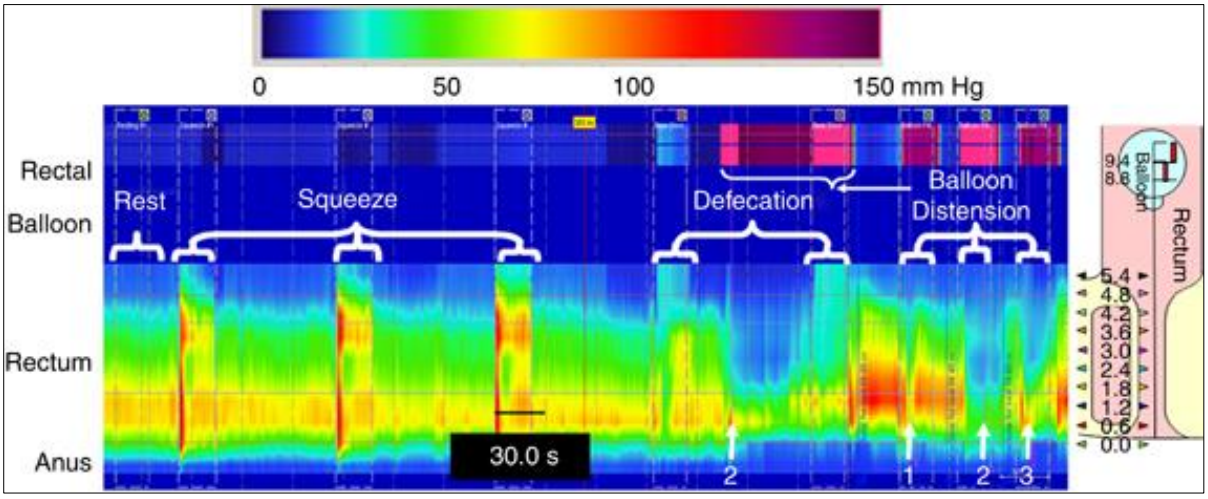


Figure 1.10. HRM trace in asymptomatic patient demonstrating changes at rest, squeeze and defecation. Reproduced with permission from Nature publishing group [239].

Despite the fact that HRAM technology provides an improved manometric and topographic analysis, there is much work to be done before it helps unravel the complexities of the anorectal disorders.

1.2.4.2.4 Endolumenal Functional Lumen Imaging Probe (EndoFLIP)

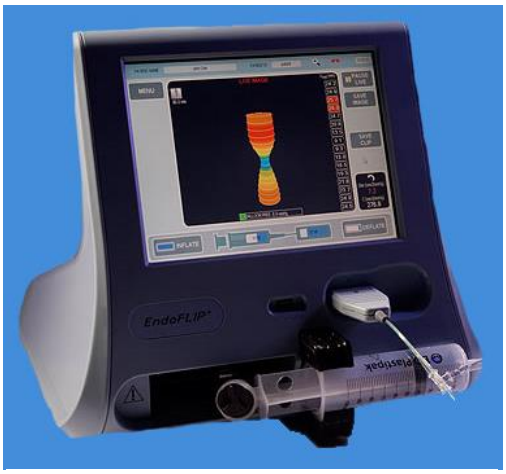


Figure 1.11. EndoFLIP device

EndoFLIP is the most contemporary technology investigating luminal physiology. As with HRAM, EndoFLIP was initially designed to study the gastro-oesophageal junction and later extended to anorectal studies [240]. It is based on the impedance planimetry technique [241,242] which measures the relationship of cross-sectional area (CSA) and pressure during constant volume distention in the GI lumen. Previously, it has been suggested that the measurement of radial force through resistance to distention, ie, distensibility, rather than pressure alone, should be used as a prime determinant of sphincteric strength [243]. By applying the law of Laplace, EndoFLIP can be used to determine the distensibility (wall tension) from the CSA and pressure data.

The EndoFLIP unit consists of a live image display screen, a data recorder and a motorised insufflator. The securing clasps on the front of the unit hold a syringe in place which is connected to the balloon catheter. The automatic insufflator, activated from the touch screen display, moves the syringe thereby inflating the balloon of the measuring probe. The probe's electrical connector is inserted into the slot at the front of the unit which allows for the data to be transferred to the unit for displaying on the screen and for recording.

a. EndoFLIP in upper gut studies

Upper GI Imaging

EndoFLIP allows assessment of mechanical competence of the gastroesophageal junction (GEJ). With the aid of a distensibility graph, it provides information on how wide the GEJ opens for a given distension pressure. This information is particularly useful in patients suffering from gastro-oesophageal reflux disease (GORD). In case of GEJ, opening easily at low distension pressure, i.e. being patulous, the patient with GORD symptoms is likely to benefit from the fundoplication surgery, which reduces the distensibility of the GEJ. However, if the GEJ is found to be very tight, fundoplication surgery would require further consideration [244,245].

Upper GI Surgery

Real-time measurements by EndoFLIP can allow a surgeon to measure the GEJ during laparoscopic fundoplication surgery, both after crural repair and during and after the fundoplication wrap procedure. EndoFLIP also allows for measurement of small hiatal hernias pre and post repair. During Heller Myotomy for achalasia, it helps assess whether sufficient muscle has been cut and the desired distensibility of the GEJ has been achieved. In addition, it has also been used as a tool to measure oesophageal lumen size and thus aid in oesophageal stent sizing or dilatation. [244-246]

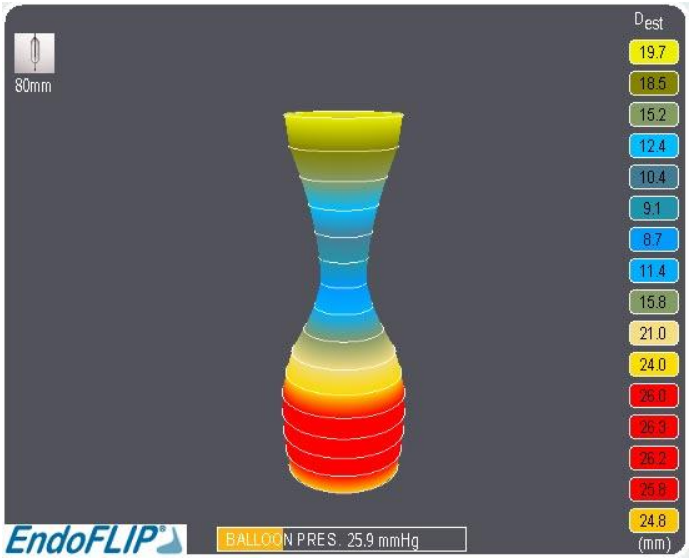


Figure 1.12. Snapshot of EndoFLIP screen after fundoplication. Diameter of points across lumen shown on right side of screen and balloon pressure at bottom.

Bariatric Surgery

During gastric band surgery, EndoFLIP allows the surgeon to set a consistent band stoma diameter thereby minimizing the risk of the band being too tight. It also helps in assessing whether sufficient peri-gastric fat has been removed to create an adequate stoma size [247-249]. Additionally, stoma size during Roux-en-Y gastric bypass has been shown to affect weight loss post-surgery. EndoFLIP allows for measuring of the stoma size in order to achieve the optimal outcome [250,251].

EndoFLIP can also be used to measure sleeves created during gastric plication procedure since the diameter of the sleeve can be observed changing in real time as successive plications are performed [252-254].

b. Applying EndoFLIP to the Anorectum

The EndoFLIP has been used in anorectal studies in the recent years. The potential of EndoFLIP to overcome the limitations of conventional manometry in the anorectum are similar to those described in Upper GI imaging using EndoFLIP. Real-time display of the whole anorectum allows disorders of co-ordinated defecation to be more readily identified. There is no requirement for movement of the catheter. This may reduce involuntary recruitment of external sphincter fibres during resting pressure measurement. It also reduces the number of squeezes required and has the potential for reducing patient discomfort.

However, the key advantage of EndoFLIP lies in its ability to provide information about the distensibility of sphincter besides displaying the real-time images. This major difference between EndoFLIP and ARM allows the former to determine the opening pressures of the anal canal or resistance to distension offered by anal canal while the former evaluates the anal canal on the basis of pressure generated in its closed state. This information about opening pressures is quite significant in patients with FI, especially those who have normal pressures on ARM.

Luft et al. used EndoFLIP to evaluate the biomechanical properties of the anal canal in their study on fifteen healthy volunteers. They divided the anal canal into three anatomical segments, upper anal canal (surrounded by the IAS and the puborectalis), middle anal canal (surrounded by the IAS and the EAS) and the lower anal canal (surrounded by the EAS). With the help of a 12 cm long probe they were able to obtain 16 CSA points. They reported that the biomechanical properties of the anal canal are not uniform with the mid anal canal was significantly less distensible than the upper ($p < 0.01$) and the lower anal canal ($p < 0.05$) [18]. A separate study by the same group looking at fourteen patients with systemic sclerosis found

that the mid anal canal in this patient group was easily distensible. They concluded that this was due to fibrotic degeneration of the IAS secondary to systemic sclerosis which caused it to become easily distensible rather than leading to a stiff, poor compliant anal canal [255]. Alqudah et al. studied distensibility of the anal canal in 20 healthy volunteers using EndoFLIP and found that the opening pressure for females was twice that found in males (11 mmHg vs 5 mmHg; $p < 0.001$). Opening pressure was defined as the pressure at which the CSA started to increase.

Luft et al. state that the balloon diameters used in the above-mentioned studies were much smaller than those found necessary to elicit the rectoanal inhibitory reflex in an earlier study from the same centre. This previous study primarily compared rectal wall properties in acute and chronic spinal cord injury patients with those of healthy volunteers by means of rectal impedance planimetry [256]. It also aimed to obtain additional information about the rectoanal inhibitory reflex in these groups. However, in the methodology section of their previous study, only the distension pressures which activated the RAIR are mentioned with no reference to the balloon diameter used, thereby making it difficult to comment upon. Moreover, the probe used in the two studies are not directly comparable, as the earlier study used a smaller intrarectal balloon whereas the later studies used the longer 12 cm balloon with its distal end placed in the rectum, mid part in the anal canal and proximal end outside the anal verge.

It is our understanding that the foremost purpose of developing the impedance planimetry technique for anorectal studies is to obtain a more accurate assessment of the anal sphincters. However, inadvertent activation of RAIR in the aforementioned studies, due to the use of a longer probe lying in the rectum, cannot be excluded. RAIR reflex, consists of relaxation of IAS in response to rectal distension [213]. Manometric studies, using latex balloon have shown that although the balloon inflation volume, required to achieve the highest amount of pressure drop due to activation of RAIR can be between 30-40 ml, first detectable drop in pressure occurs at even a small inflation volume of 10 ml [214,257]. Although not directly comparable to the EndoFLIP catheters used by Luft et al. it is clearly evident that the distension of rectal part of the EndoFLIP catheters would have led to some degree of RAIR activation, thereby altering the results obtained. Moreover, there is no doubt that the distended probe coming out of anal canal would have also altered the biomechanical properties in the distal-most anal canal. An earlier in-vitro study using the impedance planimetry technique reported that the CSA measurements were not significantly affected if the excitation pair of electrodes was within the area being measured [242]. However, this was not possible in the studies by Luft et al. and Alqudah et al. as the probe was considerably longer than the anal canal being measured with the excitatory electrodes positioned in the rectum and outside the anal verge.

Ironically, the use of this long catheter defies the principal purpose of EndoFLIP, i.e. to achieve more reliable and accurate anal canal assessment. We believe that the use of this longer oesophageal probe is a major limitation of these studies and to overcome these limitations we developed a custom made intra-anal probe which is described in the next chapter.

1.2.5 Treatment

The rapid advancement in our understanding of physiology and pathology of IAS has resulted in the development of several new management options. These treatment options vary according to the IAS pathology and thus can be categorised into low and high-pressure management options respectively.

1.2.5.1 Treatment options for low pressure

The initial management starts with lifestyle modification, medications and coping strategies. If these steps are not sufficient to reduce the symptom severity, further treatment is chosen according to the cause of IAS dysfunction. The treatment can be classified into two main groups: Structural for morphologically damaged IAS and Physiological for morphologically intact but functionally weak IAS.

1.2.5.1.1 Initial management

a. Lifestyle modification

This is considered to be the first step in managing FI starting with the dietary advice in order to promote an ideal stool consistency and predictable bowel movements. In addition to the dietary advise patients should also be encouraged to develop good bowel habits, including adopting a good toilet posture, avoid straining and emptying bowels after a meal to utilise the gastrocolic response [258].

b. Medications

Anti-diarrhoeal medications such as loperamide can be offered to patients with FI once other causes of diarrhoea (such as bowel ca, laxative abuse, dietary factors and medications etc) have been excluded. Patients who cannot tolerate loperamide can be offered codeine phosphate or co-phenotrope.

c. Coping Strategies

NICE guidelines recommend that patients should be made aware of coping strategies as a part of their initial management. This includes providing information about continence products such as disposable pads, anal plugs, odour control products and disposable gloves. Patients should also be provided information about where to get emotional and psychological support in the form of counselling or psychological therapy where appropriate.

1.2.5.1.2 Physiological management

a. Biofeedback

Described originally as an “operant conditioning therapy” by Engel et al. in 1974 [259] biofeedback has become an important tool in the management of faecal incontinence. In its present state, it has been described as “complex combination of patient-therapist interaction, patient education and formal or informal advice on a range of related issues” [260]. Different centres use varying techniques, but the three main modalities are - rectal sensitivity training to enable the patient to detect small volumes of stool in order to prevent leakage, strength training to enhance squeeze strength and endurance and co-ordination training to teach the patient to squeeze external sphincter on activation of RAIR reflex. Allied to this is the aspect of lifestyle modification including dietary optimisation, advice on the use of antidiarrhoeals and habit training. Although different studies have measured varying outcomes measures, the mean success rate has been reported as ranging from 40-100% in the treatment of FI [261-263].

b. Topical therapy

The clinical trials using topical therapies for improving sphincter function gained popularity with our increased understanding of anal sphincter physiology. Several different topical ointments have been studied in the last two decades, phenylephrine being the commonest one.

Phenylephrine gel, an α1-adrenergic agonist has been tried, based on its properties of producing IAS contraction. Although an increase in maximum resting anal pressure was noted [264,265] not all the studies demonstrated an improvement in incontinence score. Carapeti *et al*. found an improvement in continence in phenylephrine group as compared to placebo [264] whereas Parks *et al.* did not find a significant improvement in faecal incontinence [266]. Topical oestrogen and Zinc aluminium ointment have also been used in the treatment of FI as detailed in Table 1.3. A recent trial of the novel agent, methoxamine hydrochloride, a selective α1 adrenoreceptor agonist, in a form of a suppository found that it increased the IAS

contractions [57]. However, phase II trial failed to demonstrate a significant improvement with different dosing regimens [267].

As evident from the trials, there is no effective topical therapy that exists so far for managing FI. Phenylephrine has been the most promising therapy but is required in high concentrations to be effective. However, due to its potential cardiac side effects, it has failed to gain widespread popularity. One possible reason for the failure of topical therapeutic agents is that a damaged and scarred anal sphincter will not respond to these pharmacological agents as a healthy sphincter would. The search for an effective topical agent is ongoing and one can only hope that future studies will find a pharmacological agent which will not only be effective in managing FI symptoms but will also have a low-risk profile.

Table 1.3. Effects of pharmacological agents on sphincter pressures and FI epsiodes; PNLM = pudendal nerve latency measurement, ARM = anorectal manometry

Author	Agent	Type of study	Patients analysed (n)	Follow up	FI epsiodes	Resting Pressure	Significant points
Giulio A [268] (2000)	Amitryptiline 20 mg nocturnal	Open label case study	18 patients with idiopathic FI	4 weeks	Significant improvement in incontinence score (p=<0.001)	⟺	1st clinical study using amitryptilline, Non randomised and open labelled, hence carries a potential of expectation bias. Only patients with functionally and morphologically intact sphincters included
Read M [269]	Loperamide	Double-blind cross-over trial	26 with chronic diarrhoea, FI and urgency	1 week	Significant reduction in number of FI episodes (p=<0.01)	⬆	1st study to establish effects of loperamide on anal sphincters. Non case controlled study
Carapeti EA [270] (1999)	Phenylephrine gel varying doses	Case study	12 healthy volunteers	None	Not mentioned	⬆	Study only on healthy volunteers with non-diseased sphincters
Carapeti EA [264] (2000)	Phenylephrine gel 10% bd	Double-blind random order placebo-controlled crossover study	36 Passive FI patients, intact sphincters on eaus	8 weeks	No change	⟺	

Carapeti EA [271]	Phenylephrine gel 10%	RCT	12 patients with ileoanal pouch , intact sphincters	8 weeks	Improvement in Wexner FI score (15 to 9, p=0.01)	↑	Study only included patients with ileo anal pouch so its debatable whether these findings could be extended to those with idiopathic FI. Only patients with normal pressures (on ARM), PNLM measurement and pouch sensitivity included . Low statistical power due to small sample size
Cheetham MJ [265] (2001)	Phenylephrine gel 0%, 10%, 20%, 30%, and 40%	RCT	10 Passive FI patients, intact sphincters on EAUS	Not mentioned	Not studied	↑	Significant perianal burning noted at higher concentrations of 30 & 40%. Concerns of systemic effect at high concentration. Low statistical power due to small sample size Long term results unknown

Simpson JAD [272] (2014)	Methoxamine suppository 1,2,5 & 10 mg	Randomised, double-blind, three-way crossover study	24 healthy volunteers	12 weeks	Not studied	↑	Trial funded by pharmaceutical company. Duration of maximum MRP effect were greater for NRL001 than placebo at all doses and increased with dose.
Pinedo G [273] (2012)	Zinc–aluminium based topical ointment	RCT	44 (24 in treatment arm and 20 in placebo arm)	4 weeks	Improvement in Wexner FI score (16.6 to 8.5, p=<0.001)	Not studied	Improvement in wexner scores in both arms. Improvement in QOL seen only in treatment arm
Pinedo G [274] (2009)	Topical oestrogen	RCT	36 (18 patients in each arm)	6 weeks	Improvement in Wexner FI score (11 to 7, p=0.002)	Not studied	Improvement seen in both arms for wexner scores none for qol

c. Neuromodulation

Sacral Nerve Stimulation (SNS)

SNS involves percutaneous placement of an electrode, connected to a pulse generator, against one of the sacral nerve roots (usually S3). It was initially developed to treat urinary disorders [275] and has been shown to be an effective therapy for FI. Several studies have proven its efficacy in managing faecal incontinence [276-279] and as a result, it has been incorporated in the FI management algorithm [280,281].

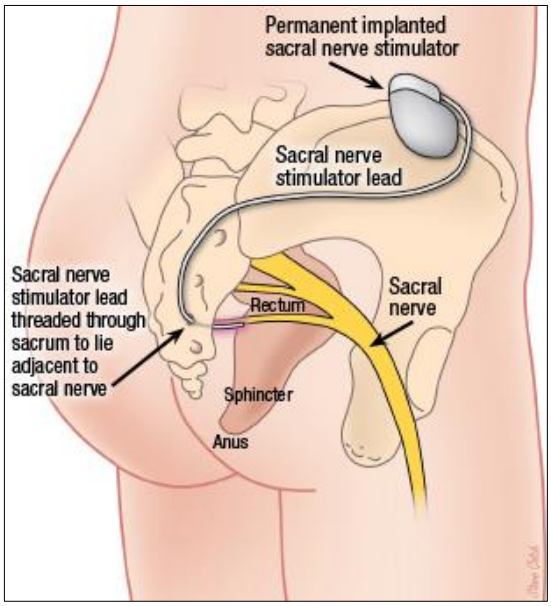


Figure 1.13. Schematic of sacral nerve stimulator in situ

The mechanism of action of sacral nerve stimulation is likely related to a range of physiological changes. It is hypothesised that stimulation of afferent fibres leads to altered anal and rectal sensation and modification of reflex pathways at the spinal cord level. Improvement in rectal evacuation may also be a contributing factor, and reduced cortico-anal activity has also been described [27]. With regard to the effect of SNS on IAS, there is no real consensus with some studies showing an increase in resting pressure and others reporting no effect on the IAS tone, as detailed in Table 1.4. Despite this conundrum, the fact remains that SNS is effective in managing FI in patients with either functional or structural weakness of IAS or a combination of both. A double-blind crossover study reported a reduction of passive soiling and a decrease in FI episodes in the patients with IAS dysfunction [282]. Similar results were reported for patients with scleroderma, having IAS dysfunction, who had a decrease in weekly episodes of incontinence with SNS use [283]. SNS has also been reported beneficial in patients with structurally weak IAS [284,285]. A study from St Mark's reported a decrease in faecal incontinence episodes per week from a mean (SD) of 9.9 (10.9) to 1.0 (2.4) ($p = 0.031$), and decrease in soiling episodes per week from 6.1 (1.6) to 1.7 (2.4) ($p = 0.031$), with use of SNS for patients with IAS disruption [286]. Another study concluded that SNS was an effective treatment in patients with FI who have anal sphincter defects, and the outcome was not associated with severity of sphincter disruption [285].

Percutaneous Tibial Nerve Stimulation (PTNS)

Percutaneous Tibial Nerve Stimulation (PTNS) initially described for managing urinary incontinence [287] involves stimulating the afferent and efferent fibres of the tibial nerve via a fine gauge needle inserted percutaneously just above and medial to ankle. It obviates the need for a permanently implanted sacral nerve stimulator thereby reducing the associated costs and risk of morbidities associated with SNS device.

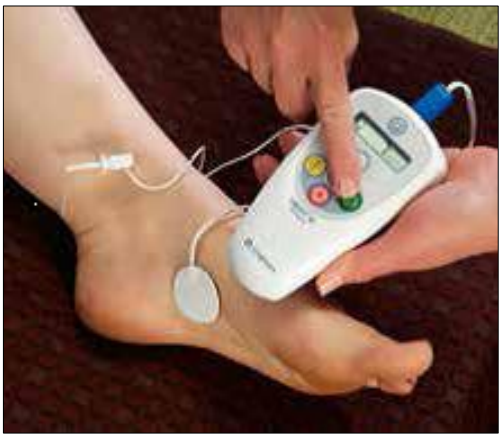


Figure 1.14. PTNS device in use

Mechanism of action of PTNS is not completely understood. It has been postulated to affect the IAS tone but the manometric data looking at anal canal pressure shows quite a bit of variation amongst studies, as shown in Table 5. However, there is no doubt that the PTNS has effect beyond the sphincters and an increase in the rectal compliance, inhibition of colorectal motility and changes in the rectal sensory thresholds for defecation have all been postulated. The supra sphincteric mechanism of action of PTNS was also confirmed by a study from our centre which found an improvement in FI episodes per week, pre & post -PTNS, across different sphincteric states (including Intact sphincters, EAS deficit, IAS deficit, Both sphincters damaged) [288]. Similar results were also seen in a study by Hotouras et al. who found an improvement in FI episodes in the patients with damaged IAS [289].

The efficacy of PTNS in managing FI has been reported by several authors. At the longest reported follow-up of 12 months, the success of PTNS was found to be 59% [290]. An RCT comparing PTNS to SNS concluded that both techniques provide clinical benefit to patients with FI [291]. However, a recent large double-blind randomised control trial (CONFIDeNT trial) having 227 patients has raised questions about the efficacy of PTNS [292]. Their primary outcome of the proportion of patients achieving ≥ 50% reduction in weekly faecal incontinence episodes was similar in both arms: 38% in the PTNS arm compared with 31% in the sham arm [odds ratio 1.28, 95% CI 0.72 to 2.28; p = 0.396].

Table 1.4. Effect of neuromodulation on anal sphincter pressures.

Author	Year	Patients (n)	Resting Pressure	Squeeze Pressure
Sacral Nerve Stimulation Studies				
Leroi AM [293]	2001	6	⟺	↑
Rosen HR [294]	2001	20	↑	↑
Kenefick NJ [295]	2002	15	↑	↑
Uludag O [296]	2002	38	⟺	⟺
Matzel KE [297]	2003	16	↑	↑
Uludag O [298]	2004	75	⟺	⟺
Sheldon R [299]	2005	10	⟺	⟺
Michelson H [300]	2006	29	↑	⟺
Melenhorst J [277]	2007	100	⟺	↑
Holzer B [278]	2007	29	↑	↑
Munoz-Duyoz A [301]	2008	47	⟺	⟺
Jarret ME [302]	2008	8	⟺	⟺
Moya P [303]	2014	50	⟺	⟺
Madbouly KM [304]	2015	24	↑	↑
Percutaneous Tibial Nerve Stimulation Studies				
López-Delgado et al. [305]	2014	24	↑	↑
George et al.[306]	2013	30	⟺	↑
Moreira et al.[307]	2013	10	⟺	⟺
Boyle et al.[308]	2010	31	⟺	⟺
de la Portilla et al.[309]	2009	16	⟺	↑

Opinion on Neuromodulation

The emergence of neuromodulation techniques in the management of faecal incontinence has helped bridge the gap between medical management and major surgery. The minimally invasive SNS and even less invasive nature of PTNS in addition to the low-risk profile makes these options attractive from a patient's and clinician's perspective.

The evidence base for the effectiveness of SNS comprises of randomised control studies and other robust studies with long-term follow-ups. The RCT's have helped provide some answers and clarifying usefulness of SNS in managing FI. A comparison of SNS to placebo reported a significant improvement in both symptoms and QOL scores and anal physiology with active treatment [310]. Another RCT comparing SNS with supervised optimal medical therapy (including bulking agents, pelvic floor exercises, or lifestyle and dietary manipulations, but not biofeedback) demonstrated the superiority of SNS over these treatment options [311]. Apart from the RCT's, several clinical studies involving SNS have been conducted worldwide. However, it is a challenge to assess these studies due to a huge variation in regard to the inclusion criteria, outcome measures and follow-up intervals. Despite this difficulty in assessing these studies, one can still conclude that SNS is an effective technique in managing FI, based on the fact that majority of the studies report a favourable outcome with minimal side effects. Further evidence supporting the use of SNS in managing FI comes from its economic evaluation which demonstrated SNS to be a cost-effective treatment in the NHS [312].

The PTNS, on the other hand, is a newer technique which has gained popularity due to its USP of being a clinic-based treatment. It does not have a robust evidence base as SNS and although level 3 & level 4 studies have demonstrated its effectiveness (50% reduction in FI episodes per week) to be around 71% (range 63–82), this success has not been replicated in the RCTs. An RCT comparing PTNS to SNS found that PTNS was successful only in 47% of patients. Another RCT comparing it to sham had even more disappointing results showing PTNS to be no better than placebo in managing FI. Despite these dismissal results, it is difficult to write off PTNS completely. One has to take into account the fact that the primary outcome measure of success in both these RCT was ≥ 50% reduction in weekly faecal incontinence episodes. It can be argued that this is not necessarily the best outcome measure for all the patients. For instance, a patient with less than 50% reduction in FI episodes may find the improvement sufficient enough to allow him/her a better social functioning whereas someone with severe incontinence even despite obtaining a 'successful' reduction in incontinence episodes may continue experiencing impaired quality of life. Future research is still warranted for PTNS, possibly with bigger sample size, long-term follow-up and perhaps focussing on different subgroups of FI patients to achieve maximal use of this neuromodulation technique.

1.2.5.1.3 Structural management

a. Surgical repair

Direct surgical repair of IAS has been attempted in the past but, failed to achieve a successful outcome. The small size of IAS, which is normally only 2.4-3.4 mm thick [30] makes it difficult for sutures to hold and makes it difficult to achieve satisfactory healing. Two studies looking at patients post repair did not find a significant improvement in postoperative manometric findings. Moreover, sonography revealed a persistent defect in few patients despite repair and the patients failed to achieve full continence [313,314]. One case series looked at 15 patients who underwent island anoplasty, which involved filling the IAS defect with skin and subcutaneous fat. It reported an improvement in continence score at a median follow-up of 34 months, but at the cost of high rate of wound infection (23%) [315]. Another more complex surgical intervention, dynamic graciloplasty has a potential to achieve good results but is associated with a high complication and failure rate [316].

b. Augmentation techniques

After successful use of bulking agents by urologists to improve bladder neck closure, these were introduced in the treatment of FI due to IAS dysfunction [317]. Although their mechanism of action remains unclear, it has been postulated that these agents work due to the mechanical effect of filling a defect or by raising anal cushions thereby causing closure of anal canal [318]. Several studies have been done using various injectable agents. A summary of these studies is presented in Table 1.5.

Although this technique has demonstrated varying levels of success, apart from few randomised controlled trials of the newer agents like Solesta, Bulkamid and Permacol, most of the evidence base consists of a limited number of case series. A Cochrane review on injectable bulking agents concluded that the number of trials in this field was limited and most of the studies had methodological weaknesses [319]. Moreover, there is a substantial variation in terms of the procedure itself, for instance, the use of ultrasound, the volume of bulking agent used, the number of sites injected etc. Another limitation of note is that there is no long-term follow-up data thereby making it difficult to predict the long-term effectiveness.

Table 1.5: Outcomes of augmentation techniques

Studies	Type of study	n	Bulking agent	Follow up in months	Significant Results	Complications
Mellgren et al.	RCT	136	Dextranomer microspheres in nonanimal stabilized hyaluronic acid (NASHA Dx) vs Sham	36	Significant reduction in mean Cleveland Clinic Faecal Incontinen score (CCFIS) from 14 at baseline to 11 at 36 months (p<.001) A 50% reduction in faecal incontinence episodes achieved by 52% at 6, 57% at 12, and 52% by 36 months	0-6 months: Proctalgia (14%), Pyrexia (8.1%), Diarrhoea (4.4%), Rectal haemorrhage (6.6%), Pain (4.4%) >6 -36 months: Proctalgia (2.3%), Diarrhoea (1.5%), Rectal haemorrhage (1.5%), Injection site nodule (2.3%)
Dehli et al.	RCT	126	NASHA Dx vs Biofeedback	24	Significant reduction in St Mark's score from 12.9 [95% CI, 11.8-14.0] to 8.3 [95% CI, 6.7-9.8] for NASHA Dx and 12.6 [95% CI, 11.4-13.8] to 7.2 [95% CI, 7.2-8.8] for Biofeedback group	Infection, pain and prolonged defecation (in 3 patients), product leakage (7 patients) in NASHA Dx group
Maleskar et al.	Case series	100	Collagen	36	Significant reduction in CCFIS from median of 14 (9–18) at baseline to 8 (5–14) at 36 mths. A total of 68% reported subjective improvement in symptoms.	38% required a repeat injection at a median of 12 mths (4–16) from 1st injection. Another 15% required a further additional injection at a median of 18 mths (14–20)
Graf et al.	RCT	206	NASHA Dx vs Sham	12	>50% reduction in FI episodes for patients in NASHA Dx group as compared to sham group at 6 months 52% vs 31% (OR: 2.4; 95% CI, 1.2-4.5) Significant increase in mean number of incontinence-free days in NASHA Dx group vs. sham group at month 6 (3.1 vs. 1.7, respectively; P=0.0156)	Proctalgia (14%), fever (8%), and rectal haemorrhage (7%)
Maeda et al	RCT	10	Bulkamid vs Permacol	6	No sustained improvement beyond 6 weeks as measured by Patients' clinical self-assessment, Anorectal physiological testing, Bowel diary or Incont scores	Not reported
Siproudhis et al	RCT	44	Polydimethyl-siloxane (PDMS) vs. saline	3	No significant difference in successful treatment in two groups (23% vs. 27%, respectively, P = 0.73)	18 AEs in PDMS group (including pain, anal inflammation or bleeding) vs. 4 in control group

Tjandra et al.	RCT	82	PTP injection (silicone) with (Group A) & without (Group B) ultrasound guidance	6	At 3 months Group A patients achieved >50% improvement in Wexner's continence score as compared to Group B (69 % vs. 40 %; P = 0.014). 93% of Group A and 92% of Group B had >50% improvement in global quality of life scores (visual analog scale)	Minor discomfort at anal injection site
Davis et al.	Case series	18	Carbon coated beads	28.5	No significant difference up to 6 months. At 12 months, significant improvement noted in the continence grading (P=0.003) and patient satisfaction (P=0.053)	Mild anal discomfort, Pruritis ani, Passage of bulking agent
Malouf et al.	Case series	10	Ultrasound guided silicone injections	6	At 6 months 70% had no relief of symptoms. No significant change noted in anorectal manometry	Severe pain, Ulceration or infection requiring antibiotics, Leakage of bulking agent
Feretis et al.	Case series	6	Microballoons made of silicone	8.6	Improvement in mean incontinence score from 16.16 (15-18) to 5 (4-7); (P =0.027)	No significant AEs or serious post-implantation complications
Kumar et al.	Case series	17	Collagen	8	65% showed marked symptomatic improvement. 12% reported minimal and 18% reported no improvement	Nil reported
Shafik et al.	Case series	11	Polytetrafluoro-ethylene	22	63% improvement in Wexner score post injection	Mild pain
Shafik et al.	Case series	14	Autologous Fat	6	85% improvement in Wexner score post injection	Mild pain

c. Neosphincters

i. Graciloplasty

This involves perianal musculoplasty using the gracilis muscle which is mobilised from the upper thigh and fixed to the contralateral ischial tuberosity after encircling the anus completely. Gracilis muscle is naturally composed of fast twitch type II muscle fibres that are easily fatigable. As a result, initial trials resulted in a poor outcome with deterioration in patient's control of continence over time due to muscle fatigue. However, in the early 1980s researchers demonstrated that application of low-frequency electrical stimulation to fast twitch type II muscle fibres could, over time, transform them to slow twitching type I fibres. This approach was applied by Baeten et al. to the graciloplasty and with the help of other studies confirming improved outcomes with the application of neurostimulation, stimulated graciloplasty (dynamic) became more popular than its unstimulated variant [320]. The effectiveness of dynamic graciloplasty in restoring continence has been shown to range between 60 to 80% at follow-ups ranging from 1 to 5 years [321-323].

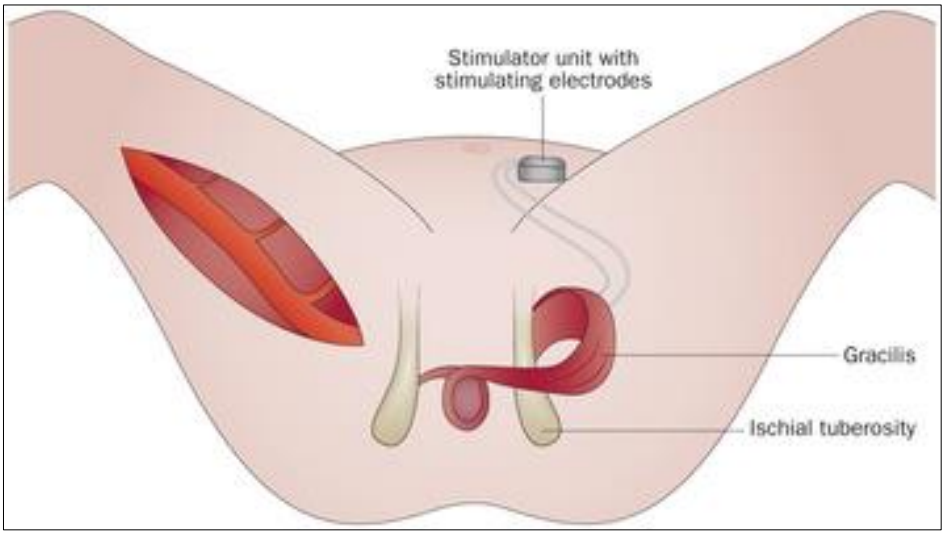


Figure 1.15. Dynamic graciloplasty; Reproduced with permission from Nature Publishing Group [1]

The main critique of this approach is its complexity, relatively high rates of complications and surgical revisions associated with it which have been reported as high as 74% respectively [324,325]. The frequent complications include infection, ischemia of the transplanted muscle, ulceration of the anal canal due to excessive tension, avulsion of the tendon fixation at the ischial tuberosity, failure of muscle stimulation due to electrode breakage or disinsertion. Apart from this, functional failure due to continuing incontinence or anal retention renders this technique less appealing. For these reasons, graciloplasty is infrequently performed at this time [326].

ii. Artificial anal sphincters

Artificial sphincter was originally developed in 1972 to treat urinary incontinence and underwent considerable technical evolution before AMS 800 device was launched in 1987. Christensen *et al.* used this as the first artificial anal sphincter in a man with severe anal incontinence. An artificial anal sphincter specifically designed for managing FI was introduced in 1996 and since then has gained considerable interest around the world. A recent meta-analysis looking at 541 patients, implanted with an artificial anal sphincter, across 21 studies reported an improvement in incontinence rate ranging between 50 – 65% in addition to a substantial increase in the resting pressure [327]. Another significant improvement was in QOL score, which was observed across all the studies.

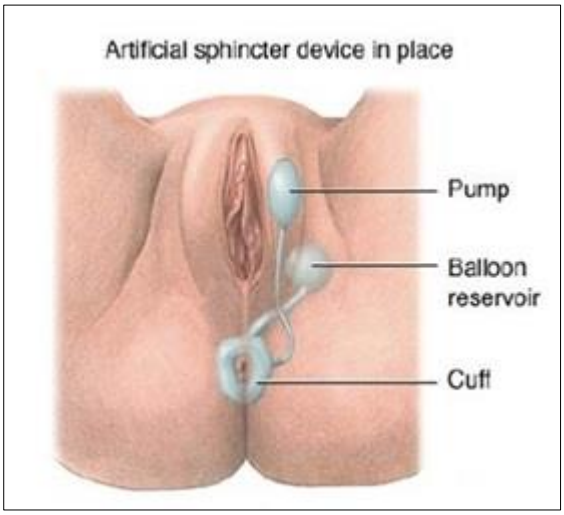


Figure 1.16. Artificial bowel sphincter

Despite the encouraging results, only a relatively small number of these devices are implanted each year owing to the associated complications and poor long-term results. Surgical revision is common with revision rates increasing with time and reaching above 90% by 5th year [327]. An interesting finding was despite an increase in the rate of surgical revision and decrease in the continence with time, the QOL of patients remained high. The commonest causes for revision were device malfunction (36%), erosion (29%) and infection (28%). Evacuatory difficulty was noted to be one of the significant problems amongst studies and it was suggested that there should be a preoperative assessment for outlet obstruction in addition to liberal use of laxatives or enemas postoperatively if required [327].

The implantation rate for artificial sphincters has fallen since the commercialization of sacral nerve stimulation (SNS) which is now an accepted treatment for patients with no sphincter defect or with a defect of less than 180 degrees [328]. However, not every patient is suitable for SNS, as is the case with patients having extensive sphincter destruction, congenital malformation, or perineal colostomy, in which an artificial sphincter might be useful.

iii. Magnetic band

The magnetic anal sphincter is an implantable device consisting of a series of magnetic beads interlinked with titanium wire. Once implanted around the anal canal the magnetic beads normally stay closed, preventing episodes of incontinence. When the patient gets an urge to defecate they simply push in a normal defecatory manner and the force generated separates the beads allowing the stool to be evacuated.

The limited amount of studies that have been conducted looking at magnetic sphincter have demonstrated a significant reduction in incontinence score and an improvement in quality of life [329,330]. A study comparing it to artificial bowel sphincter found it equally effective in short term [331]. The reported complications include infection, anal pain, rectal bleeding and device separation. This technique still requires further evaluation to determine its long-term effectiveness and understand, in particular, whether fibrosis develops around the implant thereby limiting its effectiveness.

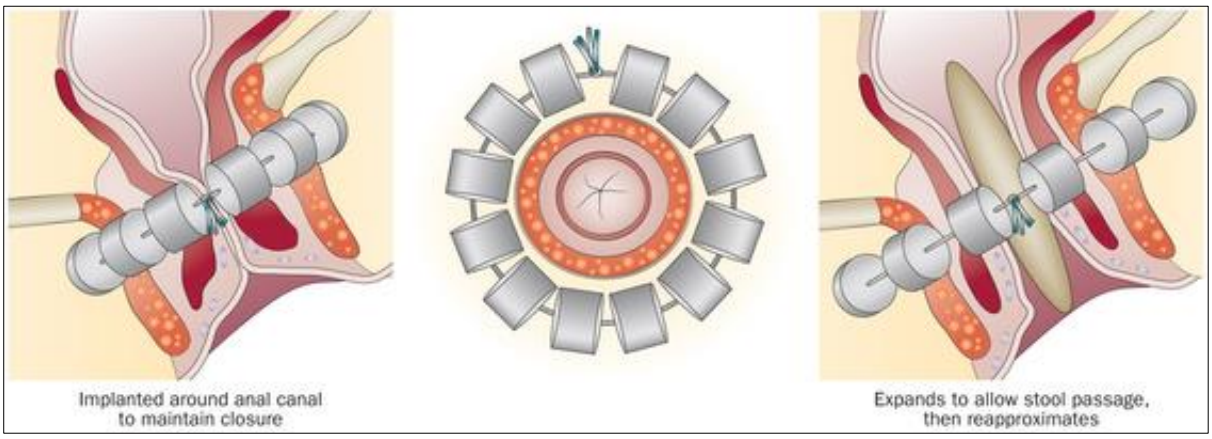


Figure 1.17. Magnetic band; Reproduced with permission from Nature Publishing Group [1]

d. Myoblasts

Myoblasts are primordial muscle cells having the potential of developing into a muscle fiber. Autologous myoblasts have been used successfully for treatment of stress urinary incontinence in the past. Frudinger *et al.* were the first to use this technique in GI setting. They injected autologous mesenchyme-derived myoblasts into the EAS of 10 female patients whose anal sphincters had been disrupted by obstetric trauma. They noted a significant and sustained improvement in the quality of life of the participants. However, the physiological changes of increased squeeze and resting pressure seen at 1 and 6 months had returned to baseline by 1 year [332].

This is a novel technique and abates the ethical debate associated with the use of embryonal cells. Moreover, the use of autologous cells would avoid any adverse effect to the foreign material. Although the short-term results from this first study were encouraging, further studies in a form of double blinded RCT with long-term follow-up will help clarify the effectiveness of autologous myoblasts in improving sphincter function.

e. Smooth muscle cells

Bioengineered human IAS reconstructs have been successfully developed from human IAS smooth muscle cells [333,334]. Rattan *et al.* were able to develop IAS reconstructs which demonstrated a much higher basal tone than seen in previous similar studies. Although the basal tone of these constructs was 1/20th of an intact IAS the authors speculated that this might increase after grafting. The reconstructs were shown to respond to different agonists and antagonists in a manner similar to the intact IAS [335].

f. SECCA

First used in Mexico in 1999, this involves administering temperature controlled radiofrequency energy to the anal canal. There is no definite consensus on its mechanism of action but several hypotheses have been put forward including neuromodulation of afferent nerve fibres, collagen deposition, fibrosis and modulation of interstitial Cajal cell function [336]. Current belief is that it's mechanism of action likely involves both structural and physiological elements.

Figure 1.18. SECCA device (on left) and in use in anal canal (on right).

A review looking at 10 studies of 220 patients concluded that SECCA improved continence for at least 6 months. Moreover, this improvement was sustained in the 39 patients that were followed up for 5 years. The complications included mucosal ulceration, minor rectal bleeding and anal pain all of which were self-limiting [336].

The main advantage of this technique is its minimally invasive nature and relatively low morbidity [258]. Although it has shown good outcomes in the short term, larger studies are required to identify the suitable subgroup of patients who will benefit from this technique. The follow-up also needs to be long enough to determine the efficacy of SECCA in longer term [337].

Table 1.6: Effect of SECCA on anal sphincter pressures.

Author	Year	Type of study / Level of evidence	Patients analysed n	Follow up (months)	Resting Pressure	Squeeze Pressure
Takahashi T [338]	2002	Single centre, Cohort study **Level 4**	10	12	⟺	⟺
Takahashi T [339]	2003	Single centre, Cohort study **Level 4**	10	24	⟺	⟺
Efron JE [340]	2003	Multicentre, Cohort study **Level 4**	50	6	⟺	⟺
Felt-Bersma RJ [341]	2007	Single centre, Cohort study **Level 4**	11	3	⟺	⟺
Lefebure B [342]	2008	Single centre, Cohort study **Level 4**	15	12	⟺	⟺
Kim DW [343]	2009	Single centre, Cohort study **Level 4**	8	6	⟺	⟺

1.2.5.2 Treatment options for high pressure

1.2.5.2.1 Topical therapy

Topical treatment has been used for high anal pressure conditions such as the anal fissures. The commonly used ointments are Nitroglycerine (GTN) and Diltiazem which reduce the anal canal pressure. A Cochrane review found them to have a successful outcome in 50% cases of anal fissures and being associated with much less adverse effects as compared to surgical treatment [344]. GTN has also been used to alleviate the post haemorrhoidectomy pain caused due to IAS spasm [345,346].

Another newer agent, sildenafil, which is a selective phosphodiesterase 5 (PDE5) inhibitor, has been shown to reduce the IAS tone during in vivo [347] and in vitro studies [348] (on human IAS). Tadalafil, another PDE5 inhibitor has also been reported as improving pain during defecation and healing of anal fissure in a small case series of 7 patients [349]. These agents are not currently the part of mainstream treatment options and further trials are awaited to study their long-term effectiveness.

1.2.5.2.2 Injectable agents

Botulinum toxin, a neurotoxin leads to temporary chemical denervation of motor end plates. When injected into the IAS it results in a reduction of resting anal pressure and an increase in local blood flow thereby promoting healing. Although earlier studies showed good initial healing (up to 80% of patients) at 6 months [350,351] later studies showed a fissure recurrence rate of 42% at 48 months follow-up [352]. A recent meta-analysis concluded that botox is equivalent to GTN in efficacy [344].

1.2.5.2.3 Surgery

A surgical procedure is usually reserved for the patient with chronic anal fissure which has failed to resolve with topical or injectable agents. Anal dilatation, which used to be one of the surgical options up to the late 20th century, has been largely abandoned due to high rates of incontinence associated with it. The procedure of choice is lateral sphincterotomy (LS), which involves division of internal sphincter to reduce the resting anal pressure. It has been shown to have better outcomes than topical agents or botox injection [353,354]. The main side-effects of LS are urgency, flatus incontinence and mild faecal soiling in early postoperative period. However, this immediate postoperative incontinence is rarely permanent and is usually mild.

CHAPTER 2

MATERIALS & METHODS

2.1. Research Proposal

This research project was developed to assess the utility of EndoFLIP as a diagnostic tool for investigating anal canal function and to evaluate if it overcomes the technical limitations of conventional manometry, while still yielding reproducible data. This project received the approval of NRES Committee London, Queen Square (Ref. 11/LO/1429) and Research & Development department at University College Hospital (Ref. 11/0373).

2.1.1 Hypothesis

1. Anal canal is a heterogeneous structure.

2. Dynamic testing of the anal canal using EndoFLIP will reveal this heterogeneity.

3. Anal canal of patients suffering with FI would differ significantly from those of healthy cohort in their biomechanical and geometric properties when subjected to EndoFLIP testing.

2.1.2 Aims & Objectives

Primary aim

1. Utilising EndoFLIP to evaluate heterogeneity of anal canal.

Secondary aims

1. Demonstrate repeatability and validity of EndoFLIP in assessment of anal canal function.

2. Obtain reference range data for EndoFLIP.

3. Ascertain the clinical utility of EndoFLIP.

2.2 EndoFLIP device

EndoFLIP has been used as a novel method to evaluate oesophageal physiology in health and disease. By providing the real time functional imaging of the oesophagus besides the data on luminal diameter, pressure and distensibility, EndoFLIP has helped evaluate oesophageal diseases such as GERD, achalasia, scleroderma and eosinophilic oesophagitis. The impedance planimetry technique, measuring the compliance and distensibility, revealed a higher distensibility of oesophagogastric junction in the GERD patients [245] and a lower distensibility in the achalasia and scleroderma patients [355,356], as compared to controls. The patients with eosinophilic oesophagitis (EO), have also been shown to have a significantly lower oesophageal distensibility, as compared to the controls, presumably due to the tissue remodelling and fibrosis in EO patients [357,358].

In addition to the diagnostic uses, EndoFLIP has also been found valuable during surgery. It has been shown to improve weight loss post gastric band and laparoscopic gastric plication by allowing adjustment of the gastric band stoma diameter [359] and gastric remnant volume [360] respectively. In achalasia patients, it has been shown to predict the symptomatic outcomes after Heller's myotomy or peroral oesophageal myotomy [361] and post pneumatic dilatation [362].

Despite being in existence for more than a decade and showing promising results in Upper GI studies, EndoFLIP's utility as a tool to investigate anorectal function has been extremely limited.

2.3 Impedance technology

EndoFLIP uses a technique called impedance planimetry that helps estimate the cross-section area of the conductive fluid contained in a cylindrical balloon. Impedance is defined as the resistance, a circuit offers to the flow of current when a voltage is applied. This resistance, when measured, provides us with a meaningful information in the context of the area it's being used. For instance, in the study of body composition, lean tissue, which is rich in water and electrolytes, offers minimal resistance to the flow of current whereas fat which is not water rich offers higher resistance. This difference in impedance offered by different tissues helps in studying the body composition [363].

The use of Impedance in GI studies has become well established over the years. The first use of impedance for determining the luminal diameter dates back to 1971 when Harris et al. used

it in a rabbit to measure the ureteral cross-sectional area (CSA) [364]. Gregersen et al. in 1991 used it to measure the cross-sectional area in the GI tract and coined the term impedance planimetry [365]. Impedance planimetry has subsequently been used in several studies to study the biomechanical properties of GI tract.

Impedance planimetry technique uses a probe containing an array of ring electrodes within a flexible cylindrical balloon. These ring electrodes are surrounded by a conductive fluid within the balloon which conforms to the shape of lumen it is placed in.

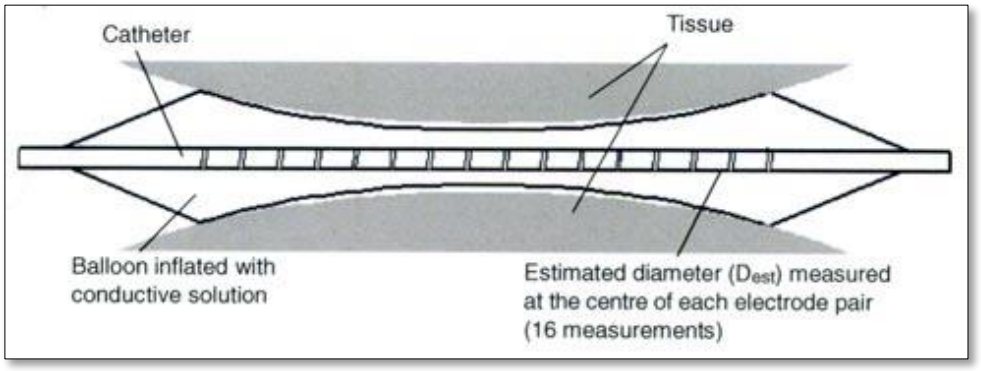


Figure 2.1: Principles of impedance planimetry; image courtesy Crospon

As the electrical current (I), conductivity of fluid in the balloon (σ and distance between the electrodes (d) stays constant, impedance (R) can be calculated using Ohm's law: V = I x R

V = I x R, where R can be expressed as $\mathrm{R} = \frac{d}{\sigma \text{ x CSA}}$

Thus $\mathrm{V} = I\ x\ \frac{d}{\sigma \text{ x CSA}}$

The above formula shows that V is inversely proportional to CSA, thus the CSA can be estimated based on the voltage reading.

EndoFLIP device, which deploys impedance planimetry technique, consists of a generator providing constant alternating current (100 mA at 5 KHz), an amplifier, an A-D converter and impedance detector. The shape of the balloon is reproduced on the screen thus providing information about geometric and biomechanical properties of the lumen in which the probe is placed. Simultaneously, the pressure within the balloon probe, representing the pressure in the lumen, measured using a solid state sensor is also displayed on the screen.

2.4 Development of probe for anorectal studies

Most of the initial work with EndoFLIP focussed on the Upper GI tract where it provided information on the functional (i.e., bolus transit) component of manometrically detected contractions. When combined with pH testing, impedance planimetry allowed for detection of gastroesophageal reflux independent of pH (i.e., both acid and non-acid reflux). As such the original probe was kept 12 cm long to facilitate its use in the oesophagus. All the published studies, using EndoFLIP in the anorectum have used this long probe. This longer probe meant that part of it laid in the rectum and on inflation of the balloon would have resulted in activation of rectoanal inhibition reflex (RAIR). RAIR reflex consists of relaxation of IAS in response to rectal distension [213]. This transient relaxation of IAS is well accepted to play a crucial part in maintaining continence [27]. Gang et al. showed that although the balloon inflation volume, required to achieve the highest amount of pressure drop due to activation of RAIR was 47mls, first detectable drop in pressure occurred at even a small inflation volume of 12 ml [257]. Therefore, inadvertent activation of RAIR in aforementioned studies, due to the use of longer probe lying in the rectum, cannot be excluded. Moreover, the distended probe coming out of the anal canal would have also altered the biomechanical properties recorded in the distal-most anal canal.

To overcome these limitations, I decided to modify the original probe to better suit the anorectal region. This was done by working together with the EndoFLIP manufacturer, Crospon, who were keen to develop such a probe thereby making EndoFLIP more marketable. The final anorectal probe was the result of a number of modifications to the original one, going through four different versions of the probe, each version developing on the previous version's flaws. The finished probe was unique as it incorporated two separate balloons, rectal and anal canal balloon with their respective inflation points. Moreover, the anal canal balloon was made in three separate lengths (2, 3 & 4 cm) corresponding to the varied functional length of the anal canal. The modification process along with the limitations of each probe is discussed below.

2.4.1 Probe V1

The foremost alteration that I suggested to Crospon, after initiation of this study, was reducing the balloon length from original 12 cm, thereby making the probe better suited for the anorectal region. A balloon length of 5 cm was initially believed to be appropriate and easily attainable according to the manufacturer. This balloon incorporated 11 electrodes, at a spacing of 3mm between them, thereby providing a total of 10 CSA measurements within the anal canal.

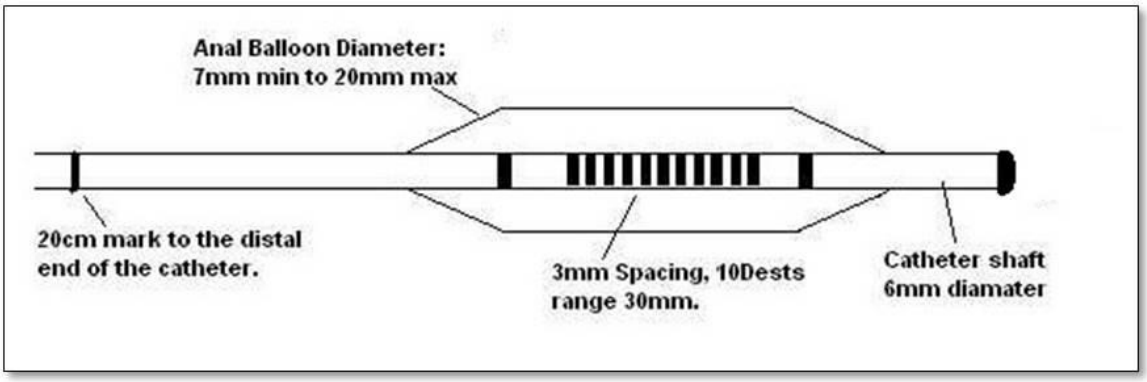


Figure 2.2: Anorectal probe V1

The limitation of this probe became obvious after a trial on patients. This probe required a separate catheter to be inserted into the rectum for initiation of the RAIR reflex. This was not only uncomfortable for the patients and logistically difficult but more importantly interfered with the anal canal measurements, acquired using the principles of impedance planimetry. To improve the accuracy of results further modifications to the probe were deemed essential. Another problem that I identified with V1 was that the 5 cm long anal canal balloon was not found to lie completely in the anal canal in all the subjects, owing to the variation of anal canal length in different individuals. In order to avoid inadvertent activation of RAIR reflex, which was the main purpose of reducing the balloon length in the first place, it was decided to get the anal canal balloon built in 3 different lengths. These lengths were chosen to be 2, 3 and 4 cm corresponding to the most commonly found functional length of the anal canal.

2.4.2 Probe V2

The idea of having two balloons in a single probe, without making the probe too bulky, was found to be feasible in discussions with Crospon. This additional balloon incorporated in the probe was a rectal balloon and had its own separate inflation point. All the other parameters were kept the same as in probe V1. Incorporation of rectal balloon allowed gaining valuable information about biomechanical properties of anal canal during RAIR without interfering with the accuracy of results.

Although the rational changes to V1 resulted in a probe better suited for anorectum, the V2 probe was not without its own flaws. Ironically, the catheter shaft, extending right up to the tip of the rectal balloon, specifically made rigid, to allow for an easy insertion was having an opposite effect. The rigid material made it almost impossible to manoeuvre the probe through the bend at the anorectal junction besides making it uncomfortable for the patient.

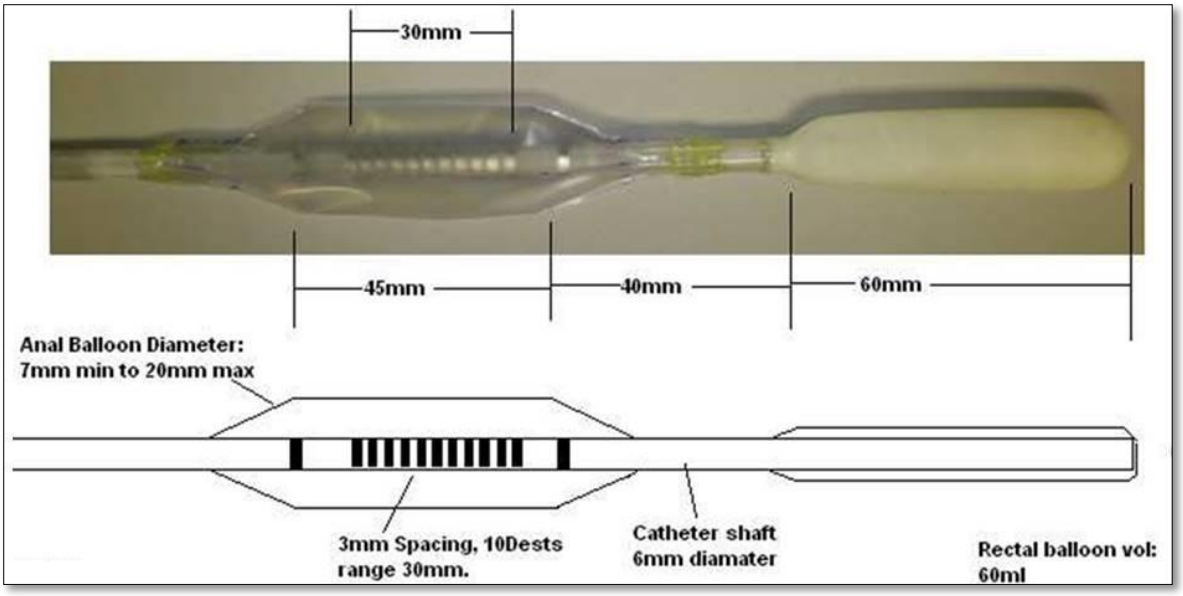


Figure 2.3: Anorectal probe V2

2.4.3 Probe V3

To overcome the limitations of V2, the manufacturers agreed to change the material of the catheter shaft to a softer and flexible alternative, the same as that of existing catheters provided by MMS for anorectal manometry.

This modification led to the development of probe V3 which had a much softer tip that did not traverse the full length of the rectal balloon, rather stopped midway. The patients were able to better tolerate the insertion.

2.4.4 Probe V4

Modifying probe V3 led to a probe that was superior in terms of patient comfort and was better suited to the anorectal region. However, further research into the impedance planimetry technique revealed that there was a possibility of improving the spatial resolution and accuracy of the probe even further.

To increase the spatial resolution, spacing in between two electrode rings in anal canal balloon was reduced from the existing 3 mm to 1 mm. This led to an increase in the number of points at which CSA was being recorded. Better accuracy was achieved by reducing the width of electrode rings from 3 mm to 1.5 mm. This final probe was near idle not only in terms of patient comfort but also for data recording. All the data collected in this current study has been recorded by this probe (Figure 2.4).

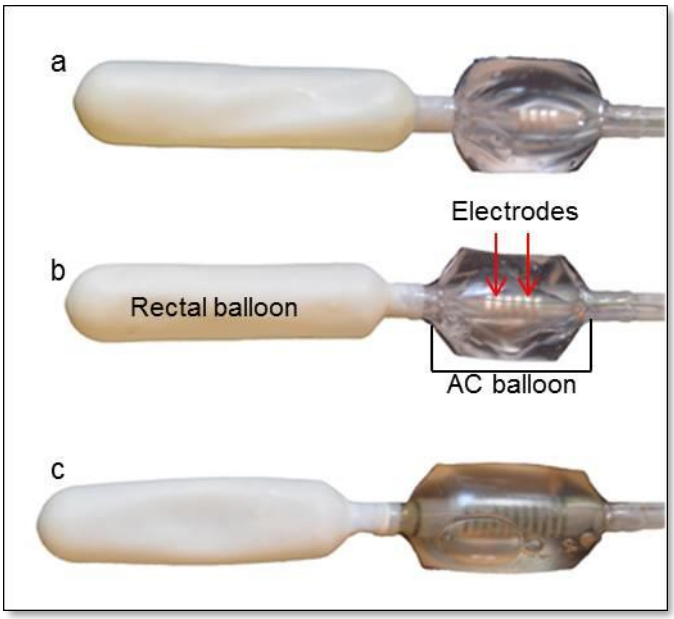


Figure 2.4: Different sized anorectal probes V4; AC: Anal canal

2.4 Repeatability and Validity

The repeatability of measurements refers to the variation in repeat measurements made on the same subject under identical conditions i.e. the same location; the same measurement procedure; the same observer; the same measuring instrument, used under the same conditions; and repetition over a short period of time. It is essential for a test to have high repeatability to be reliable, clinically useful and for comparing it with existing test modalities to determine gold standard.

The existing anorectal studies done to confirm the repeatability of a test modality have looked at parameters such as resting pressure, maximal squeeze pressure, RAIR, anal sensory thresholds and rectal volume thresholds. The table below (2.1) summarises the different studies and includes details of the test method, number of participants and study parameters. This study being more about the mechanical properties of the anal canal, I decided to evaluate the anal canal diameter rather than pressure during the resting, voluntary contraction and RAIR.

Author	Modality	n	Measured parameter
Goke, M., [366].	ARM	12	RP, SP, Rectal balloon distension
Freys SM	ARM	10	Functional anal canal, RP, SP, RAIR
Rogers, J.a	ARM	16	Functional anal canal, RP, SP, PNTML, and anal sensory threshold
Ryhammer, A.M	ARM	58	RP, SP, PNTML, and anal sensory threshold
Enrique Coss-Adame,	HRAM	16	RP, SP and SSP
A. M. P. Schizas	VVM	24	RP and SP

Table 2.1: Studies looking at repeatability of different test modalities in anorectal physiology. (ARM: anorectal manometry; HRAM: high resolution anorectal manometry; VVM: vector volume manometry; RP: resting pressure; SP: squeeze pressure; SSP: sustained squeeze pressure; PNTML: Pudendal nerve terminal motor latency)

Validity is the extent to which the measures studied in a test represent the variable they are intended to. Simply put, it refers to the credibility of a test. To be validated, a test should be sound both quantitatively and qualitatively. In this study, quantitative aspect would be the repeatability of parameters measured by EndoFLIP probe and qualitative aspect would be EndoFLIP's ability to establish the existing conceptual knowledge about anorectal physiology.

2.5 Study Groups

The study involved three groups, first group comprising of healthy volunteers, the second one having patients with faecal incontinence secondary to IAS dysfunction (without any anatomical disruption) and the third group having faecally incontinent patients suffering from scleroderma. The first group helped validate the device and establish its test-retest repeatability. It also aided establishing a normal reference range. The rest of the groups were to establish the clinical utility of EndoFLIP in anorectal studies.

2.6 Inclusion Criteria

Group 1

Male or female over 18 years of age

Normal anorectal function as ascertained by history and questionnaires

Group 2

Male or female aged over 18 years of age

Patients with faecal incontinence secondary to IAS dysfunction

Fulfil the Rome criteria for faecal incontinence

Group 3

Male or female aged over 18 years of age

Patients with scleroderma suffering from faecal incontinence

2.7 Exclusion Criteria

Group 1

Abnormal anorectal function as ascertained by history and Wexner constipation and incontinence scores

All Groups

Patients with chronic constipation, perianal sepsis or anal fistula, inflammatory bowel disease, spinal injury, history of rectal prolapse, bowel resection or transanal surgery were excluded.

2.8 Study Design

2.8.1 In vitro

In vitro study was undertaken prior to the in vivo studies to confirm the test-retest repeatability of the purpose built anorectal probe. The tests were carried out at room temperature on two pig anorectum specimens (Figure. 2.5). The anorectum specimens with a wide margin of

perianal tissue were obtained from the abattoir from already slaughtered animals. The test was performed on each specimen using the three sizes of FLIP probe sequentially. The test was repeated after 30 minutes to obtain the second reading to determine the repeatability of each probe.

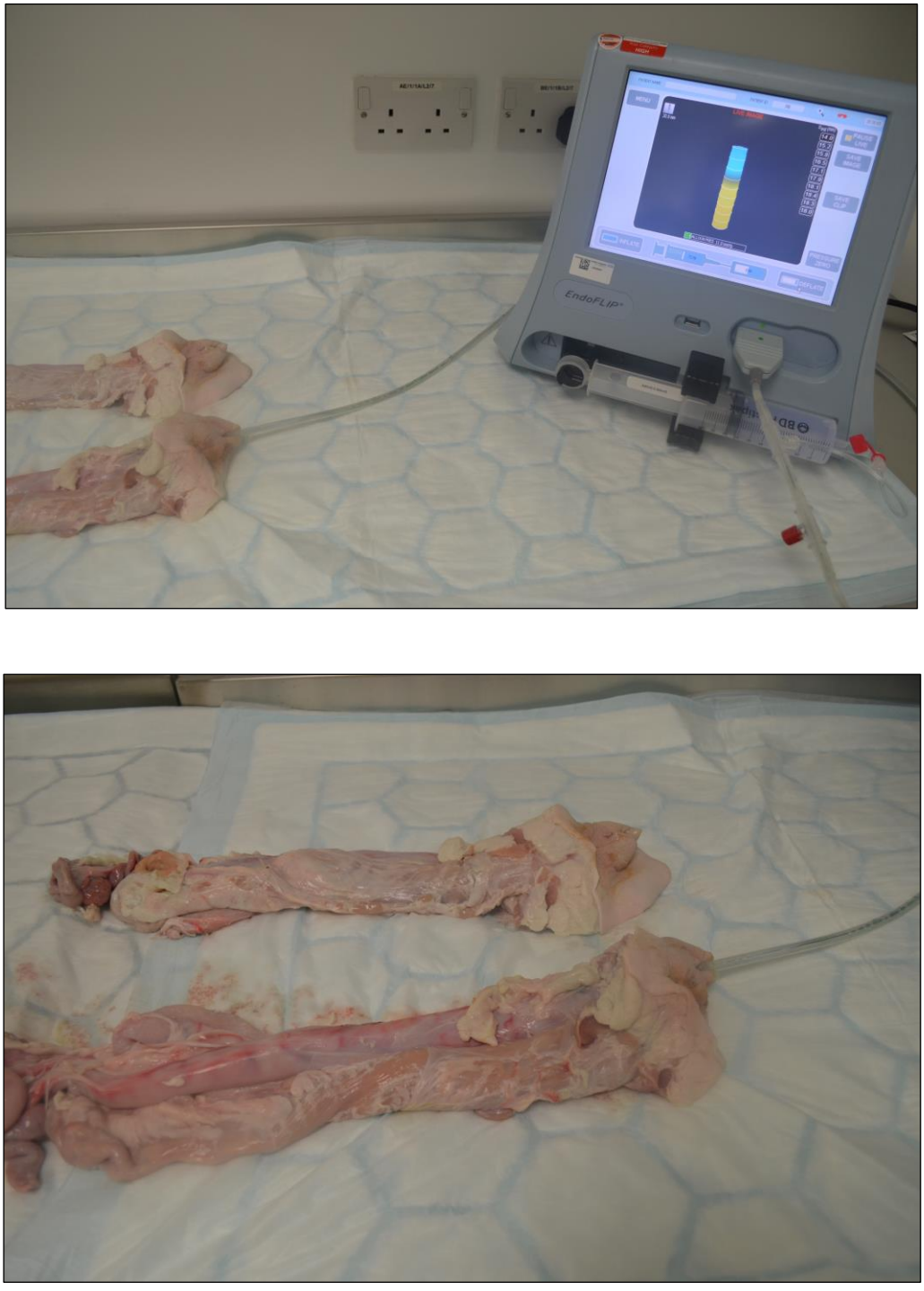


Figure 2.5: Setup for in-vitro test

2.8.2 Human study

After determining the eligibility of a participant with history and questionnaires (appendix 1 & 2), anorectal manometry was undertaken as the foremost investigation in the study. The information obtained about the functional length of the anal canal, through ARM, assisted in selecting the appropriately sized EndoFLIP probe for that individual. In vivo study had three subject groups; healthy volunteers, patients with faecal incontinence secondary to IAS dysfunction (without any anatomical disruption) and those having faecal incontinence secondary to scleroderma. The full study design is shown in figure 2.6.

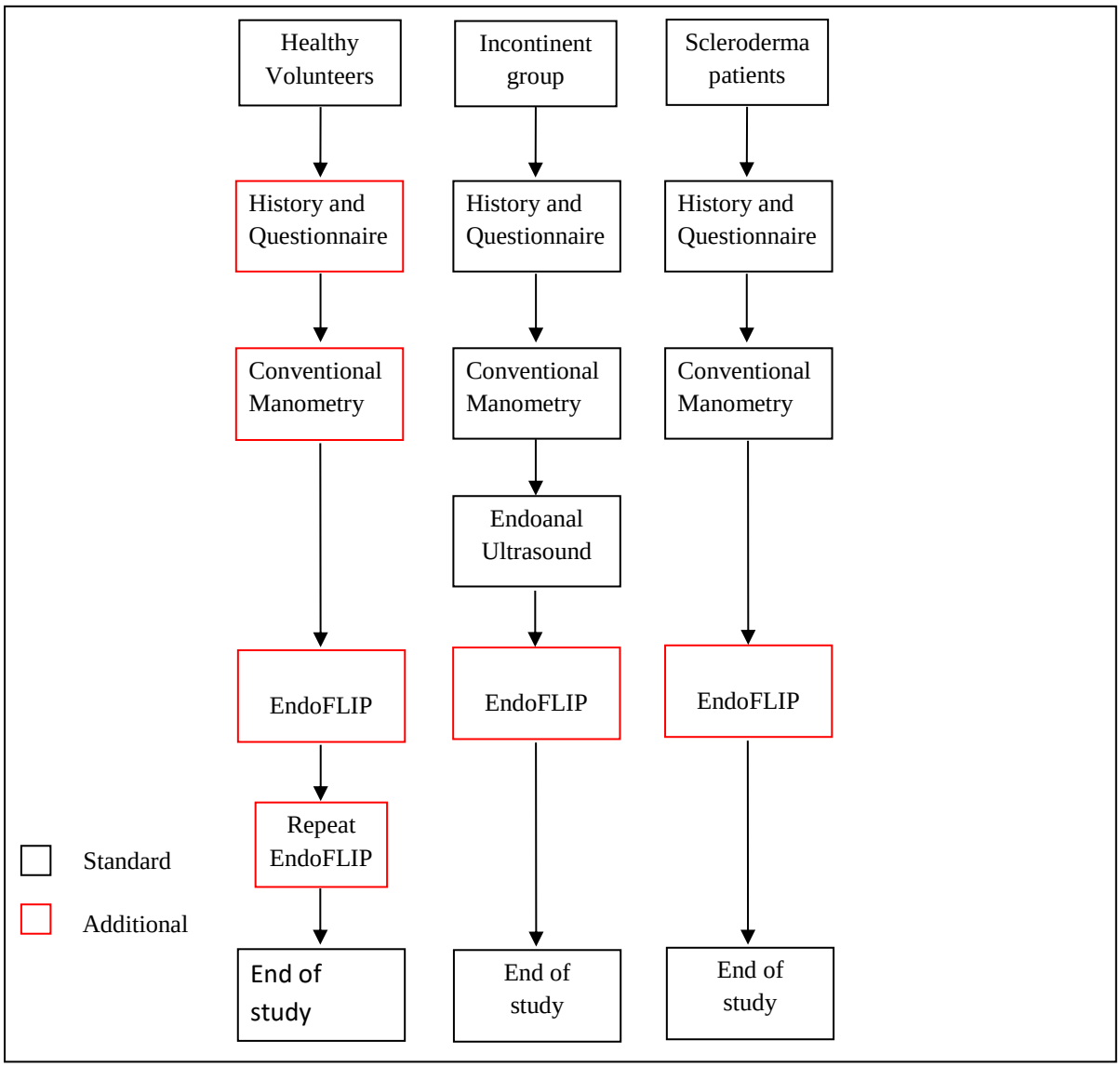


Figure 2.6: In vivo study design (red coloured box represents additional tests)

An eight-channel catheter (Ardmore Healthcare Ltd, UK, external diameter 3.9 mm) linked to a Medical Measurement Systems (MMS) eight-channel pneumohydraulic water perfusion

system (MMS, Enschede, Holland) was used for anorectal manometry (Figure 2.7). Station pull-through manometry technique was used to assess the high-pressure zone and record maximum resting and maximum voluntary squeeze pressures. The patient was placed in a left lateral position and a digital rectal examination was performed prior to inserting the manometry catheter. A well-lubricated catheter was inserted after ensuring it had been zeroed to the atmospheric pressure. Patients were not required to empty their bowels or use any sort of bowel prep prior to the test.

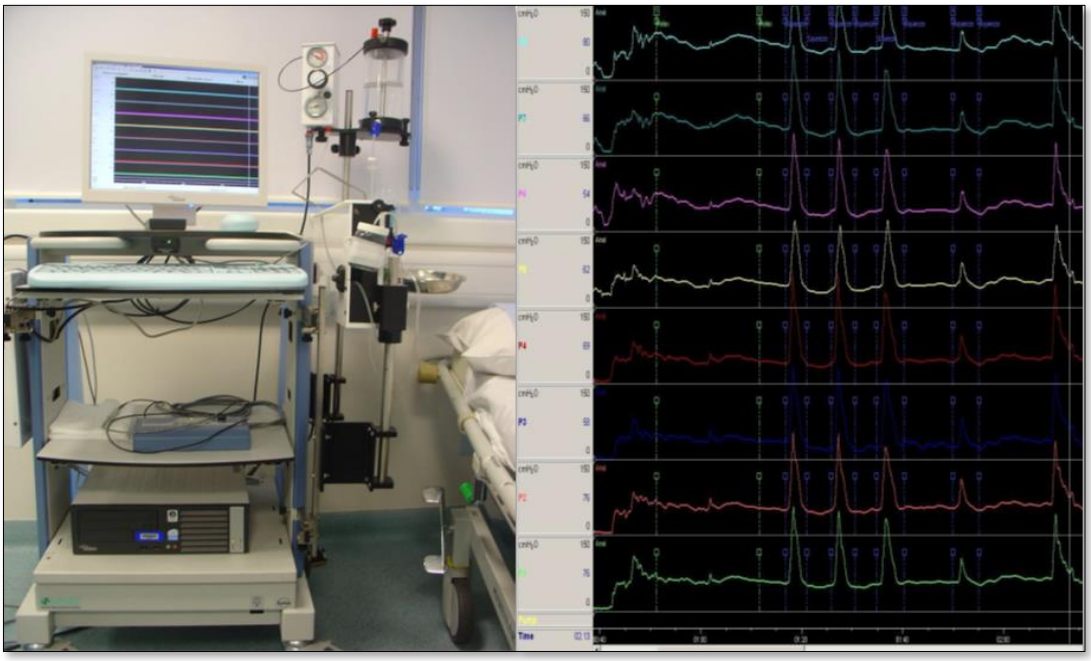


Figure 2.7: Manometry stack used in the study and manometry trace using 8 channel catheter

A time gap of five minutes was kept between ARM and EndoFLIP to allow sphincter muscles to recover. In order to study the biomechanical properties of the anal canal, incremental inflation volume was used with the EndoFLIP testing. In a pilot test on humans, the same inflation volumes, as determined by the in vitro study, were found to be suitable. A gap of thirty seconds was kept between each phase and of one minute between subsequent repetitions of the same manoeuvres at different inflation volumes (Fig. 2.8). An inflation volume of 50 mls was used to initiate the RAIR mirroring the practice followed during anorectal manometry in the unit.

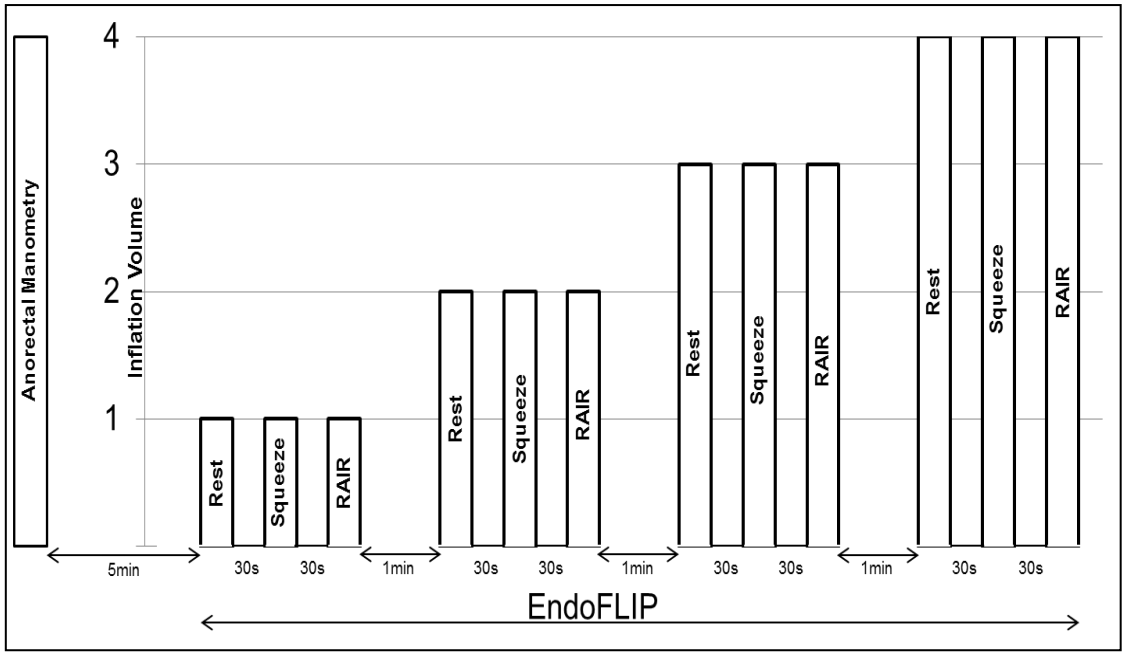


Figure 2.8: EndoFLIP study design

For EndoFLIP testing, the patient was placed in the left lateral position. The anal canal balloon was inflated twice with 10mls of fluid to ensure proper unfolding and filling of the balloon (by removing excess air). The rectal part of the probe was lubricated and gently inserted into the anal canal after setting the baseline intra-bag pressure to zero. The probe placement was done in a way that whole of the anal canal balloon was inside the anal orifice and not emerging out of it. Once the probe was appropriately positioned, it was taped onto the patient's buttock and additionally held in place by the investigator. For repeatability testing, the probe was taken out in between the two measurements and zeroed again before re-insertion.

2.9 EndoFLIP analysis

The FLIP probe recorded luminal diameter estimates (D_{est}) only in between a pair of electrodes. The total measured part of the anal canal is shown within the blue lines in figure 8. As the number of electrodes increased (with increasing length of the anal canal balloon) so did the number of recordable D_{est}. A total of 3 D_{est} points were recorded in a 2 cm probe, 5 in 3 cm and 10 in 4 cm probe respectively. For the purpose of analysis the anal canal was divided into three segments – upper, middle and lower (Fig.2.9).

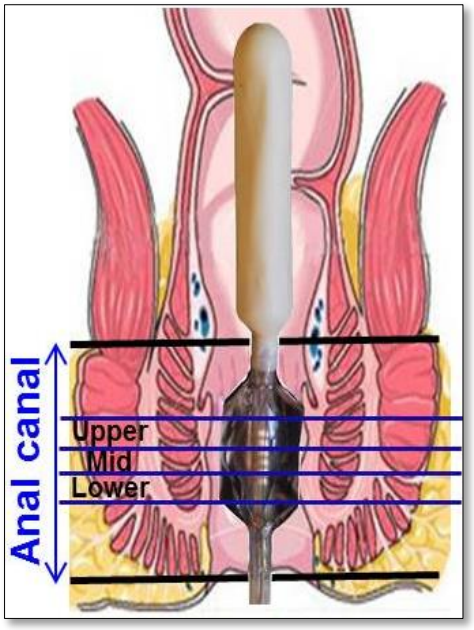


Figure 2.9: Segmental division of anal canal used for EndoFLIP analysis

Such segmental division of anal canal has previously been described in several studies on anatomical and physiological grounds. Liu et al in their study using 3D ultrasound, reported three anatomical segments of the anal canal, proximal end formed by IAS and Puborectalis muscle (PRM), mid part comprising of IAS & EAS and distal part composed of only EAS [16] (Fig.2.10). Other studies describing segmental variation in anal canal are detailed in table 2.2.

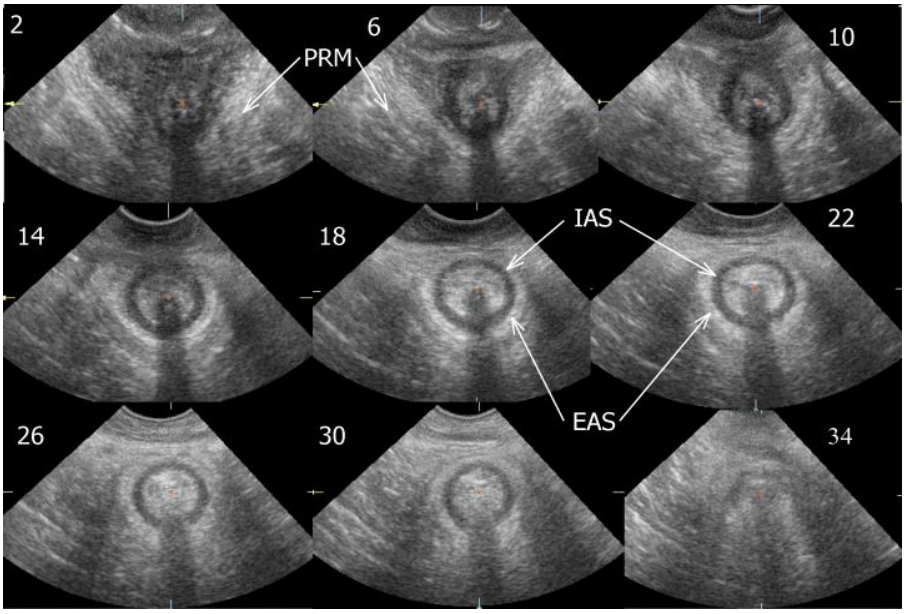


Figure 2.10: Transverse section view of anal canal seen on ultrasound at every 4 mm distance. Reproduced by permission from Nature Publishing Group.

Author & Publication	Diagnostic technique	Significant findings
Goes et al. Dis Colon Rectum (1996)	Vector manometry	High pressure zone is located distally in controls
Liu et al. Am J Gastroenterol. (2006)	3D Ultrasound & anorectal manometry	Highest pressure in mid part of anal canal
Jung et al. Dis Colon Rectum (2008)	3D Ultrasound	Anal canal has an hourglass shape
Luft et al. Tech Coloproctology (2012)	EndoFLIP	Anal canal has an hourglass shape
Alqudah et al. Neurogastro- Motil. (2012)	EndoFLIP	Lowest CSA at distal anal canal (only at lowest balloon vol)

Table 2.2: Studies describing segmental division of anal canal

The number of D_{est} points in each anal canal segment differed according to the probe length. The raw data was eyeballed for variations in these D_{est} points, which were accordingly allocated to each of the three anal canal segments as shown below in table 3.

Anal balloon length (cm)	Total D_{est} points	D_{est} points in Upper AC	D_{est} points in Mid AC	D_{est} points in Lower AC
2	3	1	1	1
3	5	2	1	2
4	10	4	3	3

Table 2.3: Distribution of D_{est} points according to different sized EndoFLIP probe

These D_{est} points were converted to the cross-sectional area (CSA) by using the mathematical formula $(A = \pi r^2)$ where A is area and r is the radius of a circle. Once the CSA was obtained, further statistical analysis was performed by calculating the mean of all the CSA points in a particular anal canal segment (upper, mid or lower), for that individual probe. For instance, in a 4 cm probe, mean of 4 CSA points was taken in the upper anal canal and mean of 3 CSA points was taken in mid & lower anal canal segments respectively.

Data was recorded for ten seconds during the resting phase of which the latter five seconds were analysed to avoid any artefacts. For squeeze, the first two seconds following initiation of squeeze and for RAIR first fifteen seconds after inflation of rectal balloon were analysed.

2.10 Statistical consideration

Sample Size

This being a pilot study, the sample size was estimated from a number of participants in the existing reproducibility studies done for the conventional anorectal manometry (Table 2.4).

Author	Number of participants (n)
Bharucha et al.[229]	19
Eckardt et al.[367]	20
Ryhammer et al.[368]	58
Rogers et al.[369]	16

Table 2.4: Reproducibility studies for anorectal manometry

The median number of participants in these studies was 19 and as such this was the number chosen as the sample size for group 1, comprising of healthy volunteers to confirm the reproducibility of EndoFLIP. There were 10 males and 9 females in this group with a mean age of 34 (20-75).

The group 2 (incontinent group) had 16 participants of which 7 were females. The mean age was 67(54-87). Group 3 comprising of scleroderma patients had 8 patients, all of whom were females.

Statistical analysis

Data was checked for normal distribution using Q-Q plots and then subjected to Analysis of Variance (ANOVA) in order to determine the significant difference between the repeated measures. The repeatability of the EndoFLIP was examined using the Bland-Altman limits of agreement method. Additionally, the intra-class correlation coefficient (ICC) was also used to examine relationship. The measured parameter was mean ± standard deviation of CSA during resting, squeeze and RAIR phase.

To obtain a reference range, ROC analysis was undertaken. This took into account the mean ratio of CSA in distal and proximal part of the anal canal.

The difference between the CSA measurements between healthy volunteers and FI patients was examined with the help of Independent T-test.

Software

Statistical analysis was performed using a software package IBM SPSS Statistics for Windows, Version 21.0. Armonk, NY: IBM Corp. MATLAB and Statistics Toolbox Release 2013b, The MathWorks, Inc., Natick, Massachusetts, United States was used as the mathematical software for analysis of data. MATLAB is an interactive program for numerical computation and data visualisation. It uses a fourth-generation language in which codes or scripts are generated for numerical computation or data visualisation. In this study MATLAB was used for data visualisation by constructing contour plots. Contour plot is a graphical technique for portraying data for three variables in two dimensions. The variables in this study were anal canal diameter (AC_{Dia}**),** distance into anal canal and time.

CHAPTER 3

IN VITRO TESTS

3.1 In Vitro tests

The foremost step after getting the custom-made anorectal probe was to test it for repeatability. In vitro tests were carried out on pig anorectum for this purpose and helped establish not only repeatability but also other test parameters, which were subsequently used for the in vivo studies. The repeatability was established with the help of three statistical tests - ANOVA, Bland-Altman plots and Intra-class correlation coefficient (ICC).

3.2 Test-retest repeatability

3.2.1 Analysis of Variance (ANOVA)

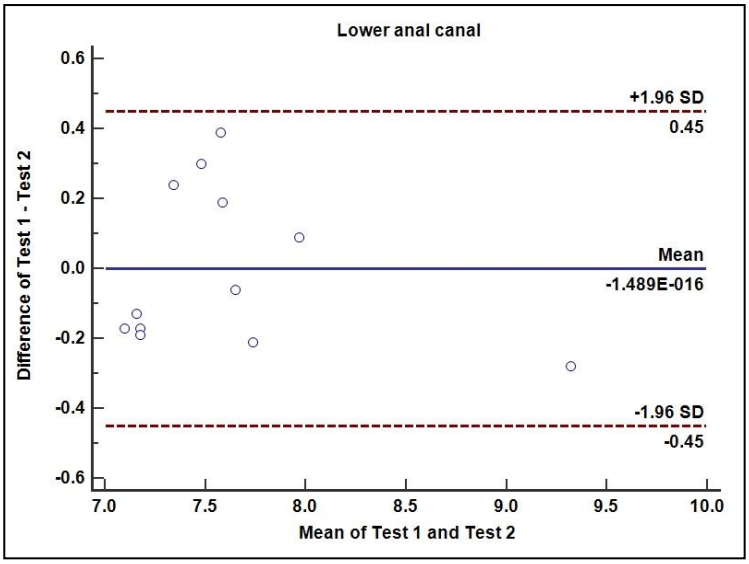
ANOVA test is generally used to test for significant differences between means of two or more groups. This was the first test to compare the repeated measurements obtained per specimen. The mean CSA, in each of the three anal canal segment, was obtained and tested for repeatability with this test. The results, shown in table 3.1, revealed a low F ratio and high p-value indicating no statistically significant difference between the repeated measurements, in either of the specimens.

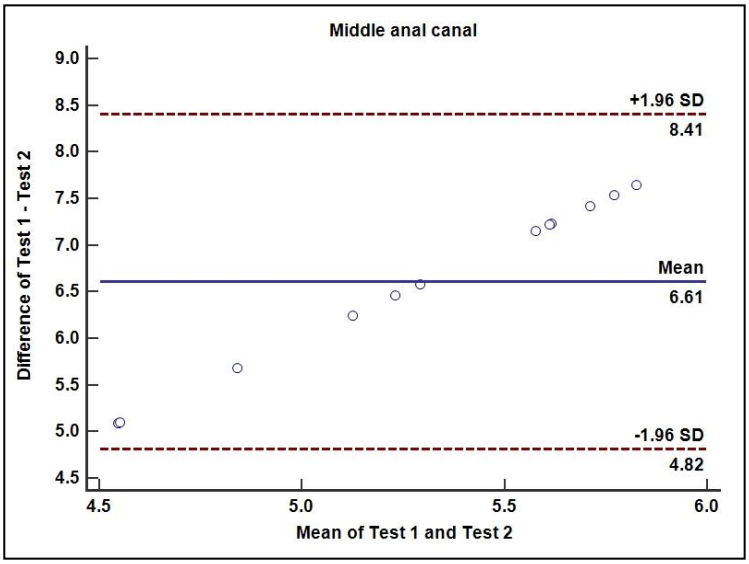
Specimen	Lower anal canal	Mid anal canal	Upper anal canal
Specimen 1	[F(1,22) = .001, p =.971]	[F(1,22) = .016, p =.901]	[F(1,22) = .000, p =.999]
Specimen 2	[F(1,22) = .423, p =.522]	[F(1,22) = .158, p =.695]	[F(1,22) = 1.24, p =.277]

Table 3.1: ANOVA results for in vitro test

3.2.2 Bland-Altman Plots

Bland-Altman plot analysis is the most widely used statistical test for assessing agreement between two quantitative methods of measurement. Mean CSA measurements, derived from the three different sized probes at all the four inflation volumes, were used to create Bland-Altman (BA) plots. The repeatability was tested individually in each of the anal canal segment. These plots, as shown below, demonstrate an excellent repeatability in both the pig anorectum specimens (Fig. 3.1 & 3.2).





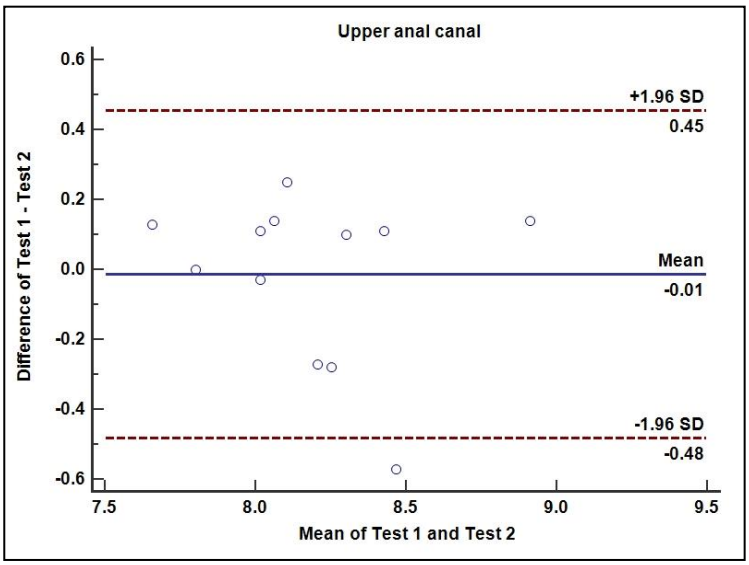
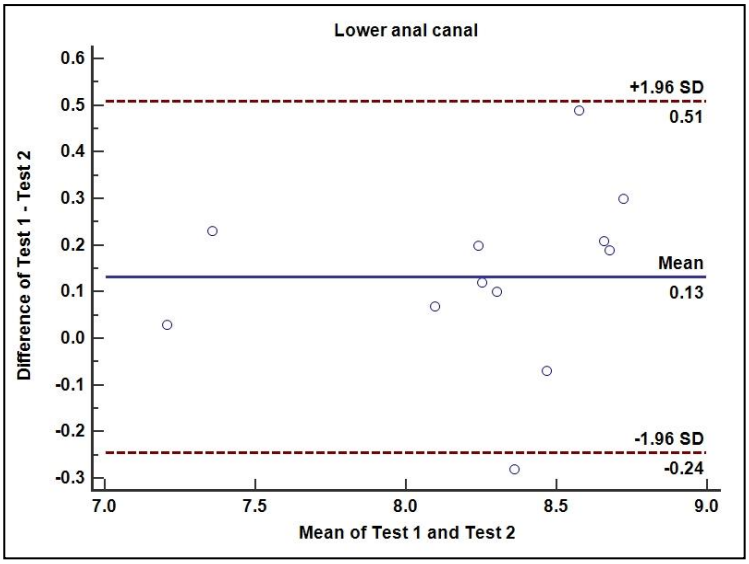
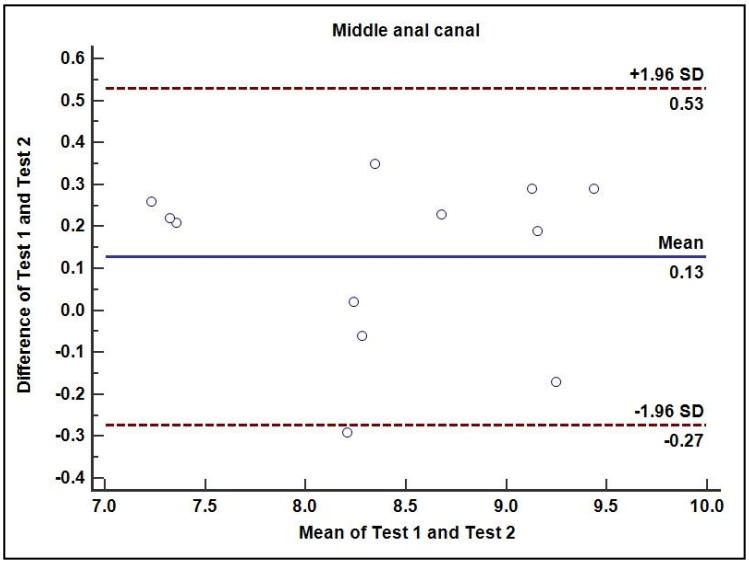


Figure 3.1: Bland-Altman plots illustrating test-retest repeatability in specimen 1





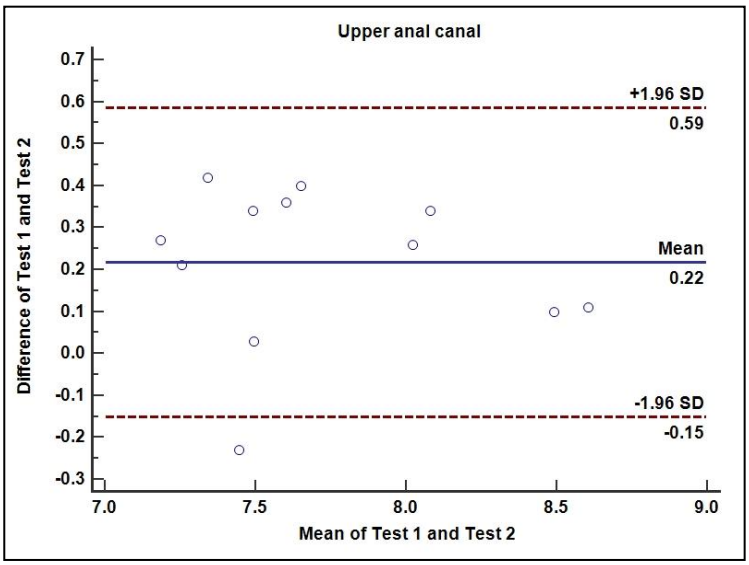


Figure 3.2: Bland-Altman plots illustrating test-retest repeatability in specimen 2

3.2.3 Intra-Class Correlation Coefficient (ICC)

There are several different correlation techniques, which help quantify the degree to which two variables are related. The Intra-Class Correlation Coefficient (ICC) was used in this study to examine the correlation between repeat measurements. It is commonly agreed that ICC value above 0.9 demonstrates excellent correlation [370]. The ICC values in this study were consistently above 0.9 in all the three anal canal segments in both the pig specimens as shown in the table below (Table 3.2).

	Specimen 1		Specimen 2	
Anal canal segment	**ICC**	**p value**	**ICC**	**p value**
Upper anal canal	.964	<.0001	.959	<.0001
Mid anal canal	.974	<.0001	.983	<.0001
Lower anal canal	.912	<.0001	.962	<.0001

Table 3.2: ICC values across three different anal canal segments

3.3 Other test parameters

In order for the FLIP to give accurate data, a balloon containing the conducive fluid is required to be touching the lumen walls. Varying inflation volumes were tried to fulfil this requirement and an initial pilot study revealed that this was not possible below 3 ml of volume, in 2 and 3 cm long balloon (anal canal) and below 5 ml in 4 cm long balloon. The maximal inflation volume was determined by undertaking incremental inflations of 1 ml until overstretching of the anal canal beyond physiological limits became apparent, both on physical examination of the specimen and by a sudden increase in pressure on the screen, indicating high resistance from the luminal wall. On the basis of these results, the volumes that helped obtain a meaningful data are shown in table 3.3. The test-retest repeatability was measured using these volumes and the data was recorded at each inflation volume.

Anal balloon length (cm)	Inflation volume (ml)
2	3,4,5,6
3	3,4,5,6
4	5,6,7,8

Table 3.3: Inflation volumes used in differently sized anal canal balloons

3.4 Conclusion

In vitro study was a significant first step before moving to the in-vivo testing. It helped establish the repeatability of the custom made anorectal EndoFLIP probe. This was important as it confirmed minimal variance in the results obtained from the probe, thereby establishing its precision.

Additionally, in vitro testing also facilitated determination of the inflation volumes to be used for the anal canal balloon. These results provided a platform for the future human tests. They helped avoid discomfort to the participants from the numerous measurements that would have been otherwise necessary to determine the appropriate inflation volumes.

CHAPTER 4

STUDY OF HEALTHY VOLUNTEERS

4.1 Human tests

This part of the study aimed to establish test-retest repeatability of the custom made anorectal EndoFLIP probes in humans in addition to determining the clinical validity of EndoFLIP technology in testing anorectal physiology.

The human tests were carried out in 19 healthy subjects, 10 males and 9 females with mean age of 34 (20-75), after determining their eligibility to participate with the help of history and Wexner constipation and incontinence questionnaires (Appendix 1 & 2). None of the subjects scored on the Wexner faecal incontinence questionnaire and the median for Wexner constipation questionnaire was 1 (0-1) with 2 subjects scoring 1 for straining rarely. This result was not considered significant enough to exclude these two subjects from this study.

4.2 Test-retest repeatability

The repeatability of the EndoFLIP probes was established using the same statistical tests as were used for the in vitro studies; Bland-Altman plots and Intra-Class Correlation Coefficient.

4.2.1 Bland-Altman plots

The repeatability was established, for each of the three measured phases – resting, maximal squeeze and RAIR reflex, by comparing the mean AC_{Dia} obtained for each anal canal segment during test 1 and test 2 with the help of Bland-Altman plots. A detailed explanation about the rationale behind using these plots and their interpretation has been discussed earlier in chapter 3 (section 3.3).

The BA plots shown below incorporate data from all the three probes across all the inflation volumes among all the healthy subjects (Fig.4.1).

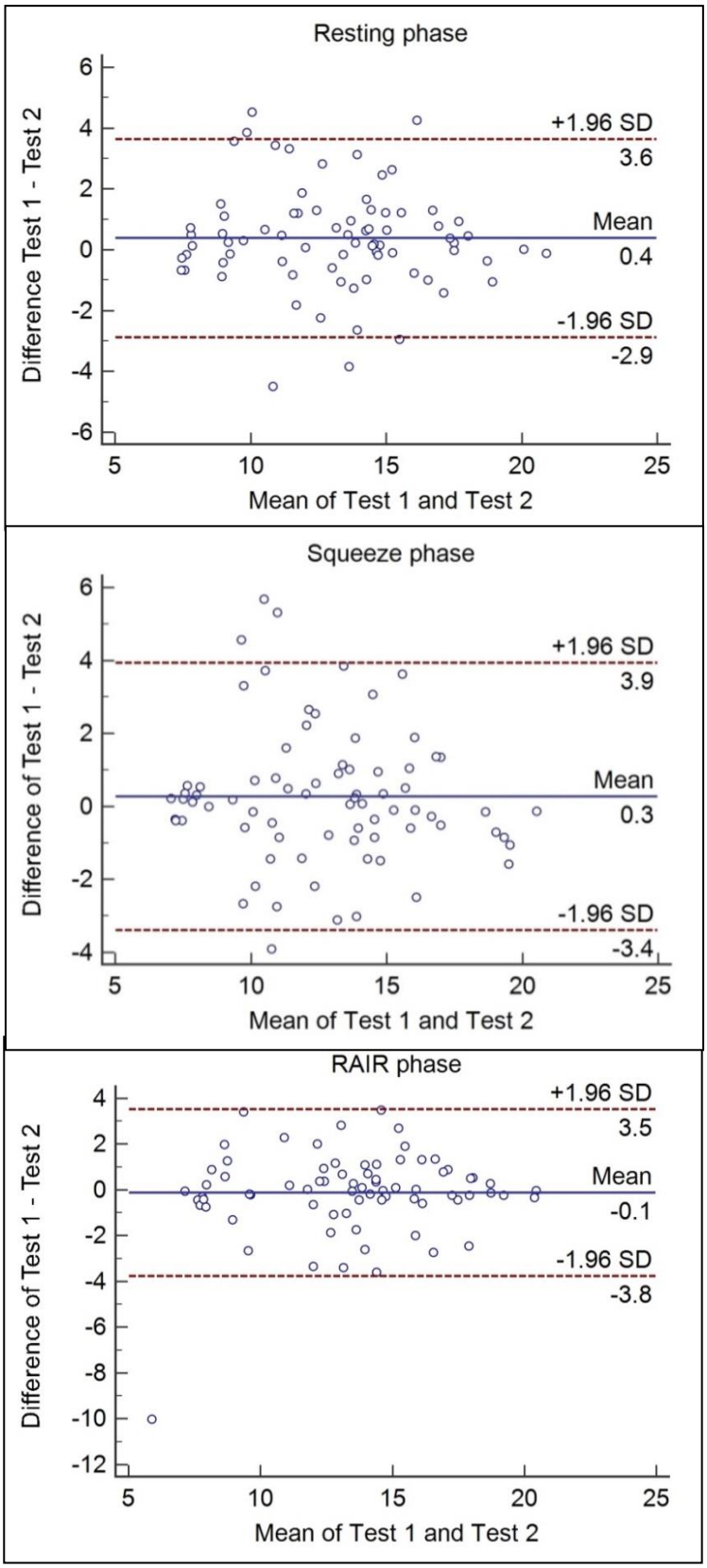


Figure 4.1: Bland-Altman plots illustrating repeatability during three different test phases in the two tests conducted in human study

4.2.2 Intra-Class Correlation Coefficient (ICC)

The repeatability was also established by the Intra-Class Correlation Coefficient. The scale from Altman, as shown below in table 4.1, was used in the classification of the reliability values [370]. The rationale behind using this test has already been discussed in chapter 2 (section 2.9).

ICC values	Analysis
< 0.20	Poor
0.21–0.40	Fair
0.41–0.60	Moderate
0.61–0.80	Good
0.81–1.00	Very Good

Table 4.1: Bland-Altman's classification of ICC values

The results showed very good repeatability among all the measured phases. This is shown in the table below.

Measured phase	Upper anal canal	Mid anal canal	Lower anal canal
Resting	.960	.960	.928
Squeeze	.978	.960	.919
RAIR	.923	.962	.957

Table 4.2: Intra-Class Correlation Coefficient (ICC) results in the three anal canal segments demonstrating a very good repeatability

4.3 Results

The MATLAB software was used for qualitative analysis of the data. The rationale behind the qualitative analysis was primarily to gain insight into the data and uncover trends in order to develop a better understanding.

A purpose-built programme in MATLAB allowed obtaining a pictorial representation of the EndoFLIP data. The colour plots represent, only the area within anal canal studied by the FLIP probe, as discussed earlier in chapter 2. In order to understand the colour plots in the subsequent pages, a sample image demonstrating anal canal in a healthy subject during the rest phase is presented below. Time (in seconds) is plotted on the X-axis and distance into anal canal (in mm) is plotted on the Y-axis so that as we move up the image we are also moving up in the anal canal. The colours in the image represent the anal canal diameter (AC_{Dia}), warm colours such as red and orange signify lower AC_{Dia}, i.e. closed anal canal and cooler colour such as blue signify higher AC_{Dia}, i.e. open anal canal.

Based on the interpretation method mentioned above, one can clearly see that the image below (Fig 4.2) shows an anal canal which is closed (red colour) in the lower-most part and open (blue colour) in its upper part. This is not to be confused with the pressure which will be higher with the warm colour and lower with the cooler colour (Low AC_{Dia} = Closed anal canal = Higher pressure & High AC_{Dia} = Open anal canal = Lower pressure).

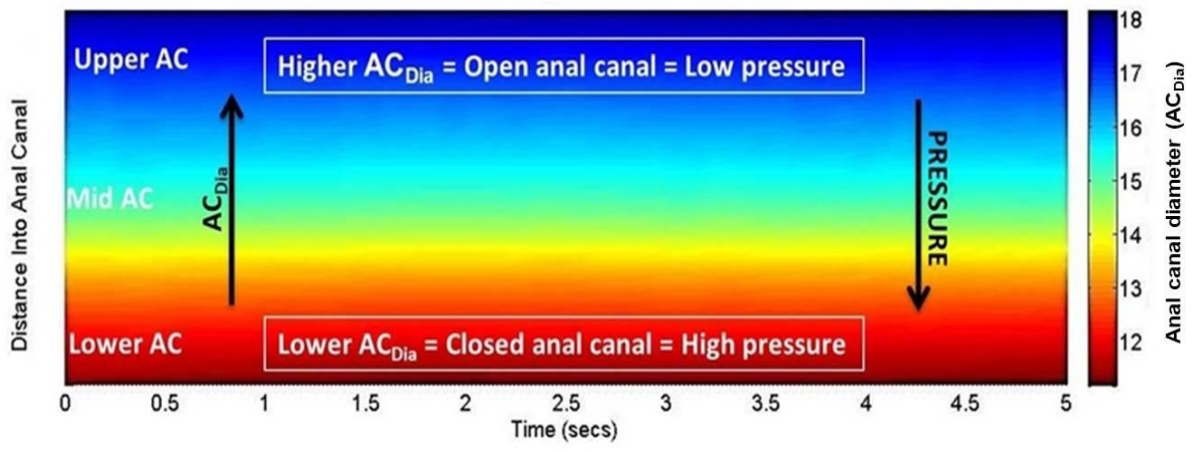


Figure 4.2: MATLAB generated image of one subject during resting phase

4.3.1 Resting phase

The results from healthy subjects revealed that during rest, most of the anal canal was closed at the lowest balloon inflation volume. However, with an increase in inflation volume, i.e. on distension, the anal canal started opening up. The lower-most part of the anal canal was found to be the most resilient to opening by an increase in inflation volume.

Figure 4.3, shows four colour plots from a single subject using a 3 cm probe. Each colour plot represents a different inflation volume (3, 4, 5 & 6 ml as mentioned in table 3.3). As we move down the image (from a to d) the anal canal is being subjected to an increasing distension (i.e. increasing balloon inflation volume). The first image shows the anal canal to be almost completely closed but as the inflation volume is increased the anal canal starts opening up, as seen by a change of colour from mostly red and orange in the first image to mostly blue in the fourth image. This was a fairly consistent pattern seen in rest of the subjects as well.

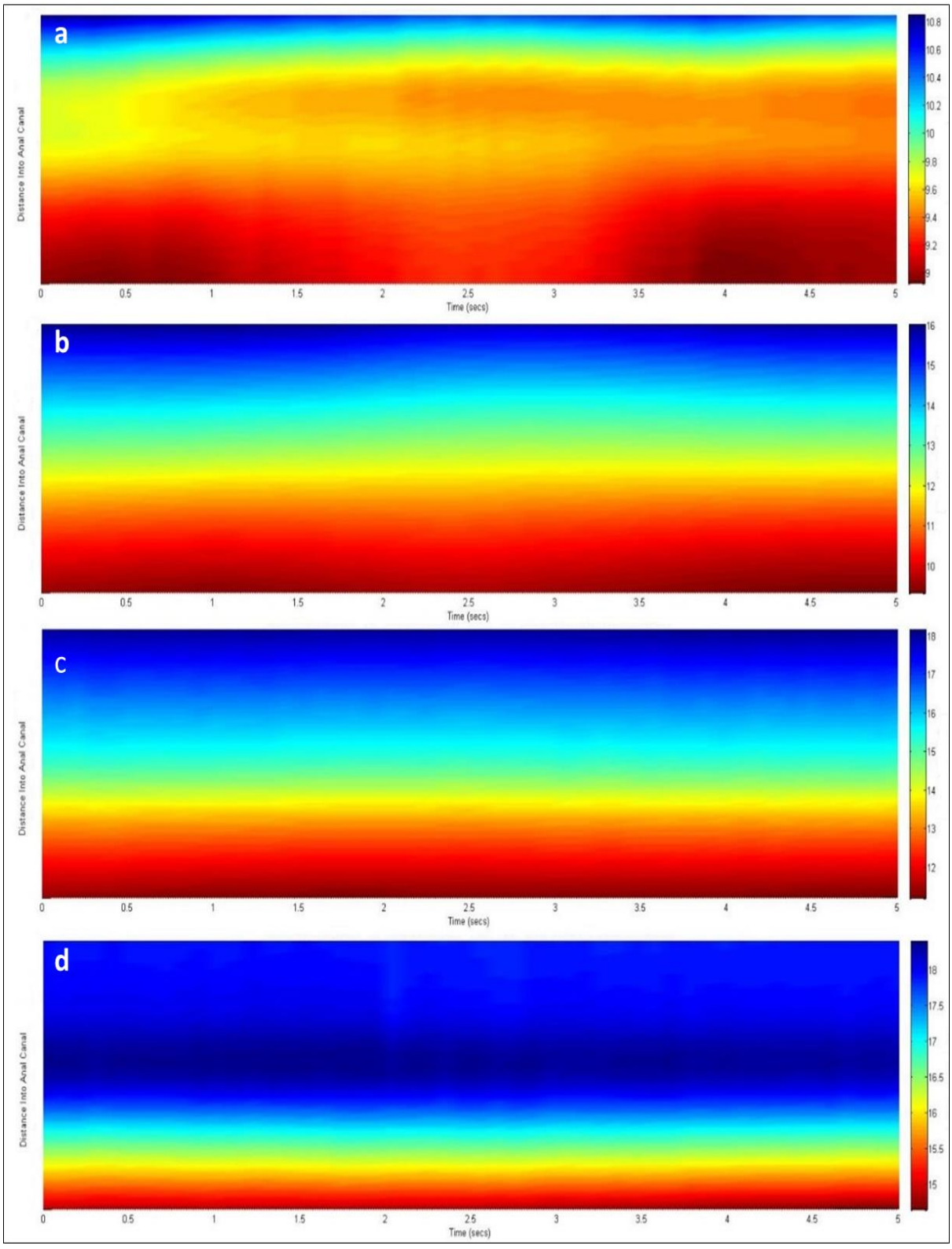


Figure 4.3: Image demonstrating changes in anal canal with distension during resting phase in a single subject using a 3 cm probe. Each panel represents a different balloon inflation volume which increases by 1ml (from 3 to 6 mls) as we move down the image from a to d.

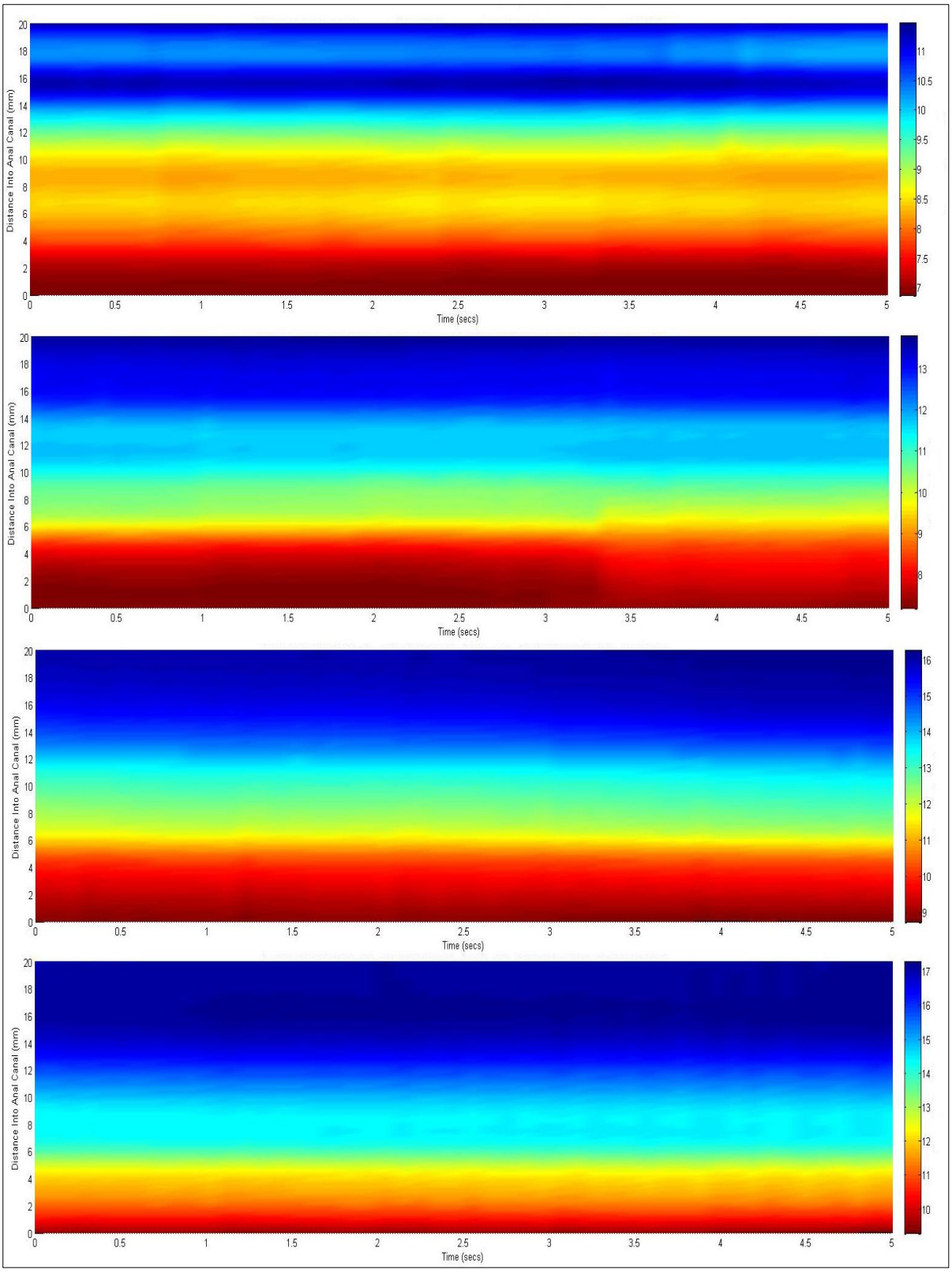
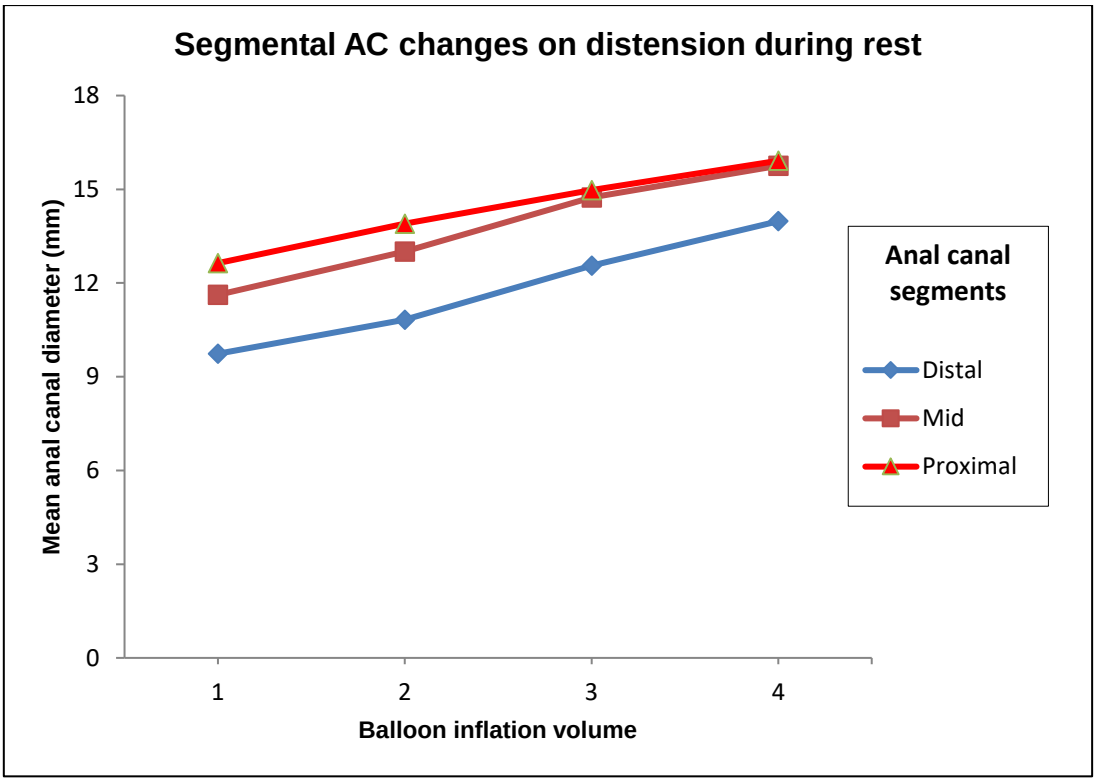


Figure 4.4: Image demonstrating changes in anal canal with distension during resting phase in a single subject using a 4 cm probe. Each panel represents a different balloon inflation volume which increases by 1ml (from 5 to 8 mls) as we move down the image.

Data for segmental changes due to distension during rest

The opening up of anal canal with distension has been discussed already. A further analysis was undertaken to study the segmental changes in the anal canal caused by the distension. The analysis revealed that an incremental distension led to progressive opening up of the anal canal in all the three segments (lower, mid and upper) as seen in the figure below displaying an increase in the mean anal canal diameter with increasing balloon inflation volume.



Anal Canal segment	Mean AC diameter (mm) at incremental balloon inflation volumes			
	1	2	3	4
Distal	9.74	10.82	12.56	13.98
Mid	11.62	13.00	14.74	15.75
Proximal	12.64	13.90	14.97	15.91

Figure 4.5: Segmental differences in the mean anal canal diameter on subjecting anal canal to incremental distension during resting phase across all the healthy subjects; AC = anal canal

4.3.2 Squeeze phase

During the squeeze phase, a decrease in the anal canal diameter was observed in the whole of the measured anal canal with the most prominent reduction being in the lower-most segment of the anal canal (i.e. greatest closure near the anal verge).

The colour plot in figure 4.6 shows the voluntary contraction in one representative subject recorded using a 2 cm probe at inflation volume of 5 ml. The graph below the colour plot displays the balloon pressure. As the squeeze is initiated, confirmed by an increase in pressure, a change in AC_{Dia} is observed in whole of the anal canal. This is noted by a change in colour from blue to warmer colours in the colour plot. Although a colour change is evident in the whole of the anal canal, the most prominent change is apparent in the lower-most segment where the colour changes to red, signifying the most decrease in AC_{Dia}. Although squeeze phase is difficult to evaluate due to the subject dependent variation in strength and duration of the squeeze, this was a common finding in rest of the cohort as well.

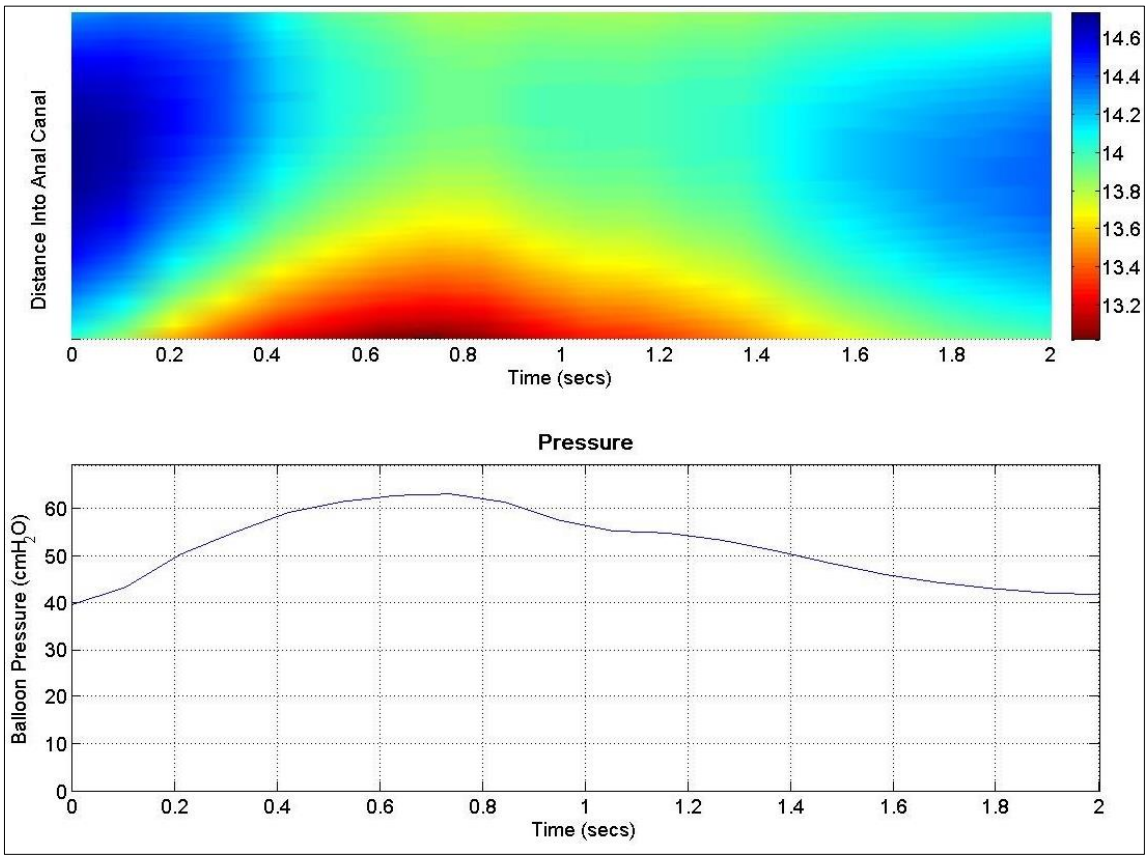


Figure 4.6: Changes in AC_{Dia} (above) and pressure (below) during squeeze in single subject

Changes during distension

With distension of anal canal (by increasing the balloon inflation) during voluntary contraction, a change in the pattern of anal closure was seen. At the least distension, almost whole of the anal canal was observed to be closing during voluntary contraction, as evident in the image below (fig 4.7 (a) & 4.8 (a)). On subjecting the anal canal to distension it started opening up progressively in all segments, apart from the lower-most segment which stayed closed even at the maximum distension, as evident by the red colour (indicating lowest CSA and highest pressure) in figure 4.7 (d) & 4.8 (d).

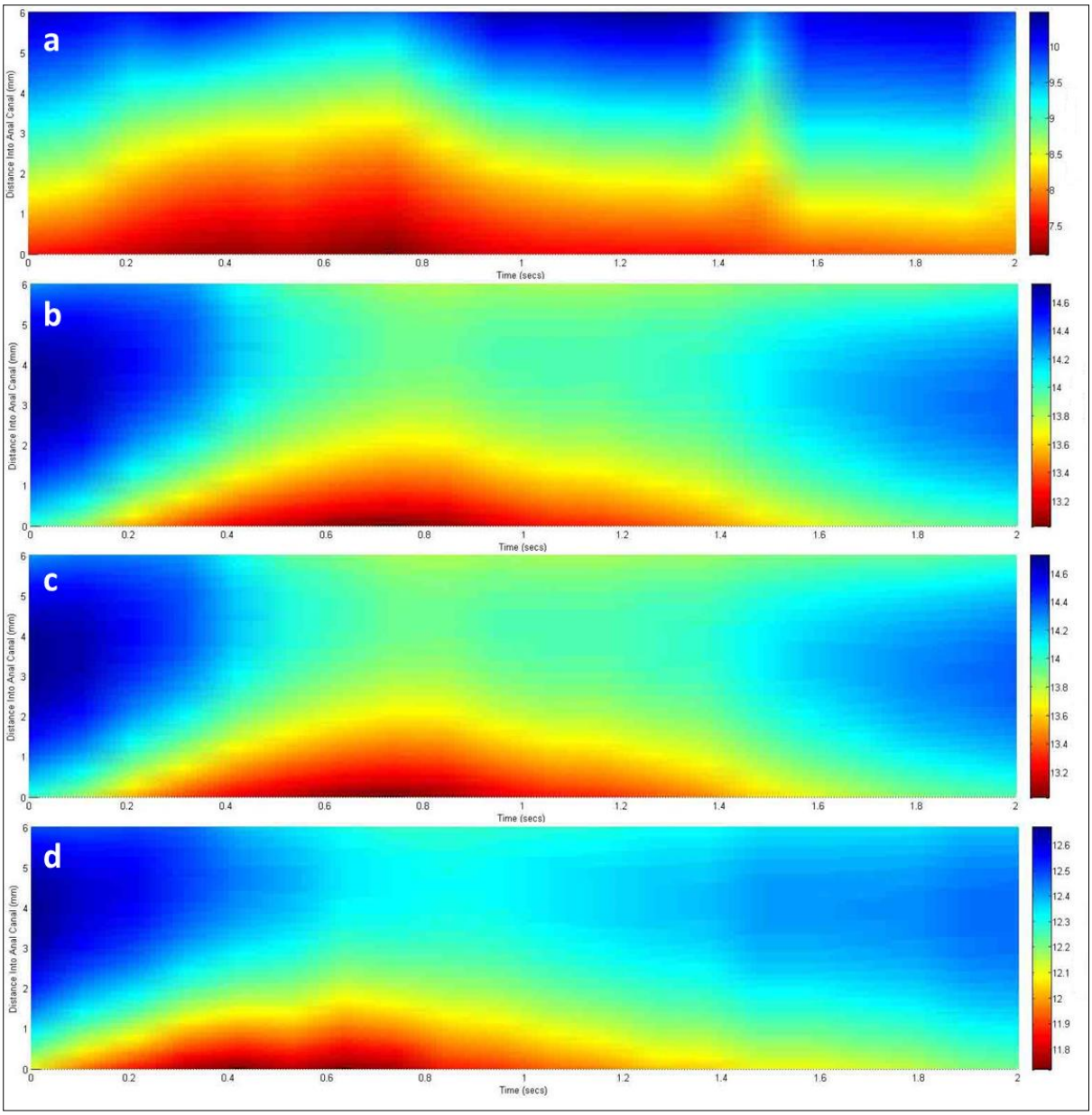


Figure 4.7: Changes in anal canal AC_{Dia} with distension during squeeze phase in a single subject using a 2 cm probe. Each panel represents a different balloon inflation volume which increases by 1ml as we move down the image (from a to d).

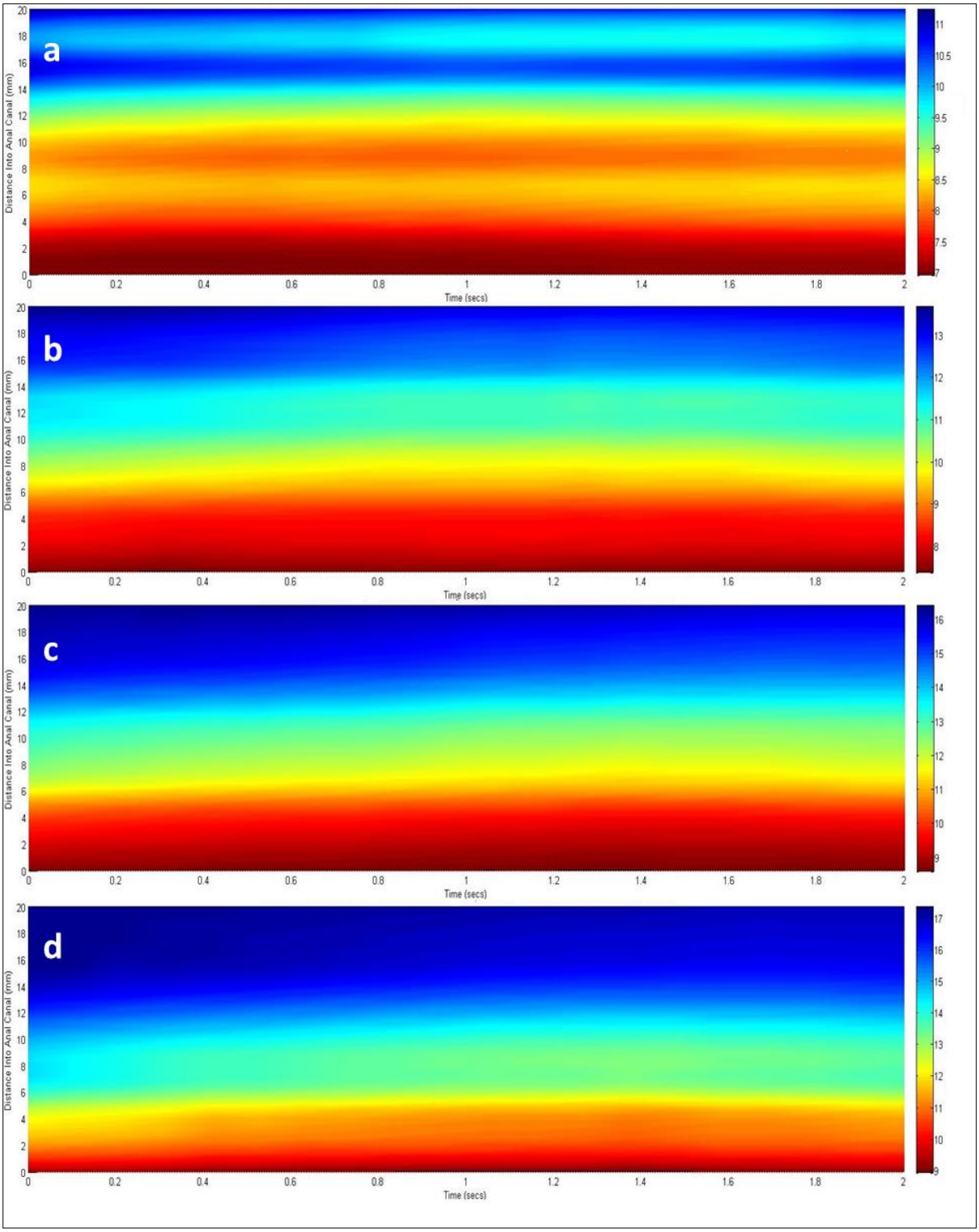
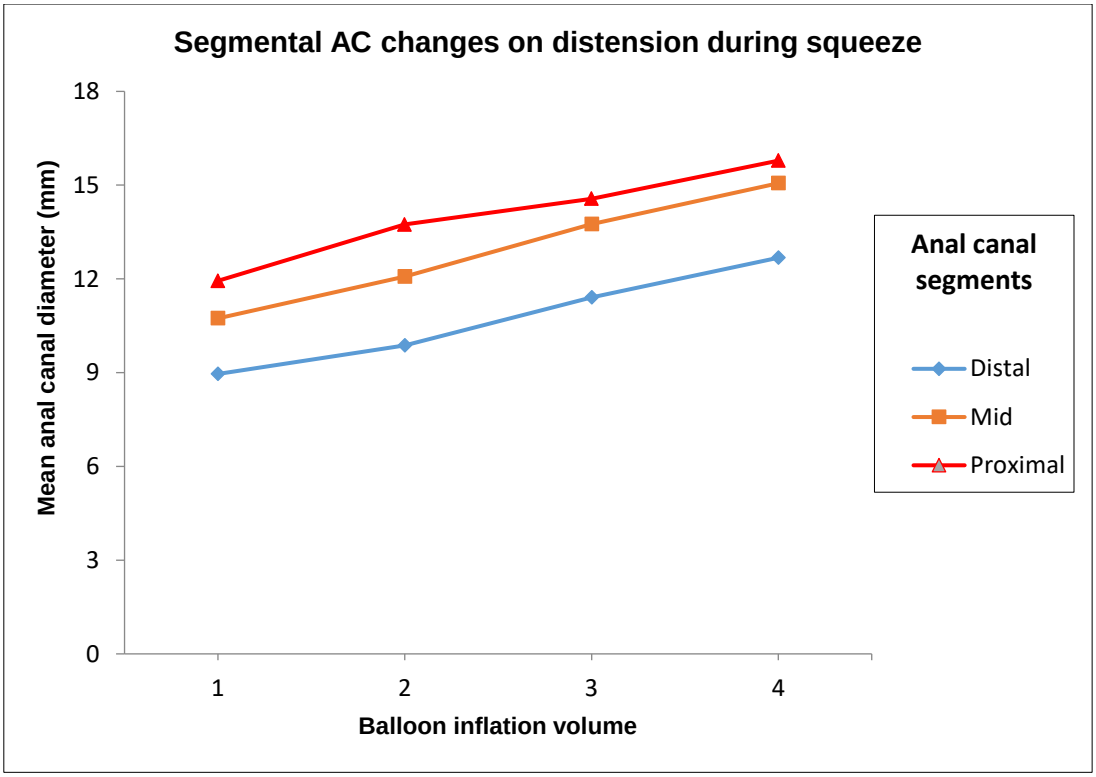


Figure 4.8: Changes in anal canal AC_{Dia} with distension during squeeze phase in a single subject using a 4 cm probe. Each panel represents a different balloon inflation volume which increases by 1ml as we move down the image (from a to d).

Data for segmental changes due to distension during squeeze

On further segmental data analysis, it became apparent that this pattern of anal canal opening to distension stood true in all the three anal canal segments (lower, mid and upper). The mean value for the AC_{Dia} for each of the four incremental balloon inflation volumes in the three anal canal segments respectively is shown below in fig 4.9.



Anal Canal segment	Mean AC diameter (mm) at incremental balloon inflation volumes			
	1	2	3	4
Distal	8.96	9.87	11.41	12.68
Mid	10.74	12.07	13.75	15.06
Proximal	11.94	13.74	14.57	15.78

Figure 4.9: Segmental differences in the mean anal canal diameter on subjecting anal canal to incremental distension during voluntary squeeze (across all the healthy subjects) ; AC = anal canal

4.3.3 RAIR phase

The rectoanal inhibitory reflex was initiated by a rapid inflation of the rectal balloon with 50 ml of air. The resultant changes in the anal canal were studied by the FLIP probe.

The colour plot below (Fig. 4.10) reveals a sudden change in the anal canal profile at the initiation of RAIR phase. The whole of anal canal appeared attempting to close as a result of sudden inflation, with the maximal closure seen in the mid and the lower anal canal. This closure lasted for few seconds and was followed by an opening of the anal canal, most prominent in the upper part, consistent with the process of sampling, of the rectal contents, by the specialised epithelium of the upper anal canal. This opening was also evident from the pressure graph which displayed a clear drop in pressure. During the opening of the anal canal, the least AC_{Dia} (i.e. closed anal canal) was noticed in the lower-most segment. This is likely to correspond to the EAS contraction, as expected during sampling, to prevent incontinence. The anal canal eventually started to return to its original state approximately 10 seconds after the initiation of RAIR reflex.

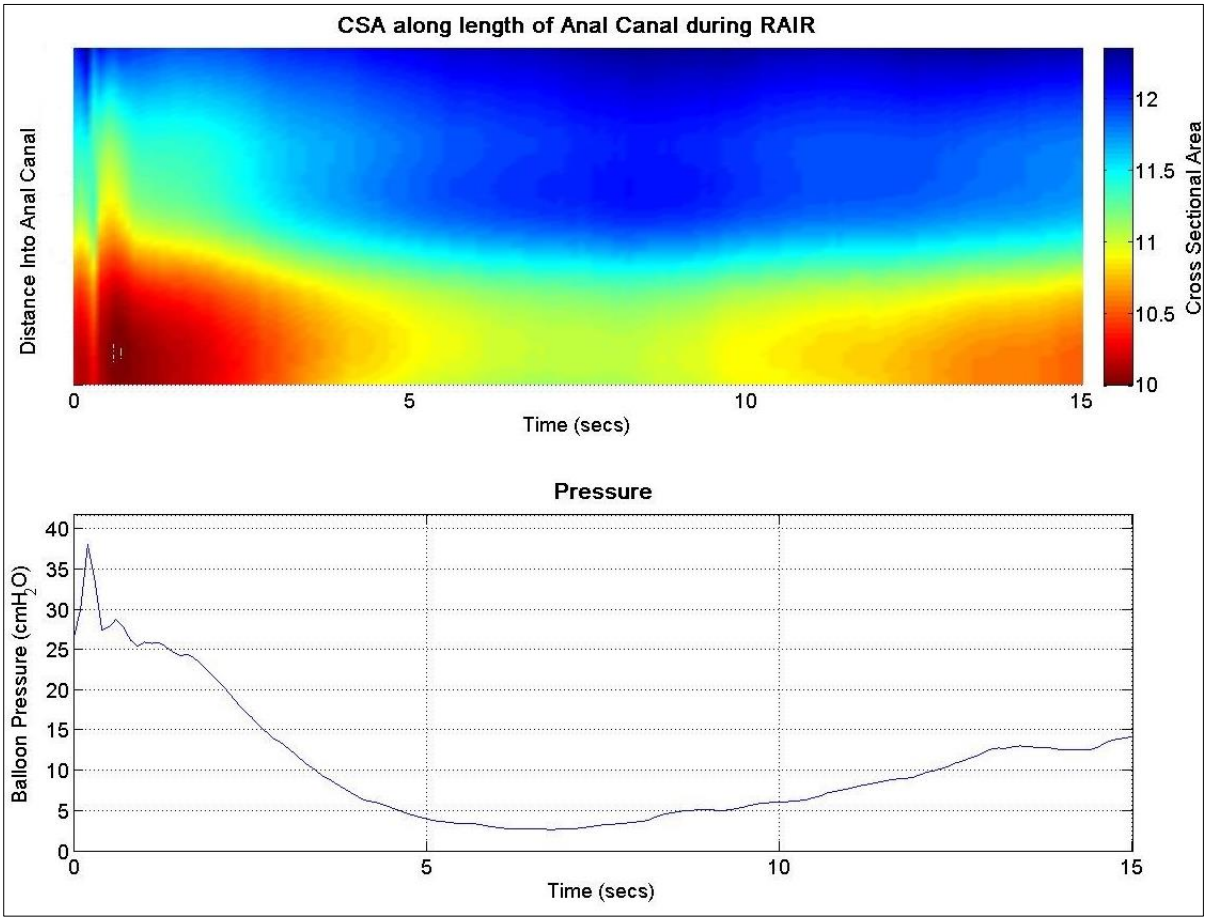


Figure 4.10: Changes in AC_{Dia} (above) and pressure (below) during RAIR phase in a single subject.

Changes during distension

Analysing the RAIR during incremental distension revealed a similar pattern, as mentioned above, at all the inflation volumes. After the initial closure of anal canal as a response to sudden inflation, it started opening up followed by closure after approximately 10 seconds. Although the lower-most part stayed resilient to opening with distension even at the highest volume, a generalised opening of entire anal canal was noted with increasing distension.

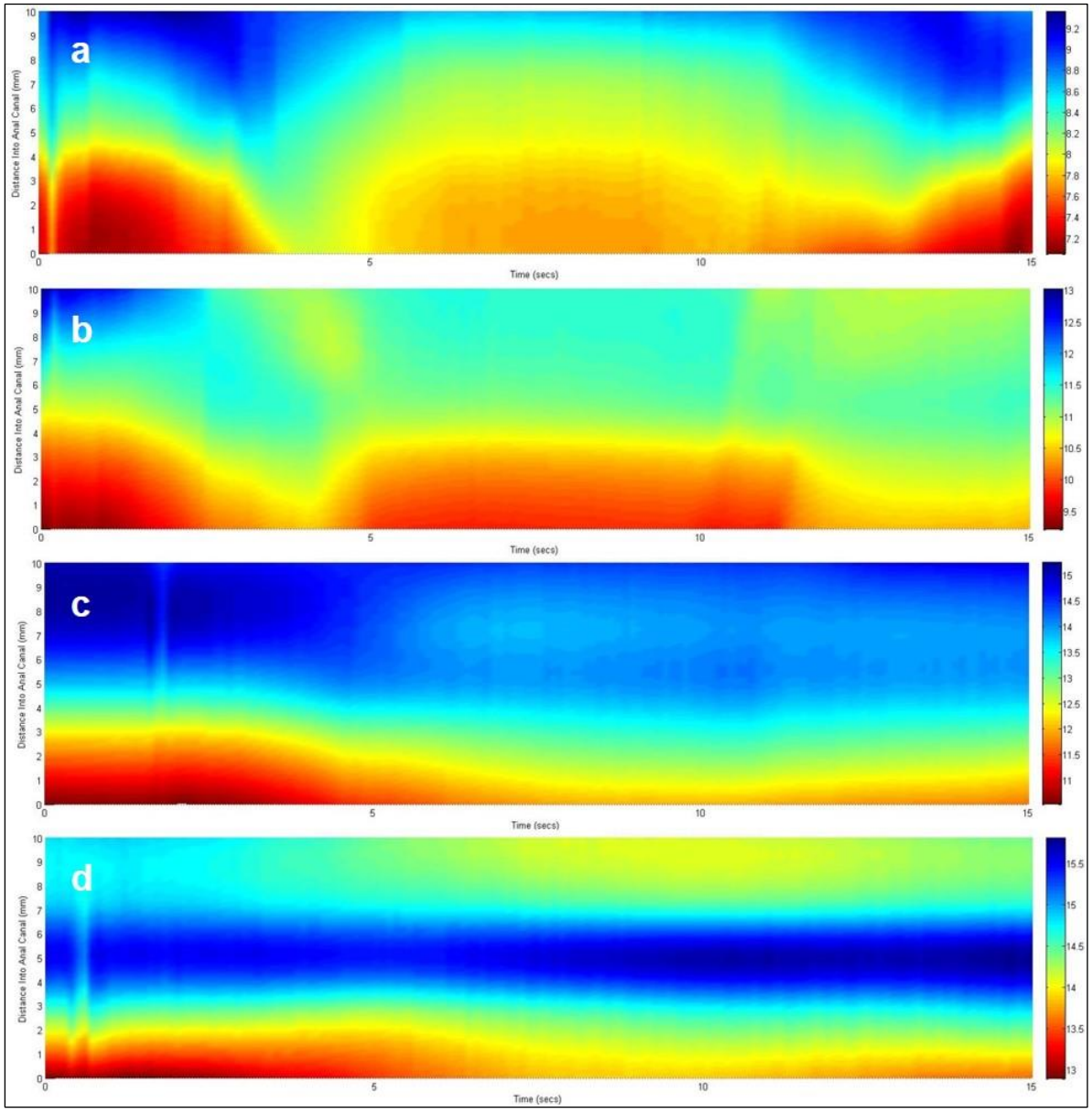


Figure 4.11: Changes in anal canal with distension during RAIR phase in a single subject using a 3cm probe. Each panel represents a different balloon inflation volume which increases by 1ml as we move down the image (from a to d).

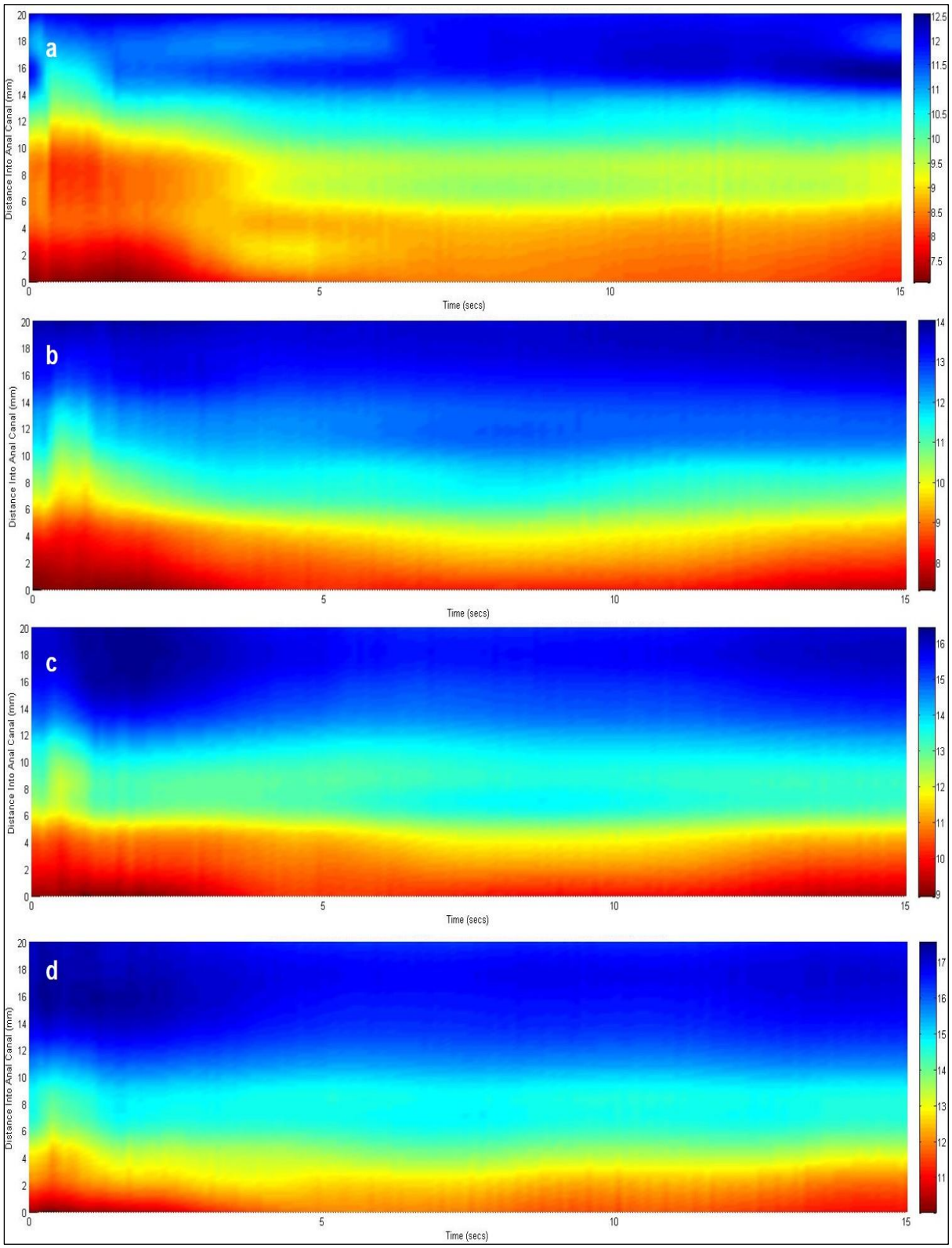
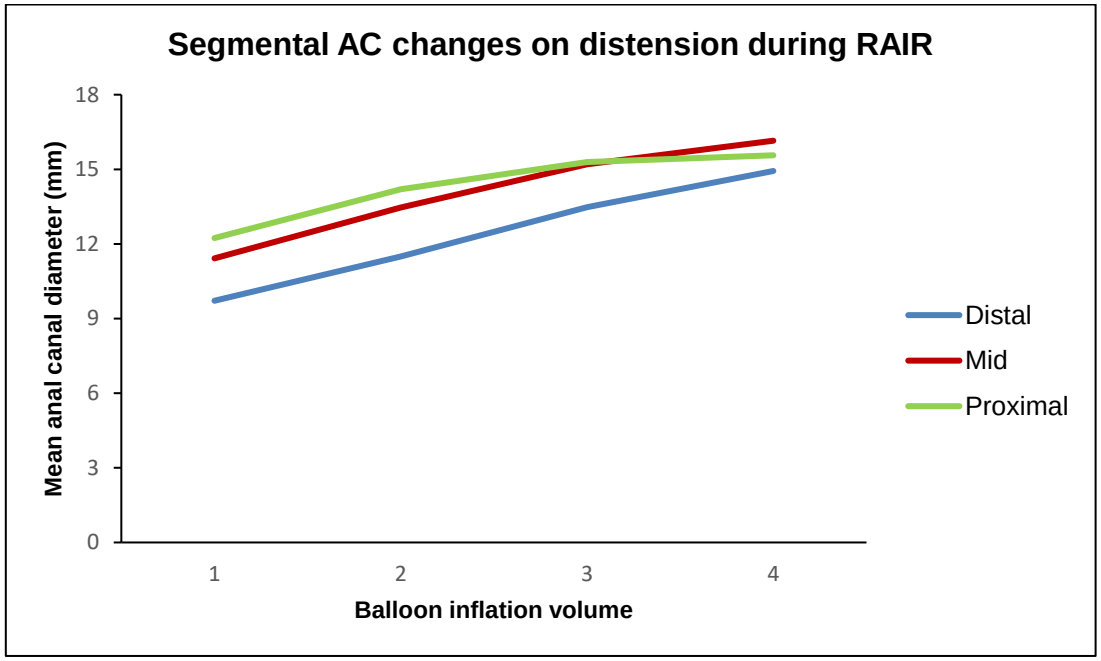


Figure 4.12: Changes in anal canal with distension during RAIR phase in a single subject using a 4cm probe. Each panel represents a different balloon inflation volume which increases by 1ml as we move down the image (from a to d).

Data for segmental changes due to distension during RAIR

The evaluation of segmental data revealed that the mean AC_{Dia} increased in the mid and distal anal canal segments with increasing balloon inflation volumes i.e. there was a progressive opening of distal and mid anal canal segments noted on distension. A similar pattern of progressive opening with distension was seen in the proximal anal canal as well. However, the mean AC_{Dia} of the proximal anal canal segment at the highest inflation volume was noted to be lower than that of the mid anal canal segment at highest inflation i.e. the proximal anal canal was more closed than the mid anal canal at maximal distension. The mean value for the mean AC_{Dia} for each of the four incremental balloon inflation volumes in the three anal canal segments respectively is shown below in fig 4.13.



Anal Canal segment	Mean AC diameter (mm) at incremental balloon inflation volumes			
	1	2	3	4
Distal	9.72	11.50	13.48	14.93
Mid	11.42	13.47	15.20	16.15
Proximal	12.24	14.19	15.30	15.56

Figure 4.13: Segmental differences in the mean anal canal diameter on subjecting anal canal to incremental distension during RAIR across all the healthy subjects; AC = anal canal.

4.4 Statistical analysis of segmental differences

The study so far has demonstrated that incremental distension of the anal canal leads to a progressive opening of all the three anal canal segments. The graphical representation (fig 4.5 & 4.9) and eyeballing the raw data revealed that the segmental differences in AC_{Dia} existed at all stages of distension (i.e at all the four balloon inflation volumes). Further statistical test were conducted to assess whether these segmental differences were significant.

ANOVA was the first test in this regard. ANOVA, stands for Analysis of Variance and is believed to be the appropriate test for comparing more than two groups. Although multiple t-tests have been erroneously used in some studies when comparing more than two groups, this is considered to be inappropriate as the repetition of multiple tests increases the probability of making Type 1 error [371]. The probability of making Type 1 error is set to 0.05 (5%) in a t-test. On conducting more than one t-test this probability keeps on increasing. However, this is not the case with ANOVA test as the probability of Type 1 error stays the same regardless of the number of groups being compared. Although a significant ANOVA ascertains that there is a significant difference between the groups, it does not identify where the differences actually exist. Hence, Post-hoc tests such as Least significant difference (LSD), Bonferroni, Tukey's etc are done to identify relationships between subgroups of sampled populations..

The results of ANOVA test are shown individually for each test phase in Table 4.6-4.8. A difference in the mean AC_{Dia} was noted in the anal canal segments and confirmed to be statistically significant by the ANOVA test (Resting phase p=<.001, Squeeze phase p=<.001, RAIR phase p=<.005). A Post-hoc test, (LSD) was done to identify the anal canal segments which differed from each other significantly. The common finding in the three different test phases (resting, squeeze & RAIR) was that mean AC_{Dia} of distal segment (closest to the anal verge) was significantly different from the mid and proximal anal canal segment (Resting phase p=.001 & <.001, Squeeze phase p=<.001 & <.001, RAIR phase p=.008 & .002). This was also the case between mid and proximal anal canal segments where the mean AC_{Dia} was found to be significantly different during the squeeze phase (p=.052) but not in resting or RAIR phase (Resting phase, p=.336, RAIR phase, p= .673), as evident below in Table 4.6-4.8.

ANOVA Resting Phase					
	Sum of Squares	**df**	**Mean Square**	**F**	**Sig.**
Between Groups	279.129	2	139.565	10.286	.000
Within Groups	3053.037	225	13.569		
Total	3332.166	227			

Post Hoc Test

Dependent Variable: Resting Phase **Test: LSD**

(I) Factor for anova	(J) Factor for anova	Mean Difference (I-J)	Std. Error	Sig.
Distal	Mid	-2.005835*	.597562	.001
	Proximal	-2.581396*	.597562	.000
Mid	Distal	2.005835*	.597562	.001
	Proximal	-.575561	.597562	.336
Proximal	Distal	2.581396*	.597562	.000
	Mid	.575561	.597562	.336

Table 4.6: ANOVA result for diameter difference in three anal canal segments during resting phase.

ANOVA (Squeeze phase)					
	Sum of Squares	**df**	**Mean Square**	**F**	**Sig.**
Between Groups	423.353	2	211.677	17.559	.000
Within Groups	2712.439	225	12.055		
Total	3135.793	227			

Post Hoc Test

Dependent Variable: Squeeze Phase **Test: LSD**

(I) Factor for anova	(J) Factor for anova	Mean Difference (I-J)	Std. Error	Sig.
Distal	Mid	-2.17781*	.56324	.000
	Proximal	-3.27945*	.56324	.000
Mid	Distal	2.17781*	.56324	.000
	Proximal	-1.10164	.56324	.052
Proximal	Distal	3.27945*	.56324	.000
	Mid	1.10164	.56324	.052

Table 4.7: ANOVA result for diameter difference in three anal canal segments during voluntary contraction phase.

ANOVA (RAIR phase)					
	Sum of Squares	**df**	**Mean Square**	**F**	**Sig.**
Between Groups	163.933	2	81.967	5.627	.004
Within Groups	3277.702	225	14.568		
Total	3441.635	227			

Post Hoc Test				
Dependent Variable: RAIR Phase			**Test: LSD**	
(I) Factor for anova	(J) Factor for anova	Mean Difference (I-J)	Std. Error	Sig.
Distal	Mid	-1.65385*	.61916	.008
	Proximal	-1.91509*	.61916	.002
Mid	Distal	1.65385*	.61916	.008
	Proximal	-.26124	.61916	.673
Proximal	Distal	1.91509*	.61916	.002
	Mid	.26124	.61916	.673

Table 4.8: ANOVA result for diameter difference in three anal canal segments during RAIR phase.

The complete spread of all the data points is displayed with the help of box and whisker plot in figure 4.14. The three different plots represent three different test phases. In each test phase, there are 76 data points in each anal canal segment, taking into account 4 different inflation points for each of the 19 healthy subjects. The whiskers show the lowest and the highest data points and therefore provide the range for the data points. The middle line in the box represents the mean and the small green lines adjacent to the mean, represent the 95% confidence interval, i.e. ± 2 SD points for the mean.

The significant point to be noted in the figure is a clear downward trend in the AC_{Dia} on moving from proximal to distal anal canal (i.e on moving out towards anal verge). This is observed during all the three phases. In other words, the lower-most anal canal (nearest to the anal verge) is the most closed part at all times.

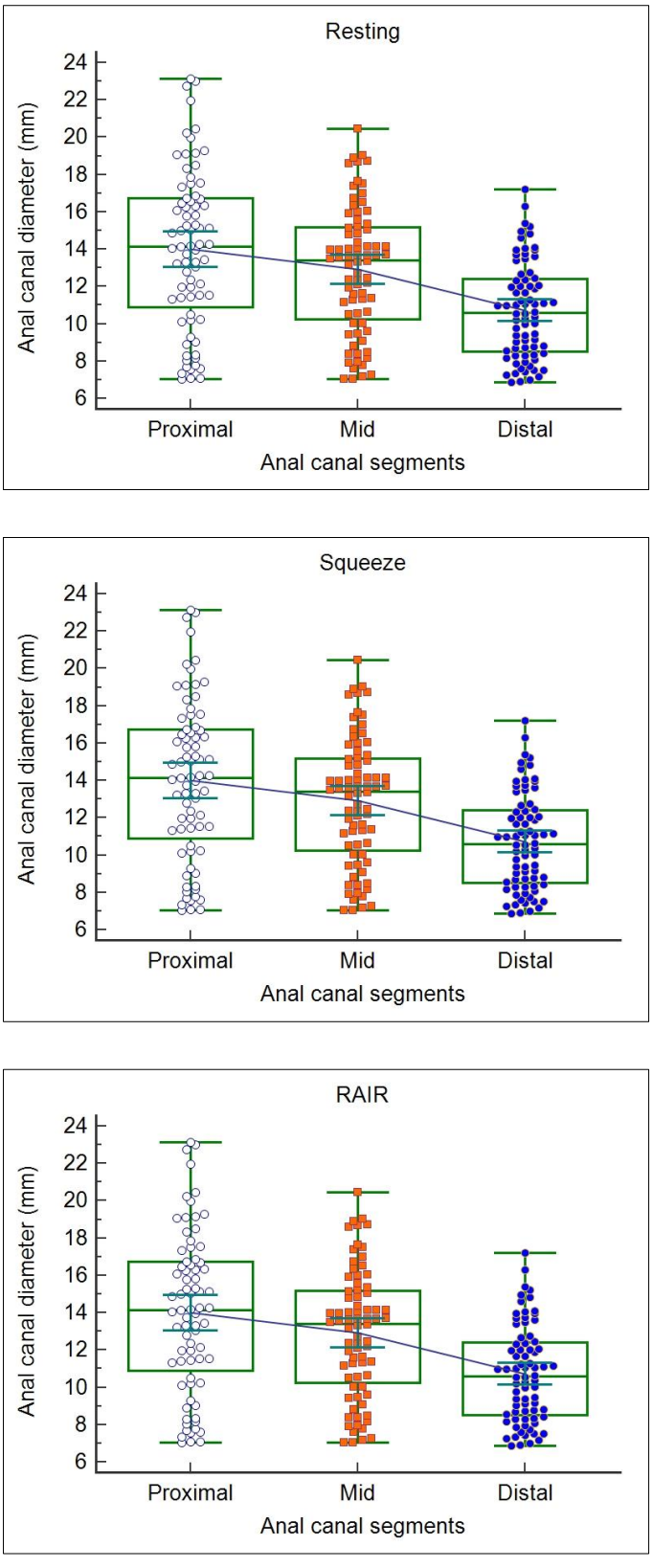


Figure 4.14: Segmental differences in the anal canal during different test phases, across all inflation volumes in the healthy cohort

4.4 Normality range

The EndoFLIP being a new technology in the field of anorectal physiology does not have a pre-existing normality range for the healthy cohort. Moreover, we used custom-made probes which have not been used in any other centre. The main purpose for establishing a normality range was to have a parameter to differentiate healthy subjects from FI patients.

The common formula to calculate the reference range is: mean ± 1.96x SD. However, with the data divided into three parts, one for each anal canal segment, and the segmental variation already confirmed it did not seem appropriate to merge the data together in order to obtain a normality range. Furthermore, having normality range for each anal canal segment would have made the process needlessly tedious. As such it was decided, to use ratio from the mean AC_{Dia} of the two anal canal segments with the highest degree of variance.

The study so far has already confirmed the highest variance to be between AC_{Dia} in the proximal (upper-most part of anal canal) and distal (lower-most part of anal canal) anal canal. As such, it was decided to calculate a ratio from these anal canal segments (during the resting phase) to obtain a normality range. This ratio was calculated for each subject in the study population (19 healthy subjects) and a mean was then calculated of all these ratios. This was called Mean Ratio $_{(Proximal: Distal)}$ (written as MR $_{(P: D)}$ in rest of the thesis).

In order to meet the rationale behind developing the normality range, i.e. to differentiate healthy subjects from FI patients, it was believed that the process would become straightforward if we used the data points from when the anal canal was subjected to maximal stress (with maximal distension). Therefore, the mean AC_{Dia} ratio between the proximal and distal anal canal segments during rest phase, at highest inflation volume only, from all the three different sized probes, was used to establish the normality range.

Analysis of the mean ratio data from 19 healthy volunteers, done using Q_Q plots in SPSS (discussed in chapter 2) found it to be normally distributed. The value of MR $_{(P: D)}$ was established as 1.18 mm^2, Min 0.92 mm^2, Max 1.82 mm^2, 95% CI, 1.01 – 1.29. The complete data points can be seen in figure 4.15. The MR $_{(P: D)}$ is shown by the long blue horizontal line and the 95% confidence interval limits are shown by the green horizontal markers in the figure.

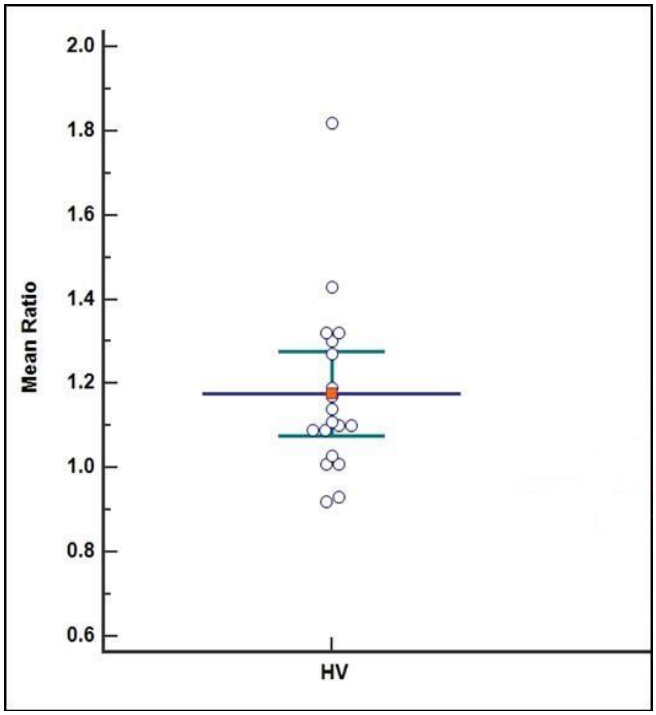


Figure 4.15: Data plot showing mean AC_{Dia} ratios (proximal : distal anal canal) for the healthy cohort

4.5 Conclusion

EndoFLIP is a relatively new diagnostic modality in the field of anorectal physiology. In the last one decade it has primarily been used in the upper GI physiology studies and as such the limited studies that used it to study anorectal physiology used the longer probe, initially intended for use in upper GI studies.

We used the custom-made probes of three separate lengths (2, 3 & 4 cm) corresponding to the shorter functional length of the anal canal. The earlier study (in chapter 3) had already proven the test-retest repeatability of these custom-made probes in in-vitro settings. However, this being a pilot study it was deemed essential to check the repeatability in humans as well. The two statistical tests, ICC and Bland-Altman's plots both helped confirm high levels of test-retest repeatability of these custom-made EndoFLIP probes, thereby paving way for their further use in this clinical study.

The next part of this study involved validating the use of EndoFLIP technology in anorectal physiology studies. The following discussion is aimed to discuss our findings in this regard as well as highlight their significance.

The most significant finding of this study was the heterogeneity of the anal canal, revealed by dynamic testing with the EndoFLIP. This establishes a key difference between EndoFLIP and anorectal manometry which assumes anal canal to have a static functionality. The dynamic nature of the anal canal was apparent both at rest and during the anal canal distension. For instance, during resting phase, EndoFLIP revealed that the anal canal was more closed in the lower and mid part than the upper part. This implies that the pressure being generated at different points in anal canal would also be different and highlights the drawback of anorectal manometry which provides a single pressure reading for whole of the anal canal. Furthermore, on subjecting anal canal to distension (by increasing the balloon inflation volume) it started opening up in the upper and mid part whereas the lower anal canal segment remained resilient to a change, clearly demonstrating that the biomechanical properties differ in the three anal canal segments.

The resistance of the lower-most anal canal segment to open with distension indicates a lower compliance of this segment. Compliance is essentially a pressure-volume relationship and can be summarised as the volume change accompanying change in the pressure [372]. A highly compliant segment will distend easily (increase in volume) for a given pressure change whereas a low compliance would mean that the segment resists distension. EndoFLIP allows determining the anal canal compliance by plotting the AC_{Dia} and the intraluminal pressure. Thus, a low AC_{Dia} and high-pressure zone seen with EndoFLIP in the lower anal canal segment (i.e. segment closest to the anal verge) indicates a low compliance as it offers higher resistance to distension. The proximal anal canal (away from the anal verge), on the other hand, was found to have higher compliance as it opened up easily on balloon inflation. Similar findings have been reported by Gregersen et al. and Jung et al. in their respective studies [373,374]. Whereas Gregersen et al. used impedance planimetry; Jung et al used 3D Ultrasound imaging to determine that the distal anal canal was less compliant as compared to the proximal anal canal during rest and voluntary contraction. This is quite a significant finding as it establishes the key role played by the distal-most anal canal in maintaining continence, by resisting involuntary opening despite an increase in pressure.

Similar results were also reported by Goes et al. in their study which used vector manometry to compare the highest mean resting pressure (HMRP) segment in the anal canal of healthy subjects and of patients with impaired sphincter function [17]. The study observed that the HMRP was located significantly more distally in controls than in the incontinent patients. EndoFLIP in the current study revealed a lower AC_{Dia} in the distal anal canal of healthy subjects thereby explaining the high resting pressure observed by Goes et al. in the distal anal canal segment of the healthy subjects.

Further to the above, significant findings were also noted during the voluntary contraction. During squeeze phase, we didn't observe a uniform geometric profile of the anal canal, again confirming the dynamic status of the anal canal. There was a decrease in AC_{Dia} in almost the entire anal canal which corresponds to the anatomical presence of puborectalis muscle (PRM) at the proximal and EAS in the mid and distal anal canal. The role of PRM in genesis of anal canal pressure and anal canal closure has already been reported in literature. Our data seems to support the finding, of these recent studies, that the puborectalis muscle plays a part in the anal canal closure [16,374,375].

In the mid part of anal canal, voluntary contraction led to a prominent closure but not as marked as in the lower-most anal canal segment. It is likely that the presence of IAS in the mid anal canal makes it less compressible and hence the closure is not as marked as in the lower-most anal canal where the only muscle present is EAS.

These findings of marked anal canal closure in the lower-most anal canal segment are clearly evident in the colour plots (Fig. 4.7- 4.8) and also confirmed by the least AC_{Dia} in the segmental variation plots (Fig. 4.11). A more pronounced CSA decrease in the lower-most anal canal segment during voluntary contraction was also observed by Alqudah et al. in their study using EndoFLIP [376]. Similar results were also observed in the study by Jung et al. who noted that the narrowest zone of anal canal moved towards the distal end of anal canal during voluntary contraction [374].

Two Danish studies from the same centre, using EndoFLIP to study anorectal physiology, reported no significant change in CSA across the different anal canal segments during voluntary contraction [240,255]. However, our study results do not agree with this finding. The ANOVA test checking for segmental variation found a significant difference in between distal and mid and distal and proximal anal canal segments but not in between mid and proximal anal canal. The likely reason for this result in Danish study is the use of longer probe which sat in the rectum proximally and outside the anal verge distally. As the liquid takes path of least resistance, the fluid in the EndoFLIP probe balloon would have been pushed towards both ends, thereby resulting in error of CSA measurements. This error was avoided in our study by the use of shorter length probe which stayed in the anal canal only.

Moving onto the RAIR phase, a brief closure of anal canal was observed as a result of the sudden rectal distension by inflation of the balloon. The brief contraction of the external anal sphincter as a result of rectal distension, rectoanal contractile reflex (RACR), is well documented in literature [377-379]. EndoFLIP clearly revealed this resultant EAS contraction, with the maximal closure seen in the mid and the lower anal canal corresponding to the

anatomical position of the EAS. This brief closure of anal canal was followed by its opening, corresponding to the well-known RAIR reflex [27] in which there is transient relaxation of the internal anal sphincter. EndoFLIP revealed opening of the anal canal to be most prominent in the upper and mid part, which is consistent with the anatomical position of IAS. This anal canal opening was also evident from the pressure graph which displayed a clear drop in pressure as a result of IAS relaxation (Fig 4.5).

During the opening of the anal canal, the lower-most segment was found to have the lowest AC_{Dia} (i.e much more closed) as compared to the rest of the anal canal. This is likely to correspond to the EAS contraction, as expected during sampling, to prevent incontinence. During sampling reflex, the transient relaxation of IAS allows the descent of rectal contents into upper anal canal. The highly specialized sensory epithelium in this region helps differentiate between gas/liquid/solid contents, thereby allowing evacuation as per the social circumstances. Continence during sampling reflex is maintained due to the reflex closure of distal most anal canal by EAS. These physiologic findings were confirmed by EndoFLIP, which demonstrated the lower-most anal canal segment (corresponding to the EAS muscle anatomically) to be the most closed part of the anal canal during the RAIR phase.

EndoFLIP clearly demonstrates a number of significant changes in the anal canal on activation of rectoanal inhibition reflex. However, all the currently existing studies that have used EndoFLIP to assess the anorectal physiology have used the longer probes, originally built for studying gastro-oesophageal junction. It is our understanding that the foremost purpose of developing the impedance planimetry technique for anorectal studies is to obtain a more accurate assessment of the anal sphincters. However, inadvertent activation of RAIR in the aforementioned studies, due to the use of a longer probe lying in the rectum, cannot be excluded and as such their results should be viewed with caution.

RAIR reflex, consists of relaxation of IAS in response to rectal distension [213]. Manometric studies, using latex balloon have shown that although the balloon inflation volume, required to achieve the highest amount of pressure drop due to activation of RAIR can be between 30-40 ml, first detectable drop in pressure occurs at even a small inflation volume of 10 ml [214,257]. Although not directly comparable to the EndoFLIP catheters used by Luft et al. it is clearly evident that the distension of rectal part of the EndoFLIP catheters would have led to some degree of RAIR activation, thereby altering the results obtained. Moreover, there is no doubt that the distended probe coming out of anal canal would have also altered the biomechanical properties in the distal-most anal canal. An earlier in-vitro study using the impedance planimetry technique reported that the CSA measurements were not significantly affected if the excitation pair of electrodes was within the area being measured [242]. However, this was

not possible in the studies by Luft et al. and Alqudah et al. as the probe was considerably longer than the anal canal being measured with the excitatory electrodes positioned in the rectum and outside the anal verge.

Luft et al. state that the balloon diameters used in their study was much smaller than those found necessary to elicit the rectoanal inhibitory reflex in an earlier study from the same centre [240]. The previous study that they refer to primarily compared rectal wall properties in acute and chronic spinal cord injury patients with those of healthy volunteers by means of rectal impedance planimetry [256]. It also aimed to obtain additional information about the rectoanal inhibitory reflex in these groups. However, in the results section of their previous study, only the distension pressures which activated the RAIR are mentioned with no reference to the balloon diameter used, thereby making it difficult to comment upon. Moreover, the probe used in the two studies are not directly comparable, as the earlier study used a smaller intrarectal balloon whereas the later studies used the longer 12 cm balloon with its distal end placed in the rectum, mid part in the anal canal and proximal end outside the anal verge.

In conclusion, the explicit demonstration of anal canal physiology has undoubtedly validated EndoFLIP technology for future studies in this field. The fundamental difference in this study was the development of a smaller probe better suited to the anorectum, allowing us to overcome the shortcomings of the previous studies. Furthermore, the three different sized probes allowed for anal canal balloon to be chosen as per the functional length of the individual's anal canal thereby allowing better accuracy. EndoFLIP's unique property of providing real-time demonstration of the anal physiology and segmental details gives it an edge over the existing investigation techniques such as anorectal manometry which consider anal canal to be a static entity. Further studies are required to determine its clinical effectiveness in different groups of patients suffering from faecal incontinence.

Chapter 5

Results: Incontinent group

5.1 Faecal incontinence group

This group comprised of 16 patients suffering from faecal incontinence despite having morphologically intact anal sphincters (confirmed on endoanal ultrasound). The main aim was to study the biomechanical properties of the anal canal in this group of faecally incontinent patients and compare the results to those of the healthy subjects.

The demographics and symptomatic severity are shown in the table 5.1. A chi squared test was undertaken to determine if there was any significant difference between males and females with regard to their age and faecal incontinence score. This failed to demonstrate any significant difference as evident by a high p value in the table

Variable	Male	Female	p value
n =	9	7	
Mean Age (Range)	66 (51-85)	64 (53-84)	0.60
Mean Wexner Score (Range)	14.3 (12-18)	15.1 (13-17)	0.27

Table 5.1: Demographics of subjects in group 2

Independent t-test was utilised to check if there were any segmental variation in the anal canal of the faecally incontinent patients as compared to the healthy subjects. T-test is a widely used statistical test for significance/hypothesis testing between two groups. The two types of t-test - an independent t-test and the paired t-test, are used according to the characteristics of the two groups under comparison, whether they are independent of each other or dependent on each other. In dependent groups, same participants are tested more than once to obtain "within-subjects" or "repeated-measures" outcome. Thus, in the paired t-test, "dependent groups" indicates that the same participants are present in both groups. On the other hand, participants in the independent groups are not related to each other in any way. Independent t-test was used in this study as the two study groups (healthy subjects and faecally incontinent patients) were completely independent of each other.

The MATLAB software was used as the next step to visualise the data with the help of colour plots and conduct an exploratory data analysis.

5.2 Results

The qualitative data analysis of FI patients with MATLAB revealed a considerably different picture in comparison to the healthy subjects. The contour plots were constructed for all the three-test phase (resting, squeeze and RAIR) and are shown below. The details of MATLAB software have previously been discussed in chapter 2.

5.2.1 Resting phase

Resting phase analysis revealed quite a different pattern to that seen in the healthy subjects. In faecal incontinence patients, the anal canal was found to be closed only in the lower-most segment as opposed to almost complete or mid and lower-most anal canal segment closure noted in the healthy cohort. Moreover, with increasing inflation volumes this lower-most segment also started opening up. This is in contrast with the healthy subjects in whom the lower-most part remained closed even at the highest inflation volume. This opening of the anal canal was quite prominent at the highest two inflation volumes. Colour plots are shown in Fig. 5.1 & 5.2 for two different subjects demonstrating these findings.

In both the figures, lower-most part of the anal canal is seen closed at the lowest inflation volume as demonstrated by the red colour. With an increase in the inflation volume the mid and lower anal canal segments start opening up, as evident by replacement of colours to lighter shades.

Another interesting thing to note is the presence of a prominent red coloured band in the top part of the images on increasing inflation volume. This is likely to represent a contraction of the puborectalis muscle. The puborectalis muscle is a part of the levator ani muscle and forms a sling around the rectum and upper anal canal. It has been shown to function along with EAS to maintain continence. As the mid and lower anal canal segments start opening with an increase in distension, a puborectalis contraction kicks in, likely as an attempt to close the anal canal. Keeping in with the anatomical position of puborectalis muscle this anal canal closure is seen in the upper and mid anal canal segment only.

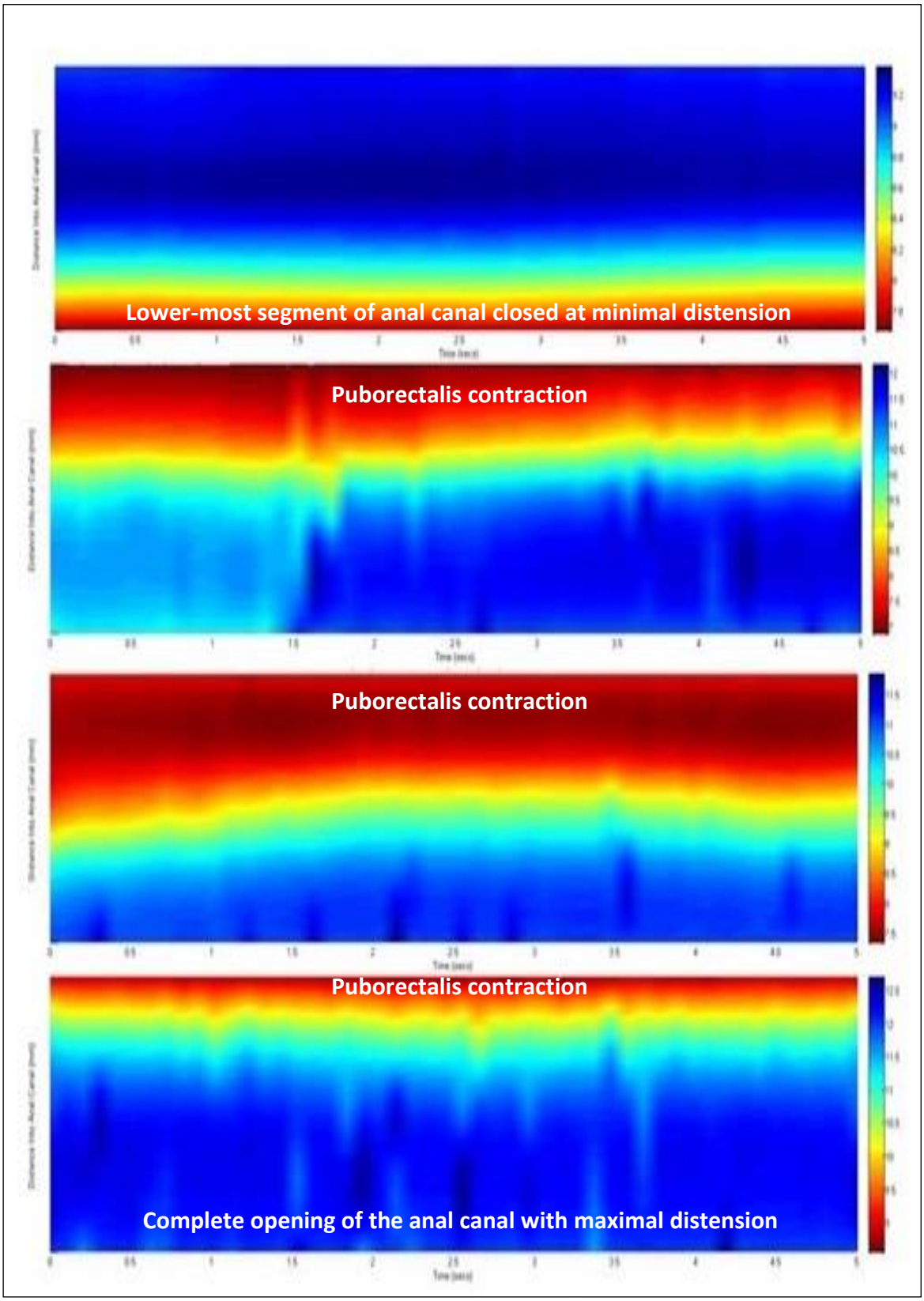


Figure 5.1 Complete opening of lower anal canal seen with increase in balloon inflation volumes during resting phase in a single subject. Upper red band in the image represents puborectalis contraction

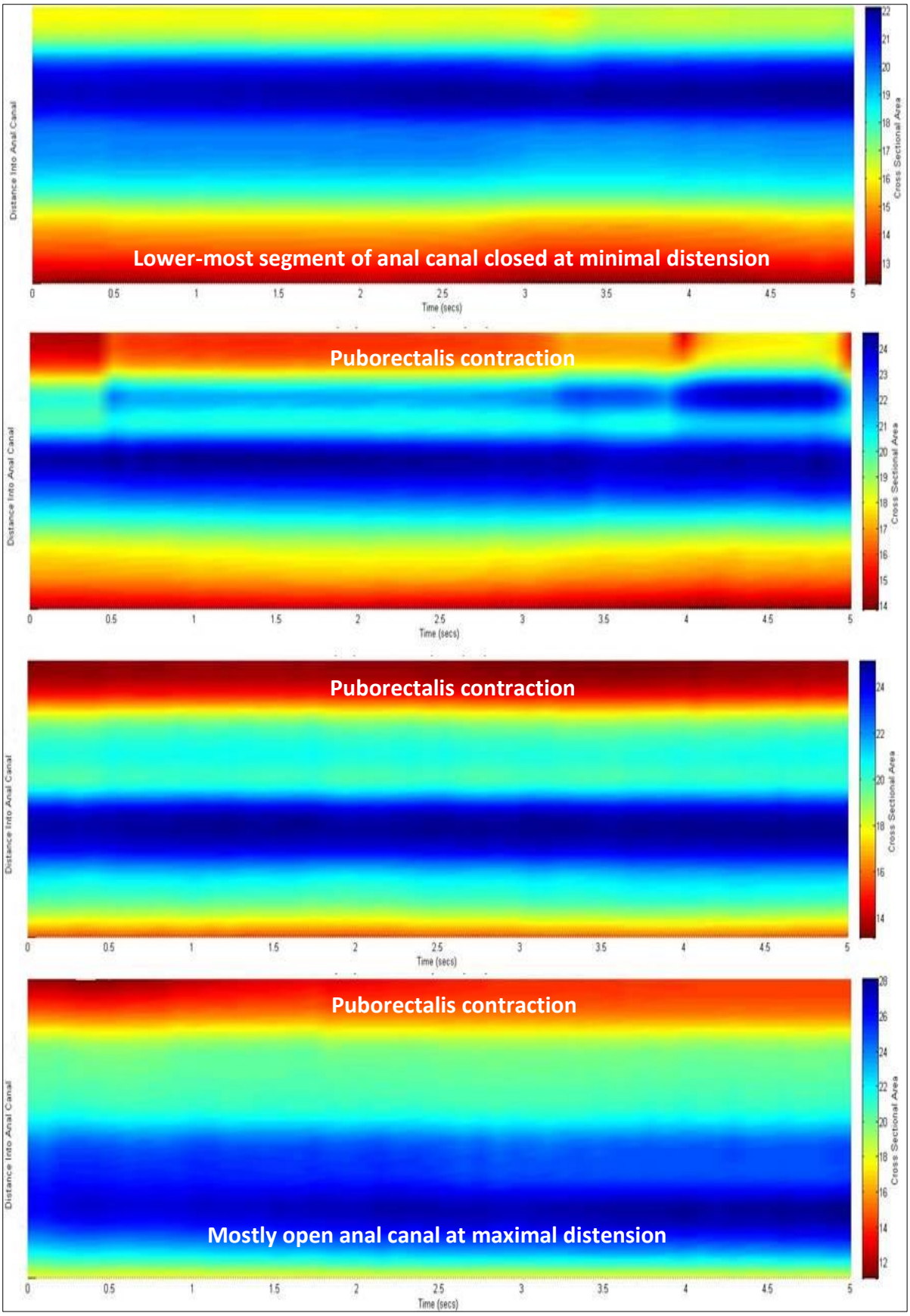
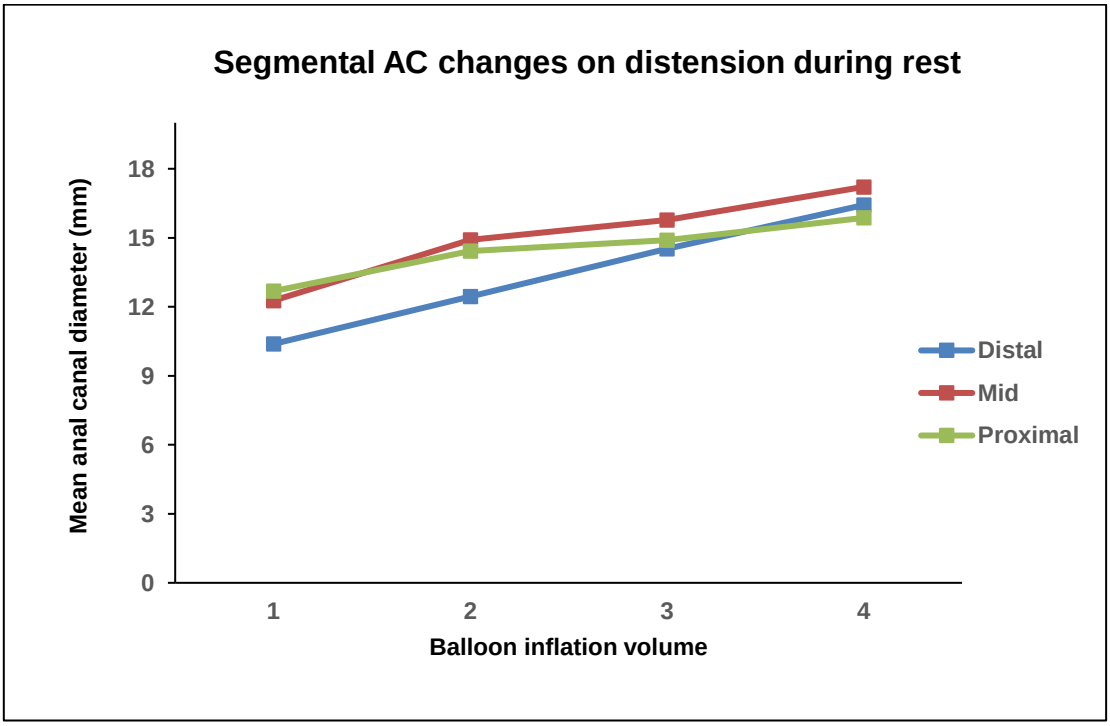


Figure 5.2: Complete opening of lower anal canal seen with increase in balloon inflation volumes during resting phase in a single subject.

Data for segmental changes due to distension during rest

The contour plots have already revealed the opening up of anal canal with distension. A further detailed analysis of segmental changes revealed that an incremental distension led to a progressive opening of the lower-most anal canal. A similar finding was seen in the mid anal canal segment but not as prominently as was noted in the healthy subjects. In the upper-most (proximal) anal canal the opening was even less prominent than in mid anal canal. Furthermore, the Mean AC_{Dia} of proximal anal canal segment reduced below that of the mid anal canal segment with an increase in distension i.e. increasing balloon inflation volume led to significantly more closure of upper anal canal in comparision to mid anal canal. At the highest distension, the Mean AC_{Dia} of the proximal anal canal was even lower than the Mean AC_{Dia} of the distal anal canal, signifying a greater closure of anal canal in the upper part (as compared to the lower-most anal canal). The graph showing these changes alongwith the raw data is displayed below in Figure 5.3.



Anal Canal segment	Mean AC diameter (mm) at incremental balloon inflation volumes			
	1	2	3	4
Distal	10.39	12.44	14.53	16.42
Mid	12.27	14.91	15.77	17.21
Proximal	12.68	14.43	14.90	15.87

Figure 5.3: Segmental differences in the mean anal canal diameter on subjecting anal canal to incremental distension during resting phase across all the subjects in group 2; AC = anal canal

5.2.2 Squeeze phase.

The standard pattern of closure of the lower-most anal canal segment during voluntary contraction seen in the healthy subjects was not replicated in this group. In fact, there was no single pattern that was seen amongst all the subjects in this group. The contraction of sphincter muscles occurred in quite a disorganised manner as evident in Figure 5.4.

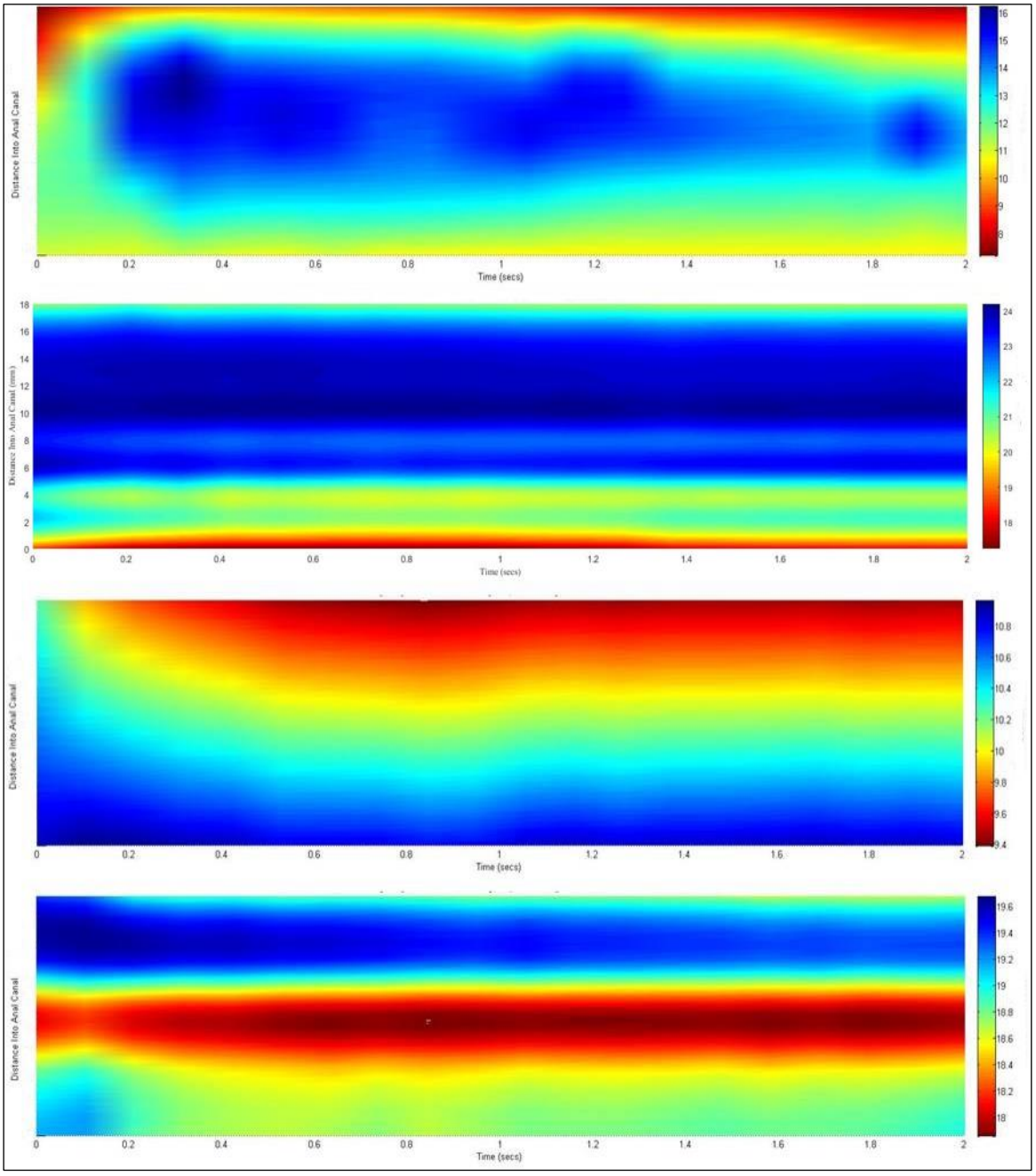


Figure 5.4: Colour plots showing diverse squeeze patterns in four different subjects from group 2

Changes during distension

Although no single pattern was noted in all the subjects, a cluster of patients was noted to have similar trends. In eleven subjects (69%) there was a complete or near complete opening of lower-most anal canal noticed with incremental distension (Fig 5.5, Fig 5.6). This was different from the healthy cohort in which anal canal opening was seen with incremental distension but not to this extent.

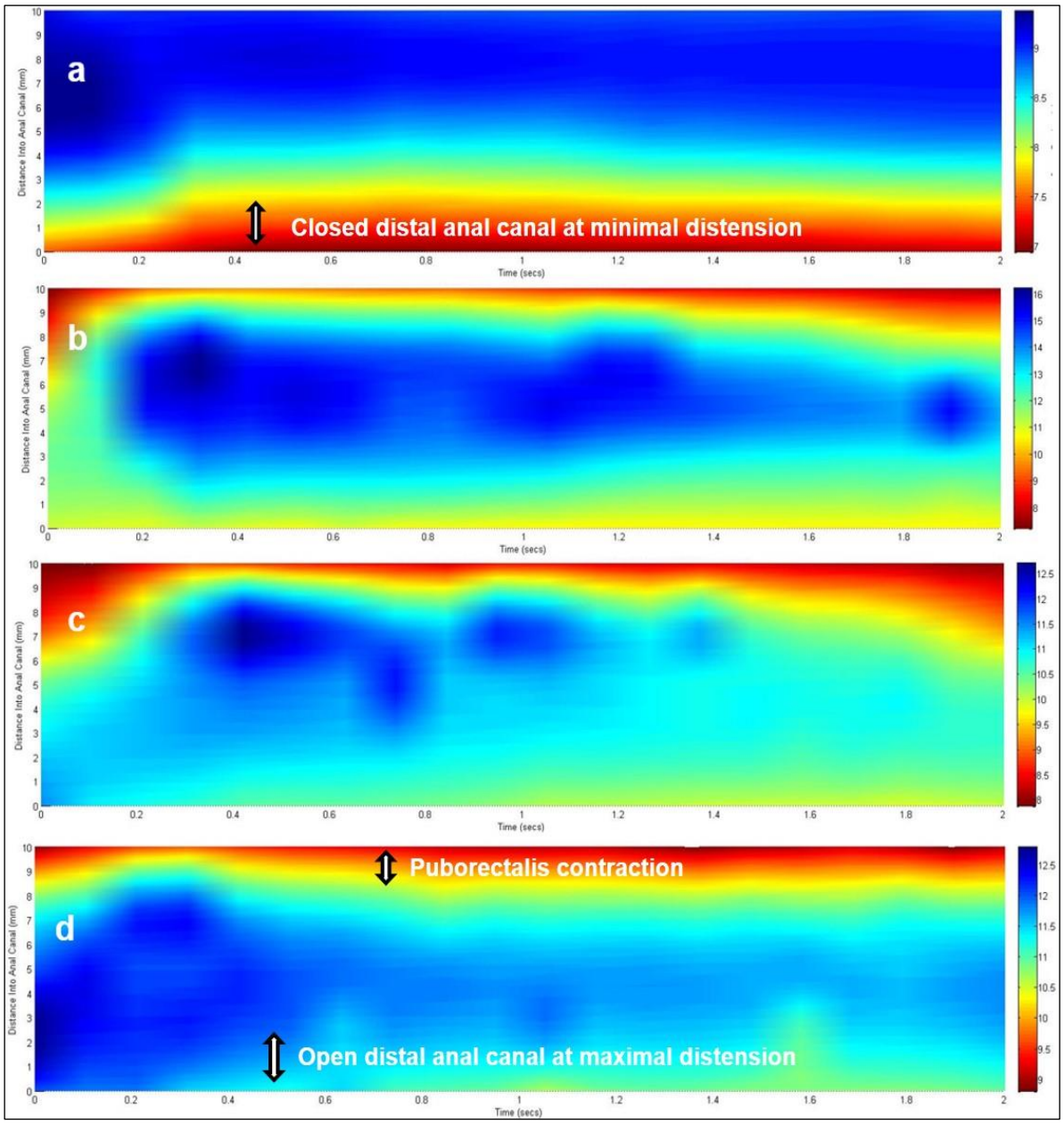


Figure 5.5: Opening of distal anal canal with incremental distension during voluntary contraction in a single subject using a 4 cm probe. Each panel represents a different balloon inflation volume which increases by 1ml as we move down the image (from a to d).

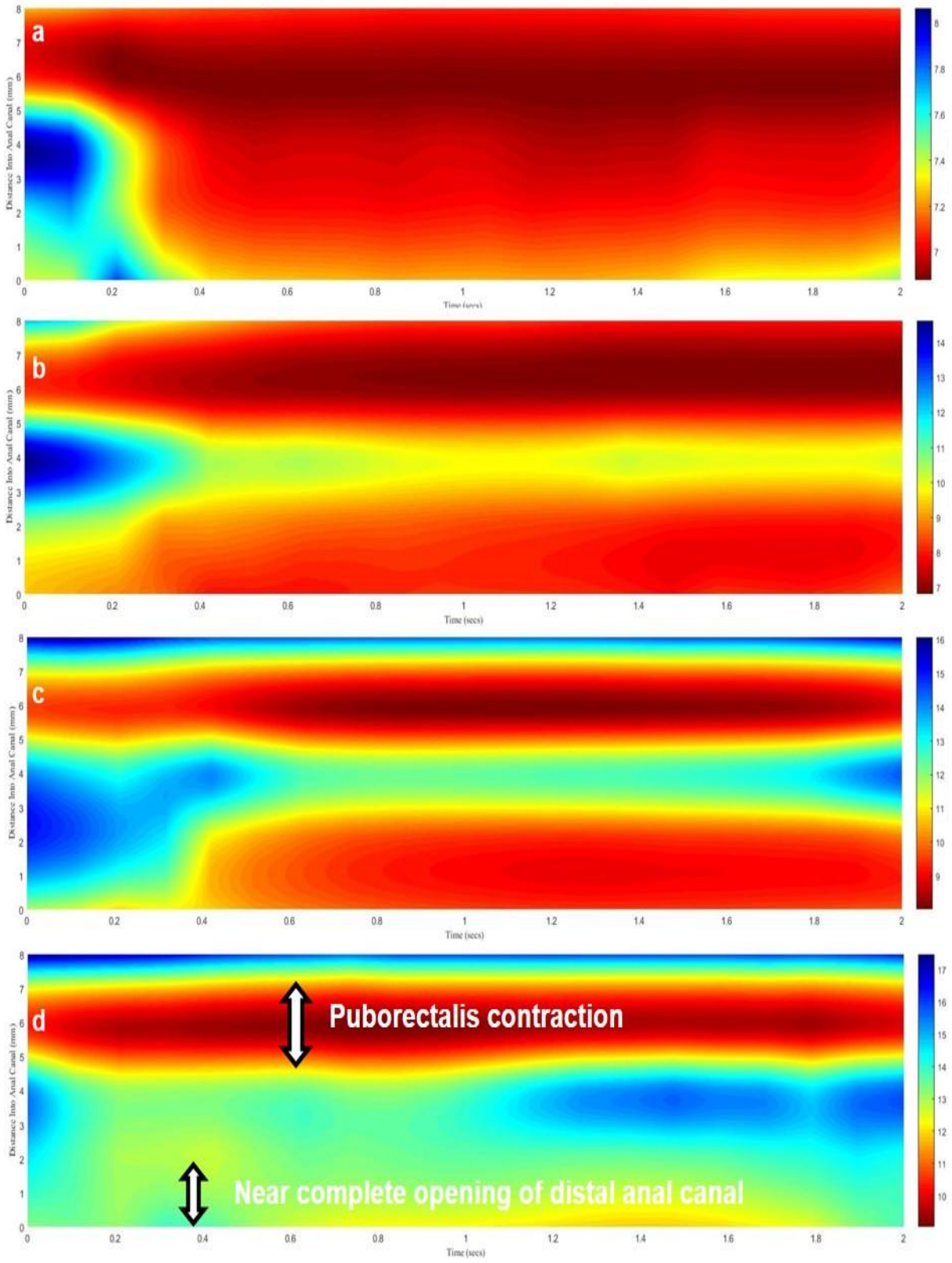


Figure 5.6: Near complete opening of distal anal canal with incremental distension during voluntary contraction in a single subject using a 3 cm probe. Each panel represents a different balloon inflation volume which increases by 1ml as we move down the image (from a to d).

An interesting point to note was that this pattern of proportionate opening of anal canal on subjecting it to incremental distension was not evident in all the patients. Instead there was a sudden and disproportionate complete opening of the anal canal noted in several patients with just a minimal increase in distension (by 1ml). This is displayed in the plots below from two different patients (Fig. 5.7).

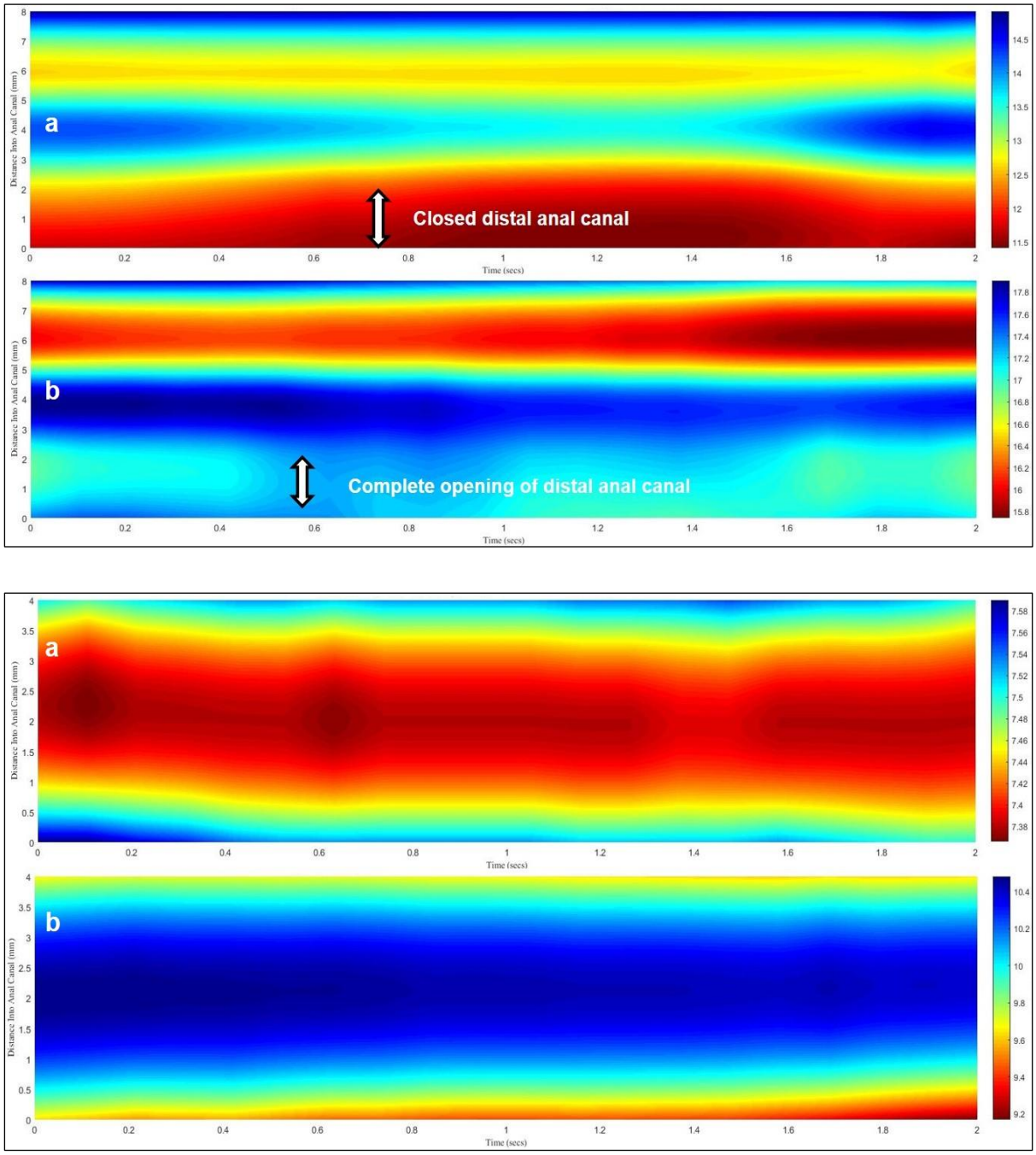


Figure 5.7: Sudden and complete anal canal opening, disproportionate to distension in two separate patients. The balloon inflation volume only increases by 1ml as we move down the image (from a to b).

Another interesting pattern picked up during voluntary contraction was closure of upper-most and mid anal canal segment on distension in 63% of the subjects (ten of sixteen). This is evident as change to warmer colours in the upper and mid portion in Fig. 5.8 & 5.9. This represents a possible puborectalis contraction which was also seen on distending the anal canal at rest in the FI patients.

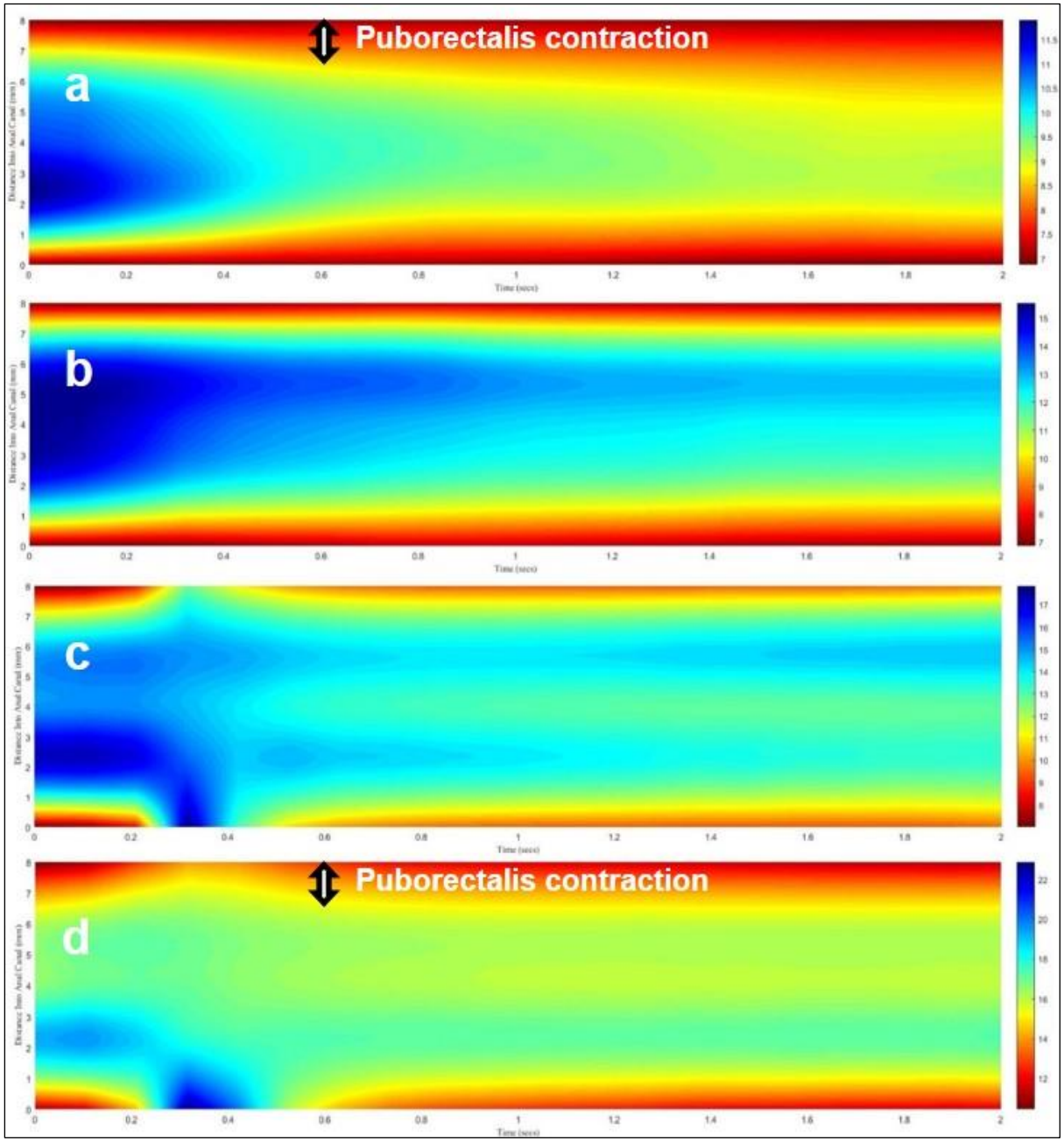


Figure 5.8: Closure of proximal and mid anal canal with distension due to puborectalis contraction during voluntary contraction in a single subject using a 4 cm probe. Each panel represents a different balloon inflation volume which increases by 1ml as we move down the image (from a to d).

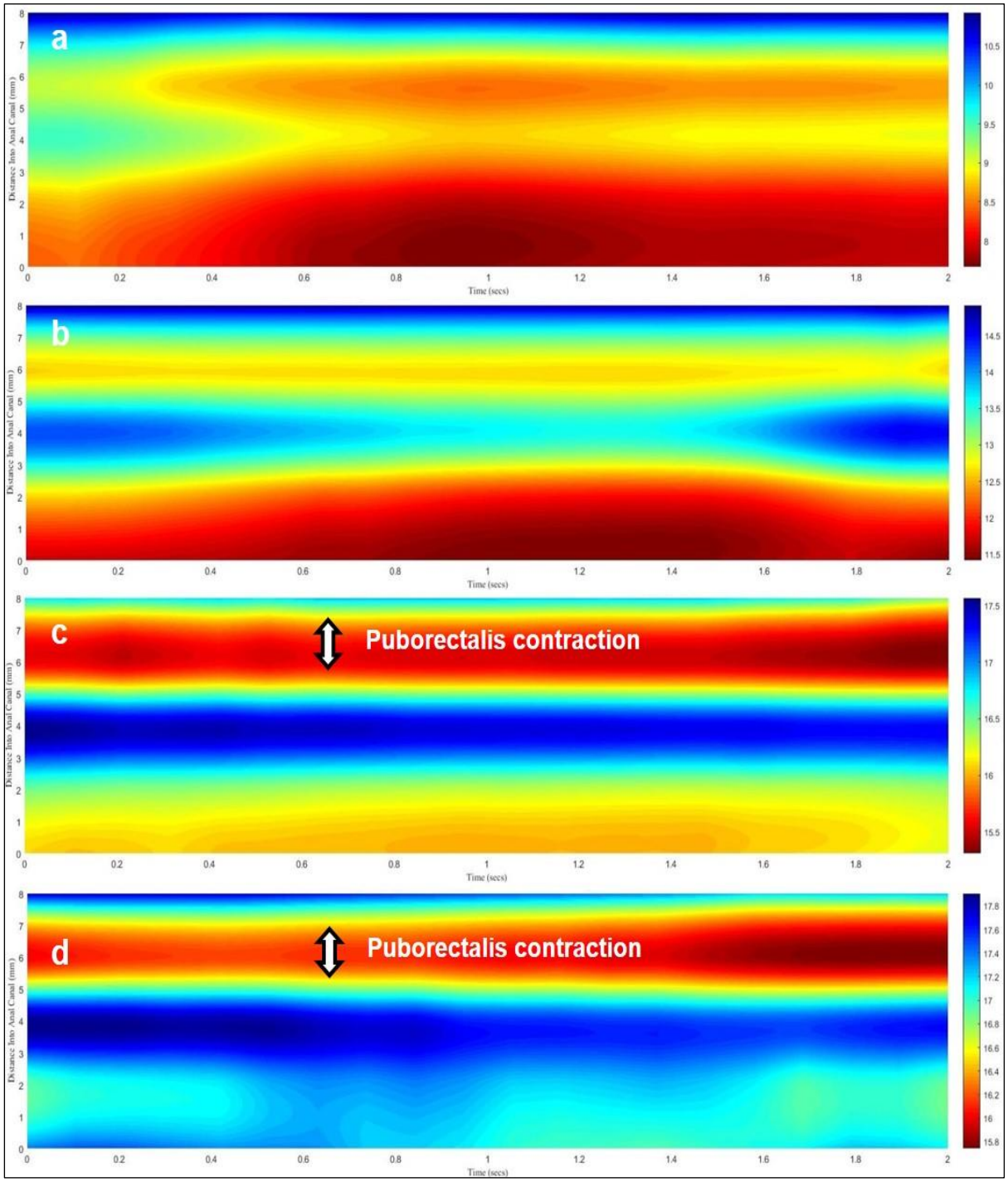
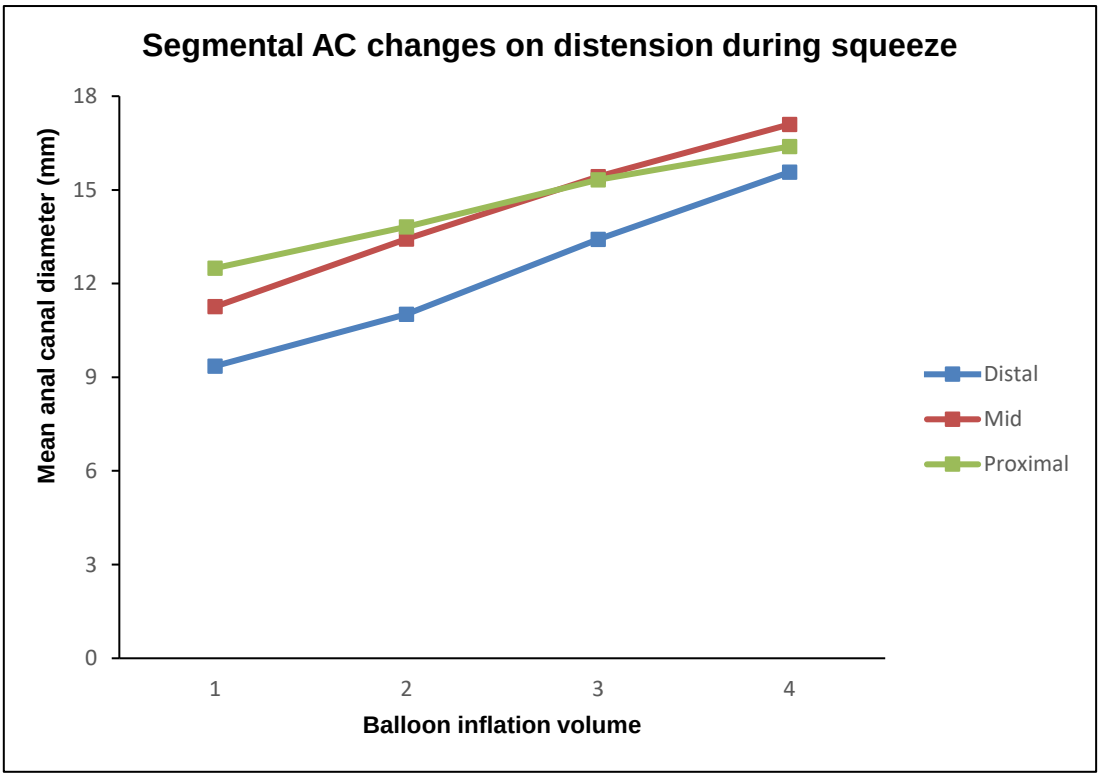


Figure 5.9: Closure of proximal and mid anal canal with distension due to puborectalis contraction during voluntary contraction in a single subject using a 3 cm probe. Each panel represents a different balloon inflation volume which increases by 1ml as we move down the image (from a to d).

Data for segmental changes due to distension during squeeze

The segmental data analysis of all the subjects in group 2, during squeeze, revealed a progressive opening of all the anal canal segments with increasing distension. However, the opening did not seem to be proportional to distension in the proximal segment. Furthermore, the mean AC_{Dia} at the highest two inflation volumes was lower in the proximal anal canal as compared to the mid anal canal, i.e the upper-most anal canal was more closed than the mid anal canal. The mean value for the AC_{Dia} for each of the four incremental balloon inflation volumes in the three anal canal segments respectively is shown below in Fig. 5.10.



Anal Canal segment	Mean diameter (mm) in incremental balloon inflation volumes			
	1	2	3	4
Distal	9.36	11.02	13.42	15.57
Mid	11.26	13.42	15.42	17.10
Proximal	12.49	13.82	15.33	16.39

Figure 5.10: Segmental differences in the mean anal canal diameter on subjecting anal canal to incremental distension during voluntary squeeze (across all the subjects in group 2) ; AC = anal canal

5.2.3 RAIR phase

In keeping with the other two phases, RAIR phase also differed in FI patients in comparison to the healthy subjects. Although the pressure graph during RAIR showed the pressure changes similar to those seen in the healthy cohort and as would be expected in a normal anorectal manometry, the pattern of segmental variation observed in the healthy cohort was not replicated. In fact, multiple varying patterns (during RAIR) were seen amongst the FI patients as was also the case during voluntary contraction in this group.

A common finding in FI patients was a complete or near complete opening of the lower-most anal canal during RAIR, even at minimal distension. This finding is in contrast to the healthy subjects where the lower-most anal canal had the least AC_{Dia} and was found to be closed even at maximal distension. The two colour plots (Fig. 5.11 & 5.12) from two different subjects demonstrate this observation. There is an initial contraction at balloon inflation causing a decrease in AC_{Dia} in the lower and mid anal canal (seen as red colour). This is followed by a complete opening of the lower canal as evident by the blue colour.

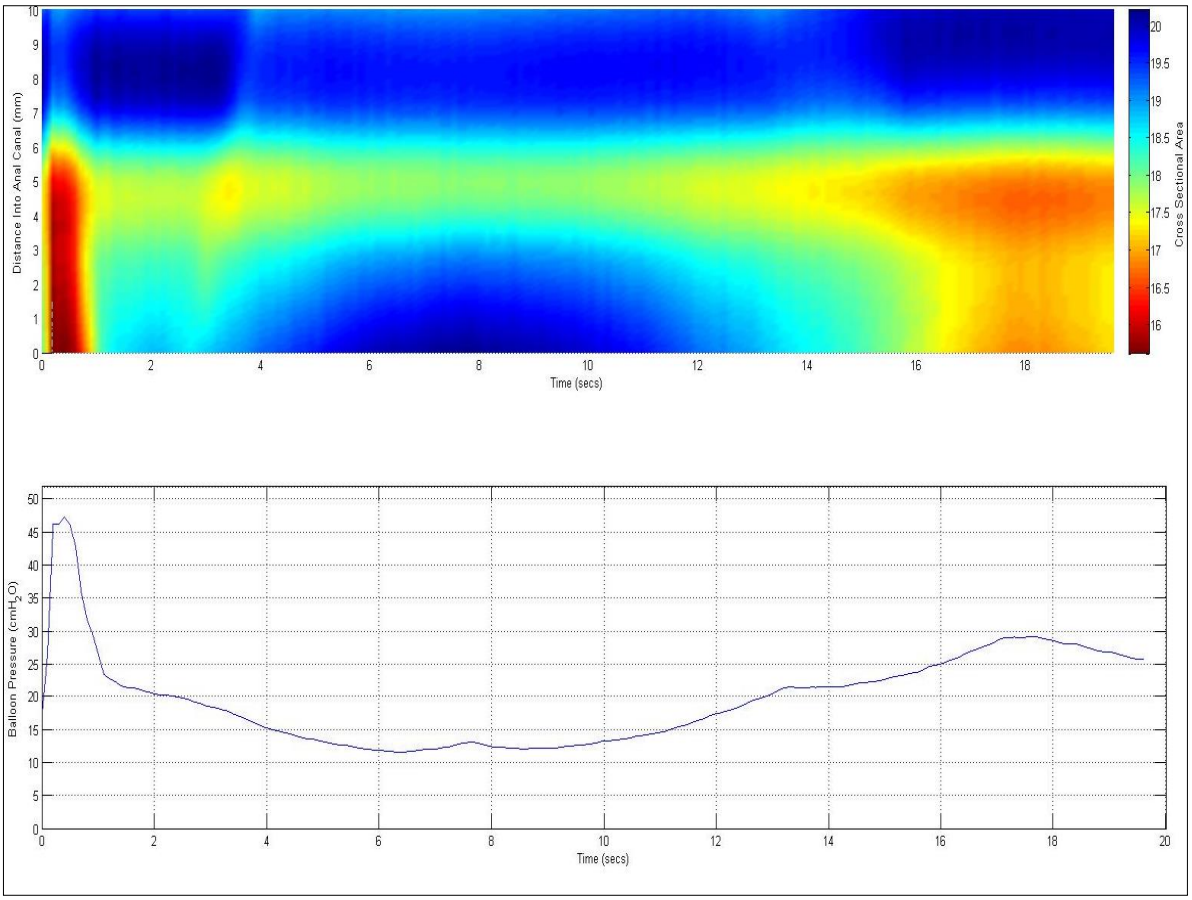


Figure 5.11: Colour plot and pressure graph of a single subject during RAIR phase

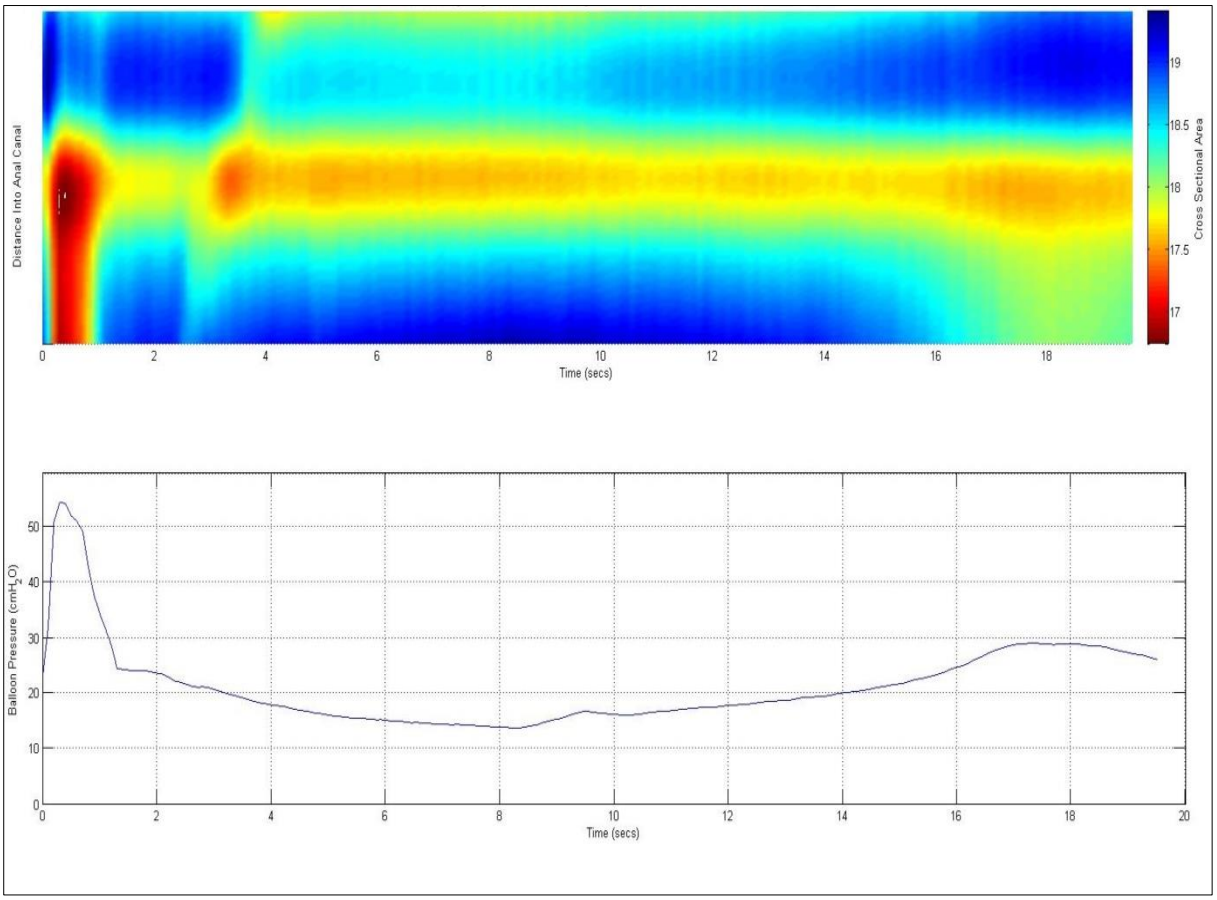


Figure 5.12: RAIR phase in a single FI patient showing opening of lower-most anal canal after the initial contraction of lower and mid anal canal. The lower panel displays a pressure graph for the same patient demonstrating pressure changes similar to those that would be expected during a normal anorectal manometry.

Changes during distension

The segmental changes in the anal canal due to incremental distension during the RAIR phase are displayed in Figure 5.13 & 5.14. With incremental distension the distal anal canal started opening up as shown by change of colour from red to blue in the figures below. A complete opening of the distal anal canal at the highest distension was seen amongst 88% (14/16) of the patients in FI group. Interestingly, 44% (7/16) patients displayed a complete opening of the distal anal canal even at the minimal distension. A puborectalis contraction was also noted in 75% (12/16) of the patients in this group. This was evident as a red band in the proximal anal canal (similar to that seen during the voluntary contraction) and was usually noted at the higher two distensions.

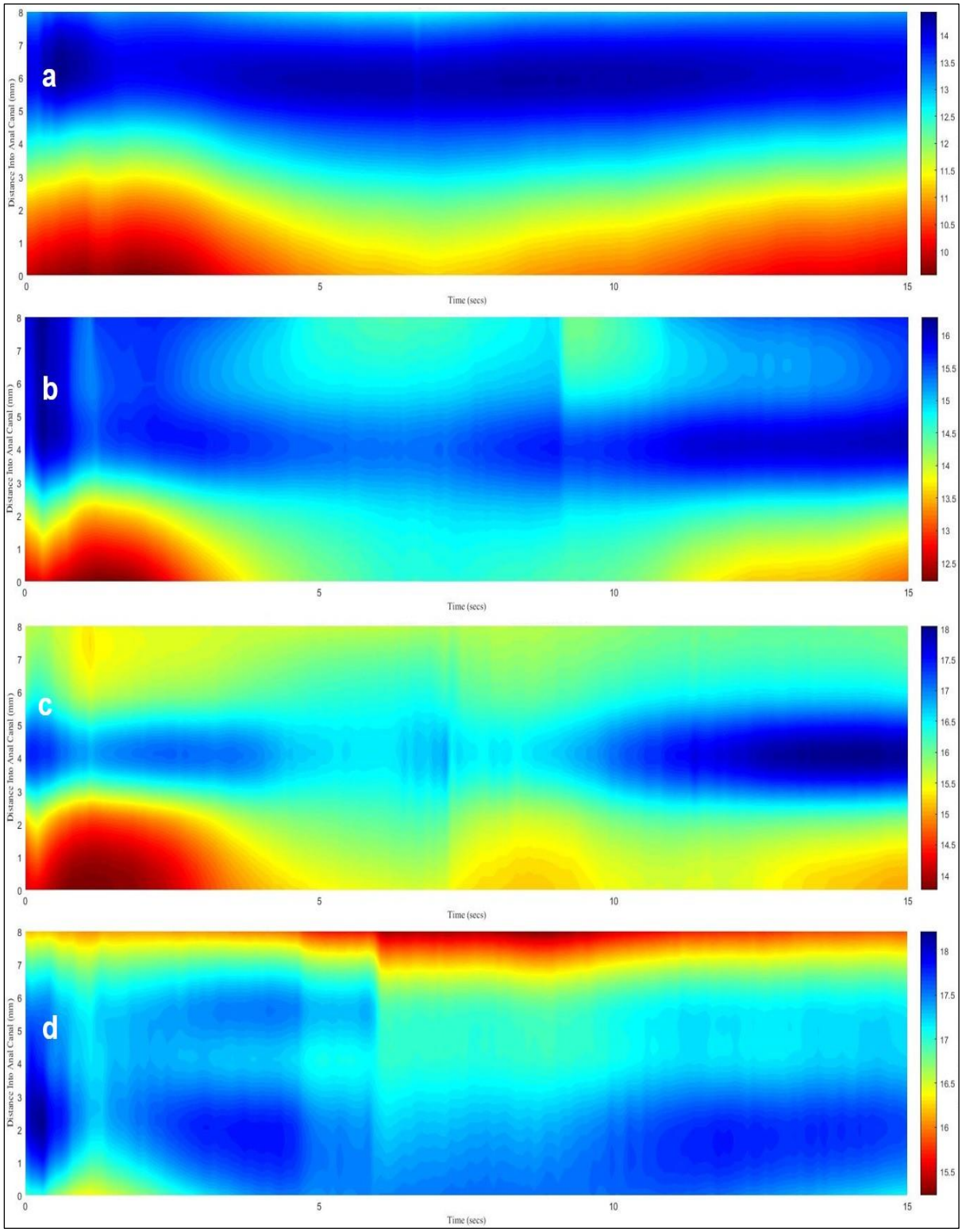


Figure 5.13: RAIR phase in a single FI patient showing complete opening of lower-most anal canal with incremental distension using a 3 cm probe. Balloon inflation volume increases by 1 ml in each panel as we down the image (from a to d).

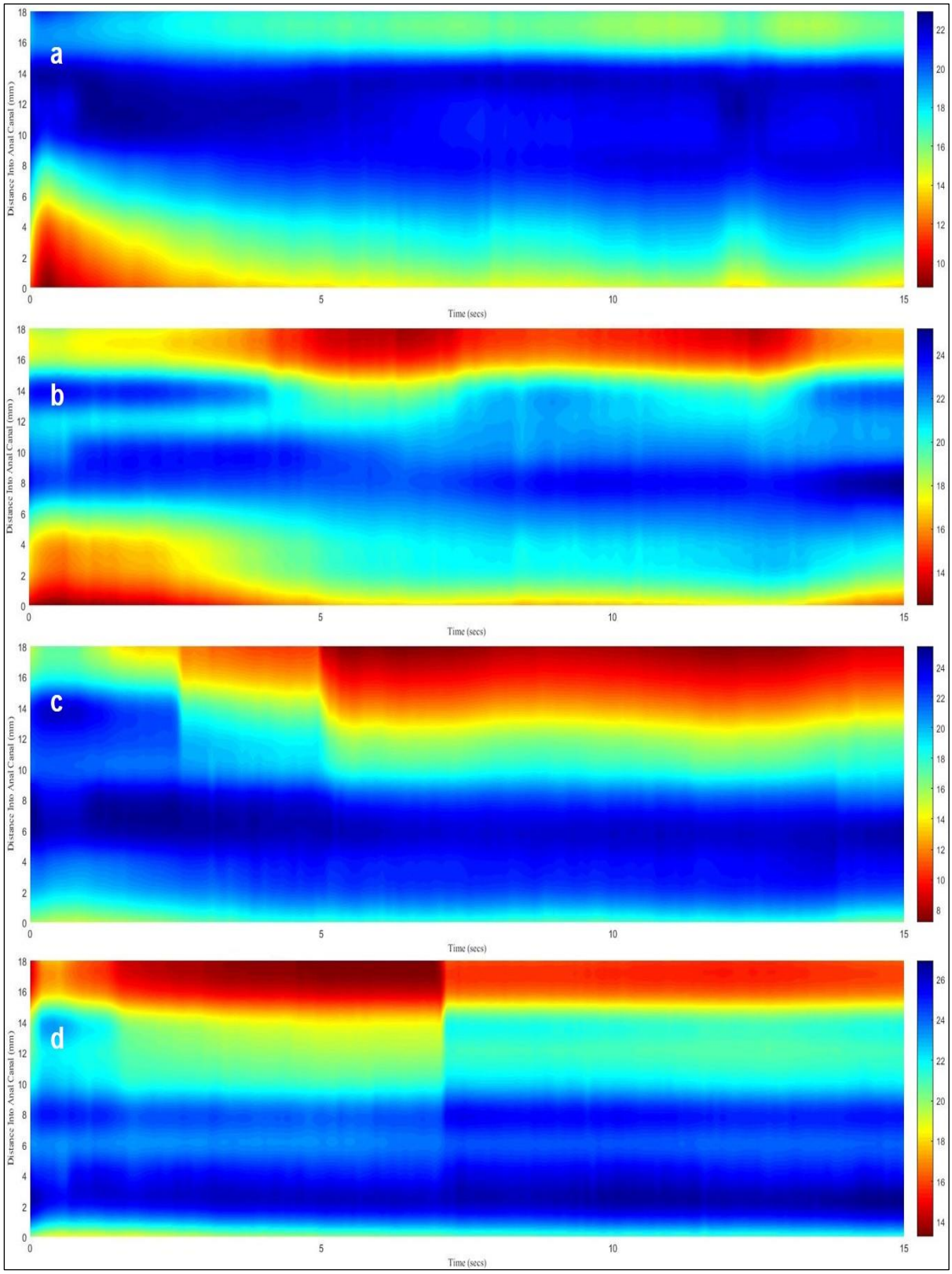
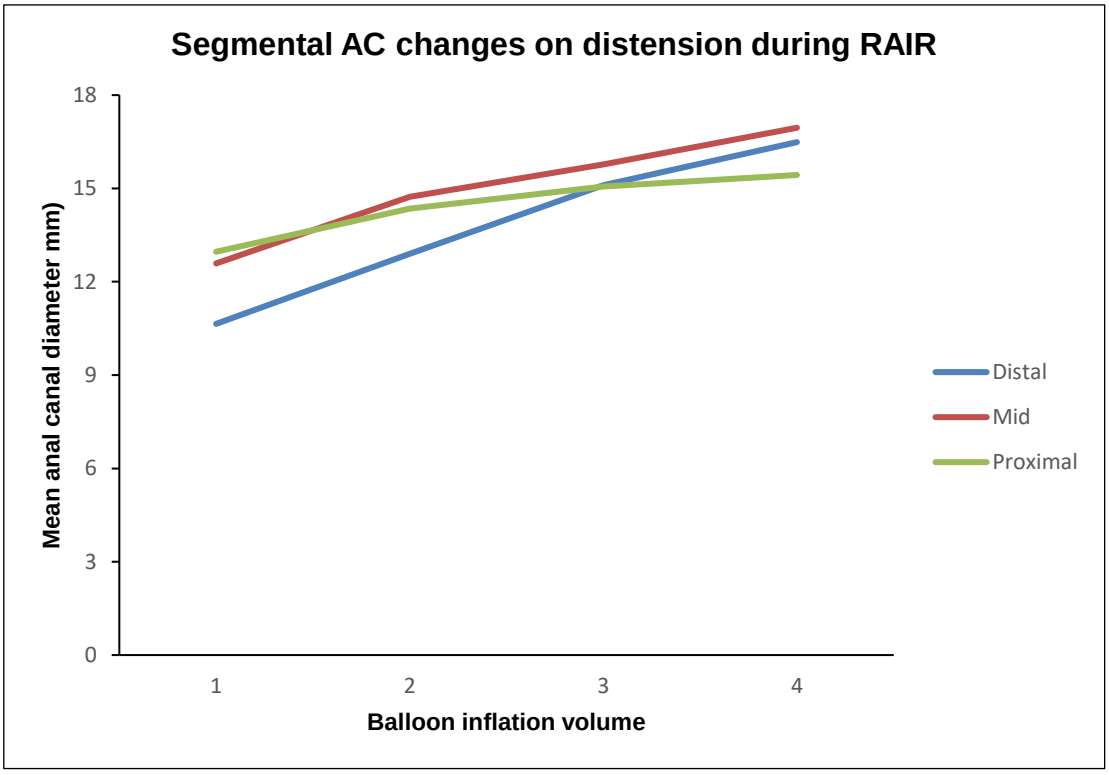


Figure 5.14: RAIR phase in a single FI patient showing complete opening of lower-most anal canal with incremental distension using a 4 cm probe. Balloon inflation volume increases by 1 ml in each panel as we down the image (from a to d).

Data for segmental changes due to distension during RAIR

The segmental data analysis of all the subjects in group 2, during RAIR, revealed a progressive opening of all the anal canal segments with increasing distension. However, the opening did not seem to be proportional to distension in the proximal segment. Furthermore, the mean AC_{Dia} at the highest two inflation volumes was lowest in the proximal anal canal, i.e the upper-most anal canal was more closed than the mid and lower-most anal canal at the highest two inflation volumes. The mean value for the AC_{Dia} for each of the four incremental balloon inflation volumes in the three anal canal segments respectively is shown below in Fig. 5.15.



Anal Canal segment	Mean AC diameter (mm) at incremental balloon inflation volumes			
	1	2	3	4
Distal	10.65	12.90	15.10	16.49
Mid	12.59	14.73	15.77	16.95
Proximal	12.97	14.35	15.06	15.43

Figure 5.15: Segmental differences in the mean anal canal diameter on subjecting anal canal to incremental distension during RAIR across all the subjects in group 2; AC = anal canal

5.3 Statistical analysis of segmental differences in FI patients

The analysis of faecally incontinent patients revealed segmental differences in the anal canal on subjecting it to distension. Further statitical testing to assess whether these segmental differences were significant was done with the help of One-Way ANOVA test. The details of ANOVA test and the rationale behind its use have already been discussed in chapter 4.

The results of ANOVA test are shown individually for each test phase in Table 5.2-5.4. There was no statistically significant difference noted in the mean AC_{Dia} of the different segments during resting and RAIR phase (Resting phase p=<.087, RAIR phase p=0.254). As such, this data was not subjected to further Post Hoc tests which are usually reserved for significance testing between the groups.

ANOVA Resting Phase					
	Sum of Squares	**df**	**Mean Square**	**F**	**Sig.**
Between Groups	83.660	2	41.830	2.475	**.087**
Within Groups	3193.819	189	16.899		
Total	3277.479	191			

Table 5.2: ANOVA result for diameter difference in three anal canal segments during resting phase.

ANOVA RAIR phase					
	Sum of Squares	**df**	**Mean Square**	**F**	**Sig.**
Between Groups	48.188	2	24.094	1.380	**.254**
Within Groups	3282.623	188	17.461		
Total	3330.811	190			

Table 5.3: ANOVA result for diameter difference in three anal canal segments during RAIR phase.

Unlike the resting and RAIR phase, there was a statistically significant difference noted in between the anal canal segments during voluntary contraction (p=.006), as shown in table 5.4. A Post-hoc test, (LSD) was done to identify the anal canal segments which differed from each other significantly. This test revealed a significant difference between the mean AC_{Dia} of the distal anal canal segment when compared to mid or the proximal segment (p=.008 & .004 respectively). However, there was no difference between mid and proximal anal canal segments (p=.780).

ANOVA

Squeeze phase

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	182.965	2	91.483	5.283	**.006**
Within Groups	3272.743	189	17.316		
Total	3455.709	191			

Post Hoc Test

Dependent Variable: Squeeze Phase **Test: LSD**

(I) Factor for anova	(J) Factor for anova	Mean Difference (I-J)	Std. Error	Sig.
Distal	Mid	-1.96008	.73561	.008
	Proximal	-2.16613	.73561	.004
Mid	Distal	1.96008	.73561	.008
	Proximal	-.20605	.73561	.780
Proximal	Distal	2.16613	.73561	.004
	Mid	.20605	.73561	.780

Table 5.4: ANOVA result for diameter difference in three anal canal segments during voluntary contraction phase.

5.4 Statistical evaluation of differences between Group 1 & 2

The raw data and contour plots have so far revealed quite a few differences between the FI patients in group 2 and the healthy cohort in group 1. In order to determine whether these segmental differences within the anal canal were statistically different, the data comprising mean AC_{Dia} of the three anal canal segments from whole of the study population (in group 1 and group 2) across all the balloon inflation volumes taken together was subjected to t-test.

The t-test revealed a significant difference between the distal anal canal segments of the two groups (Resting, p=.008; Squeeze, p=.002; RAIR, p = .023). A significant difference was also noted in the mid anal canal during the squeeze phase but not in the lower or upper-most anal canal segments. The t-test results are displayed below in table 5.5.

	t-test for Equality of Means		
	t	**df**	**Sig. (2 –tailed)**
Resting			
Lower anal canal	-2.700	138	.008
Mid anal canal	-1.787	138	.076
Upper anal canal	.206	138	.837
Squeeze			
Lower anal canal	-3.164	138	.002
Mid anal canal	-2.133	138	.035
Upper anal canal	-.359	138	.720
RAIR			
Lower anal canal	-2.297	138	.023
Mid anal canal	-1.367	138	.174
Upper anal canal	-.178	138	.859

Table 5.5: Independent T-Test comparing mean AC_{Dia} in all three anal canal segments of group 1 and group 2

The difference between the distal anal canal segments of the healthy cohort and incontinent patients is displayed in Figure 5.16. Mean AC_{Dia} is plotted on the Y axis and data points from healthy volunteers (HV) and faecal incontinent patients (FI) are shown on the X axis. The plots are only for the distal anal canal during the three test phases, across all the inflation volumes.

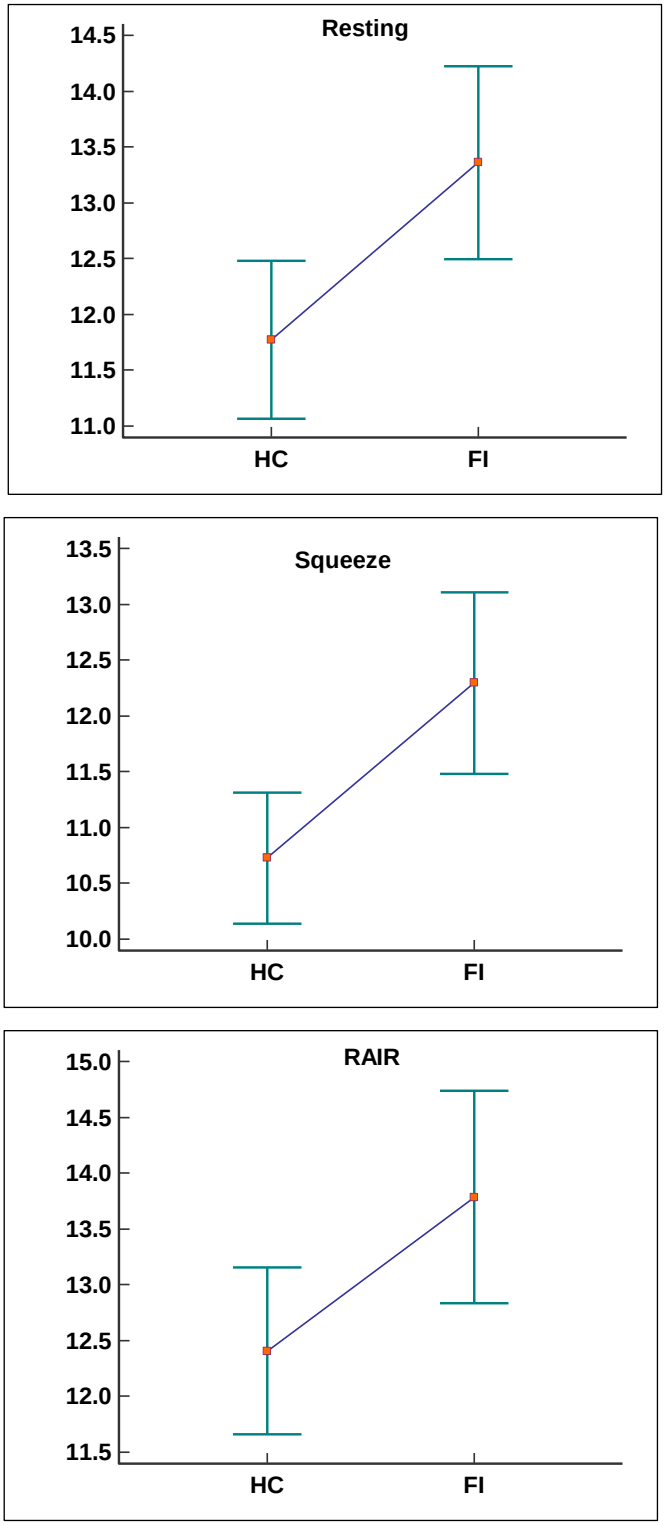


Figure 5.16: Mean AC_{Dia} difference (orange dot) and 95% CI limits (green horizontal markers) in two groups; healthy cohort (HC), faecal incontinent (FI)

The complete group statistics from the t-test are shown below in Table 5.6. The lower mean AC_{Dia} in the distal anal canal of the healthy cohort in comparison to that of the FI patients, during all the three test phase, is clearly evident. As there were 19 healthy controls and 16 FI patients who were tested at four different (incremental) balloon inflation volumes there are 76 and 64 data points for the patients respectively in each anal canal segment.

Test phase	Subjects	N	Mean	Std. Deviation	Std. Error Mean
Resting – Lower anal canal	HC	76	11.8909	3.17033	.36366
	FI	64	13.4444	3.63829	.45479
Resting – Mid anal canal	HC	76	13.8645	3.56018	.40838
	FI	64	15.0401	4.22369	.52796
Resting – Upper anal canal	HC	76	14.6190	4.21845	.48389
	FI	64	14.4685	4.42932	.55366
Squeeze – Lower anal canal	HC	76	10.7170	2.62069	.30061
	FI	64	12.3400	3.44294	.43037
Squeeze – Mid anal canal	HC	76	12.9198	3.39898	.38989
	FI	64	14.3001	4.25699	.53212
Squeeze – Upper anal canal	HC	76	14.2383	4.14381	.47533
	FI	64	14.5062	4.68749	.58594
RAIR – Lower anal canal	HC	76	12.4071	3.28133	.37639
	FI	64 n	13.7838	3.81221	.47653
RAIR – Mid anal canal	HC	76	14.0609	3.84330	.44086
	FI	64	15.0093	4.36393	.54549
RAIR – Upper anal canal	HC	76	14.3222	4.26200	.48889
	FI	64	14.4518	4.30445	.53806

Table 5.6: Group Statistics from t-test
(HC; healthy control group, FI; faecal incontinent group)

5.5 Distensibility

Distensibility has been proposed to be a better assessment marker for anal sphincter function in comparison to anorectal manometry [380,381]. EndoFLIP provides distensibility data by calculating AC_{Dia} and pressure in the lumen. This measure of AC_{Dia} divided by the corresponding intra-bag pressure serves as the distensibility index and was calculated at rest, for both, the healthy subjects and the FI patients in this study. The distensibility index was calculated for all the four inflation volumes (1 being the lowest and 4 being the highest inflation volume) as shown in Figure 5.17.

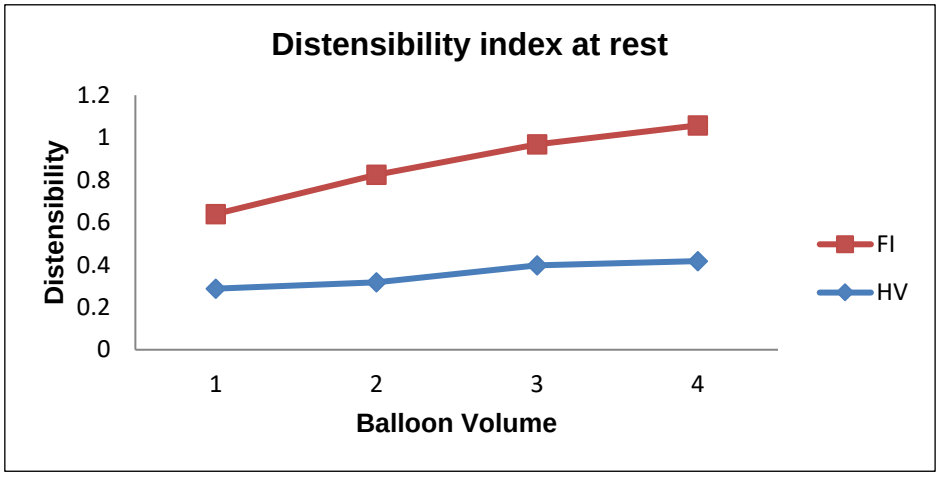


Figure 5.17: Comparison of distensibility index in healthy subjects and FI patients at rest

The distensibility index data being non-parametric, required Mann-Whitney test to check for differences between healthy subjects and FI patients. The results revealed a significantly higher distensibility in FI patients across all but the lowest inflation volume. The results are summarised in Table 5.7.

EndoFLIP anal canal balloon vol	Distensibility in Healthy subjects	Distensibility in FI patients	p value
1st (Lowest)	0.29 (0.13-1.28)	0.35 (0.14-1.63)	0.33
2nd	0.32 (0.18-0.99)	0.51 (0.21-0.92)	0.05
3rd	0.40 (0.18-0.64)	0.57 (0.21-0.92)	0.03
4th (Highest)	0.42 (0.17-0.86)	0.64 (0.20-0.86)	0.01

Table 5.7: Mann Whitney test comparing distensibility between controls & patients

5.6 Comparison of distensibility index with healthy subjects

The distensibility index, between the healthy subjects and the FI patients, was subjected to ROC curve analysis to examine its effectiveness in distinguishing healthy subjects from FI patients. ROC curve is commonly used to determine the diagnostic performance or accuracy of a test [382], with accuracy measured by the area under the ROC curve. An area of 1 represents a perfect test; values ≥ 0.7 are generally considered acceptable and an area of ≤0.5 represents a worthless test.

As the maximal difference in distensibility was noted at the highest inflation volume, it was decided to use only those values for producing the ROC curve, shown in Figure 5.18. The value of area under the curve was found to be .783.

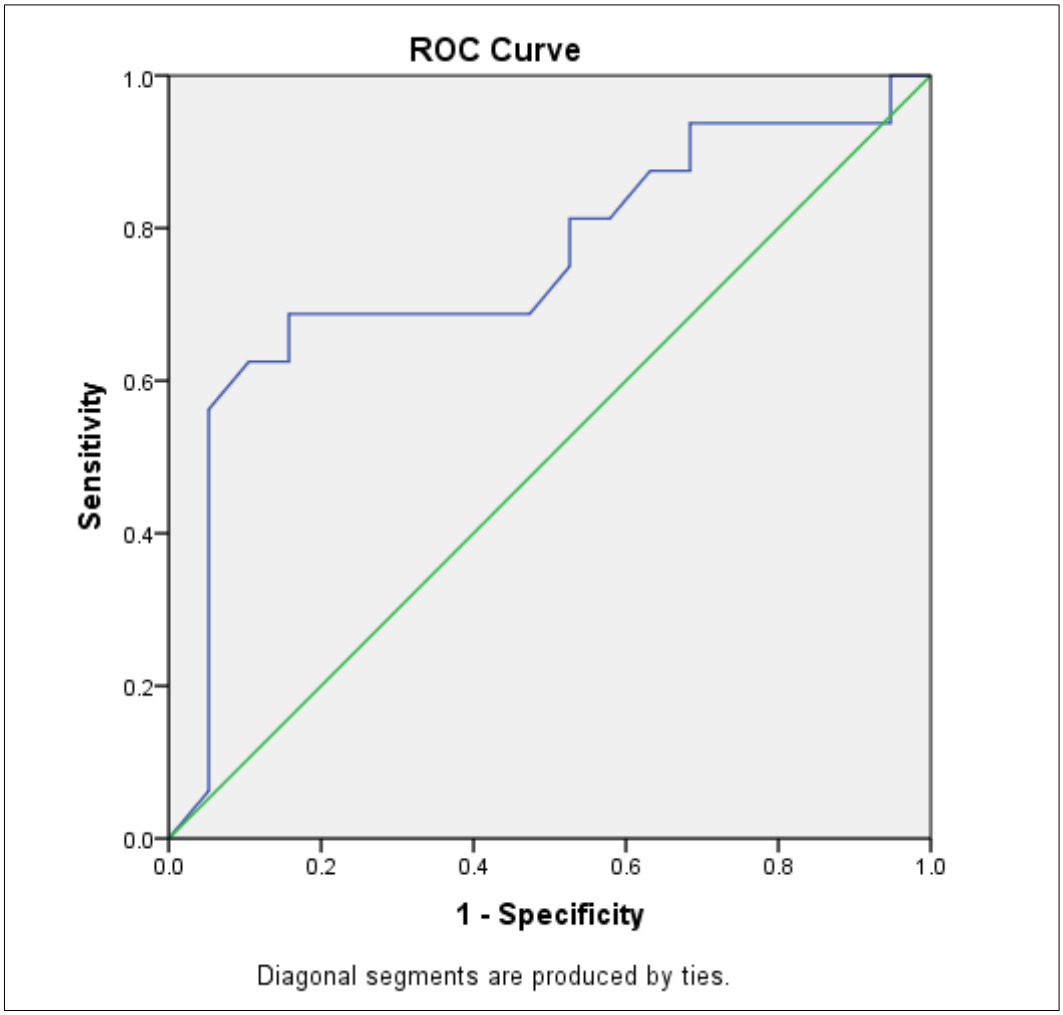


Figure 5.18: ROC curve analysis of the distensibility index

5.7 Comparison of normality range with healthy group.

The normality range has previously been calculated for the healthy cohort as described in chapter 4. The data from group 2 was analysed and established the MR $_{(P: D)}$ in FI patients to be 0.97 mm^2 , Min 0.64 mm^2, Max 1.20 mm^2, 95% CI - 0.89-1.05. This MR $_{(P: D)}$ value was lower than that found in healthy controls (1.18 (95% CI, 1.01 – 1.29)). The plot showing the comparison of healthy subjects and FI patients is shown below (Fig 5.19).

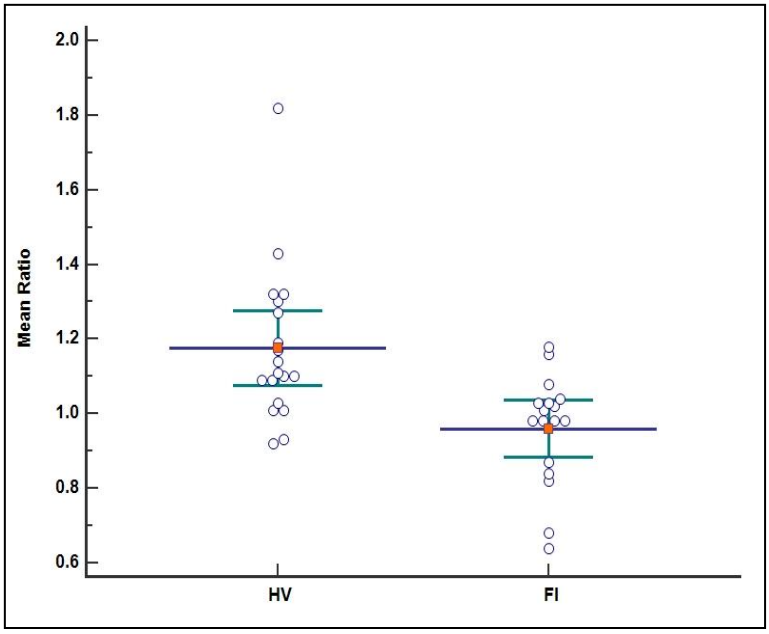


Figure 5.19: Comparison of MR $_{(P: D)}$ in healthy vs FI group

The MR $_{(P: D)}$ from patients with FI was compared to healthy controls with the help of Independent t-test which revealed a statistically significant difference in between the two values. The results are shown in the table below.

t	df	Sig. (2-tailed)	Mean Difference	Std. Error Difference	95% Confidence Interval of the Difference	
					Lower	Upper
3.555	34	.001	.21632	.6084	.09267	.33996

Table 5.8: T-test result comparing mean AC_{Dia} ratio between healthy and FI group

5.8 Conclusion

The EndoFLIP testing has helped gain further insight into biomechanical properties and segmental variations of the anal canal. Besides confirming an inter-group variance, between the healthy cohort and the incontinent patients, EndoFLIP also revealed a significant intra-group variance in the incontinent group. This was quite obvious during the voluntary contraction and the RAIR phase, where the homogeneity seen in the healthy cohort was replaced by disorganised attempts at closing the anal canal.

A common pattern of distal and mid anal canal closure seen in the healthy cohort during voluntary contraction was not replicated in the incontinent patients. Instead the FI patients displayed random patterns of anal canal closure such as closure of mid or proximal anal canal segments only or a combination of distal and proximal segment closure with an open mid anal canal segment. A consistent finding in FI patients was a minimal or no-closure of the distal anal canal during the voluntary contraction. This observation noted on the contour plots correlated well with the results of data analysis which confirmed a higher mean AC_{Dia} of the distal anal canal of FI patients in comparison to the healthy cohort (12.3 mm vs 10.7 mm, p = .002). Our findings are however in contrast with the Danish study which used a longer EndoFLIP probe (12 cm long with proximal end in the rectum and distal end coming out of the anal verge with longer probe) to evaluate the FI patients and concluded that the FI patients were able to keep significant part of the anal canal closed even at higher distension volumes [383]. The EAS is responsible for maintaining continence by generating high anal pressure in situations of increased intra-rectal or intra-abdominal pressure, such as with filling of the rectum (and consequent relaxation of IAS), coughing or sneezing. As such the non-closure of the distal segment and a generalised poor closure of anal canal observed by EndoFLIP in this study is a significant observation and goes on to explain the urge incontinence symptoms experienced by these patients. On the other hand, the comparable significant closure of anal canal in the healthy subjects and FI patients in the Danish study is difficult to explain physiologically and even more so in light of the fact that the ARM done prior to EndoFLIP in their study did confirm a low squeeze pressure. One can only speculate that those findings were due to the use of longer EndoFLIP probe in the Danish study as that was the key difference between the two studies.

The closure of proximal anal canal during voluntary contraction, seen in the contour plots, can possibly be explained as a result of puborectalis contraction. As discussed earlier in chapter 1, puborectalis is a skeletal muscle which forms a sling at the anorectal junction. Its fibres blend with the deep part of the external anal sphincter (EAS), situated in the upper anal canal, and contract along with the EAS during the squeeze manoeuvre [384]. The nerve supply for

the puborectalis muscle arises from direct branches of the anterior S3 and S4 rather than the pudendal nerve, thereby making coexistence of functioning puborectalis and non-functioning EAS a possibility. Despite the puborectalis function seen in the contour plots, there was no significant difference in the AC_{Dia} of the proximal anal canal of the FI patients and the healthy subjects (14.5mm vs 14.2 mm, p = .720), during squeeze. This is likely to be due to a comparable function of puborectalis in the healthy cohort. The puborectalis function is not a compensatory mechanism to assist a poorly functioning EAS. It is in fact a normal contributor to the squeeze pressure as has been seen in several studies, using ultrasound, anorectal manometry [385,386] and even EndoFLIP [18].

The findings on contour plots have been relatively easy to explain so far. However, it is hard to explain why the mid anal canal was open, during voluntary contraction, in few FI patients while the distal anal canal was closed. The contour plots correlated well with the statistical analysis which confirmed this finding by demonstrating a higher mean AC_{Dia} in the mid anal canal of FI patients as compared to the healthy cohort (14.3 mm vs 12.9 mm, p = .035) (Table 5.5 & 5.6). It is difficult to explain these findings especially in the absence of any demonstrable EAS disruption. However, similar findings were reported in EndoFLIP study of FI patients by Sorensen et al. [383]. They found the mid and distal anal canal opened up easily on distension. Anatomically, we know that the superficial part of EAS, surrounds the IAS in the mid anal canal. With closure seen in the distal anal canal but not in the mid part, one possibility is that there is an IAS dysfunction in these patients which renders it difficult to be compressed by the EAS in the mid segment thereby leaving the mid segment open. Besides leading to an inefficient squeeze, the open mid segment (along with open upper anal canal) would also allow the rectal contents to be constantly in contact with the sensitive anal epithelium presumably leading to failure of the anal sensory mechanism to discriminate, thereby leading to a poor control and incontinence. The IAS dysfunction in presence of functioning EAS is possible as both these sphincter muscles have different innervations (as discussed in chapter 1). Moreover, IAS has been shown to have collagen deposition with advancing age and even more so in the FI patients [124] which leads to fibrosis and might partly explain the incompressibility of IAS in our elderly study population. An increase in connective tissue [125] and fibrotic changes [126] in IAS with advancing age have also been reported in separate studies.

The haphazard attempts of anal canal closure seen, in the FI patients, during voluntary contraction were also noticed in response to inflation of the rectal balloon (during RAIR phase). The contour plots also revealed that the lower-most anal canal opened up in most of the patients with minimal distension. In the few patients that maintained closure of the lower-most

segment at minimal distension the anal canal opened up with incremental distension. This was in contrast with the healthy cohort where the distal segment closure was maintained even at the maximal distension. This finding on the contour plots correlated well with the statistical analysis which found the mean AC_{Dia} of the lower-most anal canal to be significantly higher in the FI patients as compared to the healthy cohort (13.8 mm vs 12.4 mm, p=.023). The opening of the lower anal canal in response to rectal distension points towards a possible EAS dysfunction, EAS being the muscle responsible for maintaining the distal segment closure during RAIR. However, the fact that there was no morphological abnormality of external sphincter picked up on EAUS in these patients leaves us with the possibility of aberrant physiology as the likely cause for this finding. Several studies in the past have shown neuropathic damage to EAS, in patients of idiopathic faecal incontinence, as a result of abnormal descent of pelvic floor which stretches the pudendal nerve [115,387,388]. This pudendal neuropathy is usually confirmed on pudendal nerve terminal latency testing (PNTML). However, the study protocol did not involve PNTML and as such it cannot provide an objective justification for this finding.

This abnormality in the distal anal canal discovered on EndoFLIP testing is a significant find especially in light of the fact that the pressure graph demonstrated a RAIR response that would be classed as normal during ARM (pressure dropping below the baseline after the initial rise and eventually returning back to the baseline). This finding demonstrates the edge EndoFLIP has over ARM in evaluating anal canal physiology. By demonstrating this inability of the lower-most anal canal segment to close during the process of sampling, which occurs several times a day, EndoFLIP has recognised a true anatomo-physiological problem and perhaps provided an explanation for the frequent episodes of passive soiling.

The aberrant patterns of segmental anal canal functioning as compared to the healthy cohort were not limited to the squeeze and RAIR phase only. EndoFLIP revealed a poor closure of anal canal during the resting phase in the FI patients. Furthermore, a complete opening of the distal segment with distension was also noted on the contour plots during resting phase. This finding was confirmed on the data analysis which revealed a higher mean AC_{Dia} in the distal anal canal of FI patients in comparison to the healthy cohort (13.4 mm vs 11.9 mm, p=.008). This crucial variance from the pattern observed in the healthy cohort, possibly explains the passive incontinence in this group. The distension of the lower segment via anal canal balloon replicates the physiological changes that would take place due to the excretory contents in the anal canal. Once the contents enter the anal canal, during the process of sampling, they leak out due to its inability to maintain closure in the FI patients.

In addition to the apparent differences in the lower-most anal canal, EndoFLIP also revealed a significant difference in anal canal distensibility in FI patients as compared to the healthy cohort. Distensibility is defined as the relative change in volume per unit of pressure. It is believed to provide a better assessment of anal sphincters [380,381] and has even been suggested to be considered as the principal determining factor for the sphincter strength [389]. By taking into account the pressure and the volume change at the same time, distensibility offers the crucial information about the opening forces of the anal sphincters as opposed to the existing investigative techniques, ARM and HRAM which merely provide a measure of closure forces of the anal canal. Moreover, the distensibility has already proven its edge in Upper GI studies by distinguishing non-hernia reflux patients from hernia patients, and normal subjects [244] in addition to evaluating conditions such as eosinophilic oesophagitis [390] and achalasia [391] etc.

The distensibility was calculated at the highest inflation volume as the study had already shown a significant change in AC_{Dia} at that inflation. The FI patients were found to have a lower distensibility index, portraying an anal canal which opens up at pressures much lower than those required to open anal canal in the healthy cohort. This difference in distensibility was found to be significant at all the inflation volumes apart from the lowest ($p = .05, .03$ & $.01$ respectively). Anal canal distensibility index has not been studied until recently. The limited studies that have been done in the last few years in this field have reported a lower anal canal distensibility index in females as compared to the males [19] and in scleroderma patients as compared to healthy controls [255] respectively. Results, similar to this study were reported by Gourcerol et al. who concluded that differences in distensibility index could help discriminate FI patients from the healthy controls [392]. However, further work still needs to be done in this emerging field to develop a sound knowledge before it can be put to use in the clinical practice.

In conclusion, dynamic evaluation of the heterogeneous structures of the anal canal with EndoFLIP has clearly provided new information about the functionality of anal canal. By providing the segmental evaluation, EndoFLIP has undoubtedly proven its edge above the existing investigative methods of ARM and HRAM which consider the anal canal to be a static entity. The info on segmental variation and distensibility makes EndoFLIP an exciting technology which can provide new insights into anal canal physiology, especially of passive soilers.

Chapter 6

Results: Scleroderma group

6.1 Introduction

Scleroderma or Systemic sclerosis (SSc) is a chronic autoimmune-mediated connective tissue disorder that is characterised by fibrosis of the skin and internal organs and vasculopathy. It shows a predisposition towards female sex (two to four-fold more common than men) and black population (twice as frequent than in white population). The disease prevalence is estimated to be around 8-30 per 100,000 people in Europe with an incidence of 1-2 per 100,000 people [393][394]. The uncommon nature of the disease makes it challenging not only for the patients but also the physicians to diagnose and manage the condition.

Systemic sclerosis is classified into two subsets primarily based on the extent of the skin involvement, diffuse or limited cutaneous disease with the limited disease being the commoner variant (~80% of diagnoses) and the one with a better prognosis. The skin thickening in the limited cutaneous disease is seen distal to the elbows and knees only whereas in the diffuse type proximal part of extremities and the trunk are involved. The face involvement is seen in both the subsets. In addition to the dermal changes, the classification also takes into account the visceral involvement, autoantibody profile and disease progression. Although the visceral involvement is seen in both subsets, it is early and rapid in the diffuse type as opposed to late in the disease course of limited type. Autoantibodies have proven useful not only for diagnosing the disease but also for classification. Centromere-specific autoantibodies are associated with limited cutaneous disease whereas topoisomerase-I or RNA polymerase III–specific antibodies are often associated with diffuse cutaneous type [394].

The gastrointestinal involvement is seen in both subsets and occurs in up to 90% of all patients with SSc. GI involvement is the most common cause of morbidity and third leading cause of mortality in patients with systemic sclerosis [395]. The GI symptoms are believed to be a result of fibrosis, of smooth muscles of the gut and gut vasculature, leading to dysmotility and delayed transit time in the affected part of the GI tract. Although any segment of the GI tract from mouth to anus can be affected, the oesophagus is the most commonly involved followed by the anorectum. The GI manifestations include dysphagia, GERD, delayed gastric emptying, gastroparesis malnutrition, constipation and faecal incontinence. Of these, malnutrition and oesophageal involvement have been found to be associated with an unfavourable prognosis [396,397]. The GI manifestations of the disease severely impact both the prognosis and quality of life.

6.2 Pathophysiology in Scleroderma

The systemic sclerosis is characterised by vasculopathy, inflammation and fibrosis. Although significant advances have been made in understanding of its pathogenesis, all the mechanisms are still not completely understood.

Vasculopathy is seen in the form of arterial hyper-activity and remodelling with myointimal hyperplasia which eventually lead to vascular occlusion in skin and other viscera triggering a chain of events in the pathogenesis of the disease [398]. SSc patients have been found to have elevated levels of endothelin-1 (leads to vasoconstriction, inflammation and vascular remodelling) and vascular epidermal growth factor (VEGF) (aggravates fibrosis and possibly impaired angiogenesis) [399]. Elevated levels of VEGF 165B isoform, which is an anti-angiogenic factor, have also been reported [400]. This might be responsible for lack of angiogenesis despite high VEGF levels.

There is high collagen deposition in skin and other viscera of SSc patients as a consequence of fibroblast activation. Elevated levels of transforming growth factor (TGF-β), which plays a major role in fibroblast activation, have been found in lung and dermal SSc lesions. Other factors such as platelet derived growth factor and connective tissue growth factor have also been reported and are believed to be involved in late stages of fibrosis development in SSc patients [401].

Apart from the vascular and mesenchymal elements discussed above, the immune system is also believed to play a part in pathogenesis of SSc. T cells, macrophages, mast cells and B lymphocytes have all been found in the affected skin of SSc patients [402]. The presence of specific auto antibodies also suggests a role of B cells [398]. An overexpression of IL-17, a T-cell cytokine produced by T helper cells, has been reported in peripheral blood and skin of patients with SSc. This is known to enhance fibroblast proliferation and the production of TNF-α and IL-1 (which in turn induces fibroblast production of collagen, IL-6 and PDGF). A schematic of immune system involvement in the pathogenesis of SSc is shown in figure 6.1.

It has been proposed that considering SSC as a disease of dysregulated or dysfunctional repair of connective tissue in response to an injury might be better paradigm as this leads to inclusion of all the elements of tissue repair which might be relevant to the disease [403]. The authors support this proposal by stating that “the components such as epithelium, blood derived cells and other processes contributing to wound healing, which have been shown to be involved in the pathogenesis of SSc are not accounted for by the traditional focus on a tripartite pathogenesis (linking vascular, immune, and mesenchymal components)”.

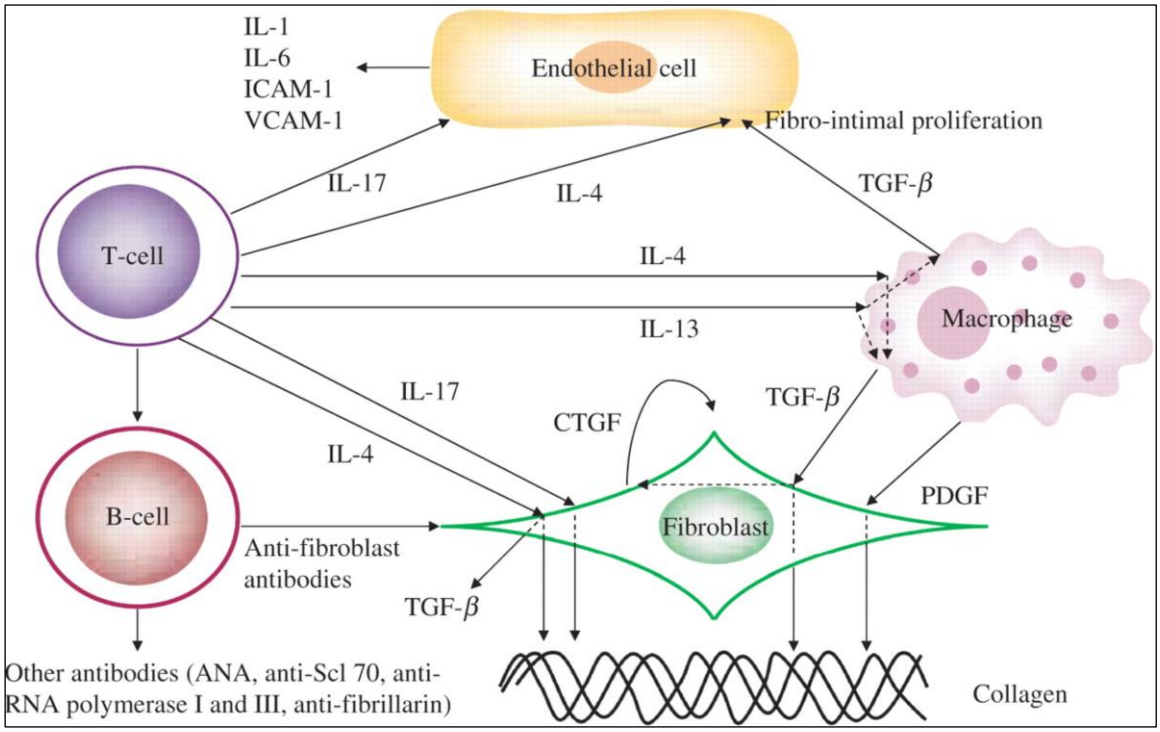


Figure 6.1: Schematic of involvement of immune system in pathogenesis of SSc (Reproduced with permission from Oxford University Press).

External influences such as an infection or environmental factors have also been implicated as the triggering events for systemic sclerosis in the genetically predisposed patients. Infection with cytomegalovirus, Epstein-Barr virus, and Toxoplasma gondii are believed to be possible triggers due to the finding of interferon signature that exists in most patients with Ssc, implying activation of innate immunity [404,405]. The environmental factors shown to be associated with Ssc include silica exposure, chlorinated solvents, trichloroethylene, welding fumes for men, aromatic solvents and ketones for women and white spirit for both genders [398,406].

The genetics has also been proven to play a role in the pathogenesis of disease. Studies have revealed an association between SSc and genes implicated in the immune system, notably polymorphisms of the interferon-regulatory factor 5 (IRF5) involved in activation of interferon targets and the ligand of CD134 (OX40L) involved in antigen presentation and T and B cells activation. The heritability of SSc has also been demonstrated by a study of 703 cases which found a 13 fold increased risk of SSc in first degree relatives [407] and a separate study which reported a 15 fold increased risk if a sibling is affected [408].

Changes in GI tract

These changes in the GI tract lead to impaired motility, digestion, absorption and excretion. There have been a number of studies which have investigated anorectal physiology in this patient group (with anorectal manometry) and found low resting anal pressure, attributing it to the involvement of IAS [130-133] whereas the squeeze pressure, generated by EAS, has been reported to be within normal limits [134-136].

With the development of better anorectal imaging modalities, such as EAUS and Endoanal MRI, the research interest moved from measuring physiological parameters towards detecting the morphological involvement of IAS in these patients. Engel et al. were the first one to report the IAS atrophy in scleroderma patients [137]. Since then two other studies by DeSouza et al. and Koh et al. have confirmed the finding of IAS atrophy in this patient group using MRI and EAUS respectively [138,139].

A study from our centre compared scleroderma patients with and without anorectal symptoms. Although one would have expected to see morphological differences in IAS in these two patient subgroups, the study demonstrated that all these patients had thinned and atrophic IAS (on EAUS) without any significant difference in the atrophy scores or IAS thickness between them [140]. The result from this study points towards other possible factors (apart from structural) at play in the IAS which lead to faecal incontinence in these patients. It can be that functional changes in the sphincter are responsible for FI in these patients, given that this has already been shown to be the case in the oesophageal involvement. The effects of scleroderma on IAS biomechanics remain unexplored and we used the novel technology using impedance planimetry, EndoFLIP, to obtain further detailed information in these patients.

6.3 Demographics

A total of eight female patients referred with proven SSc and bowel symptoms were recruited for this study. The mean age was 67 (60-70).

6.4 Results

The MATLAB analysis of EndoFLIP data is presented below. Each figure represents one particular phase (resting, squeeze or RAIR) in a single subject and as before the balloon volume increases as we move down the image.

6.4.1 Resting phase

The resting phase in scleroderma patients revealed an open lower anal canal even at the minimal distension. The mid and upper-part initially showed a closure to some extent, but both the segments started opening with distension. This is demonstrated, by a change to lighter colours on moving down the plots (i.e. increasing distension). The two figures below are from two separate patients (Figure 6.2-6.3).

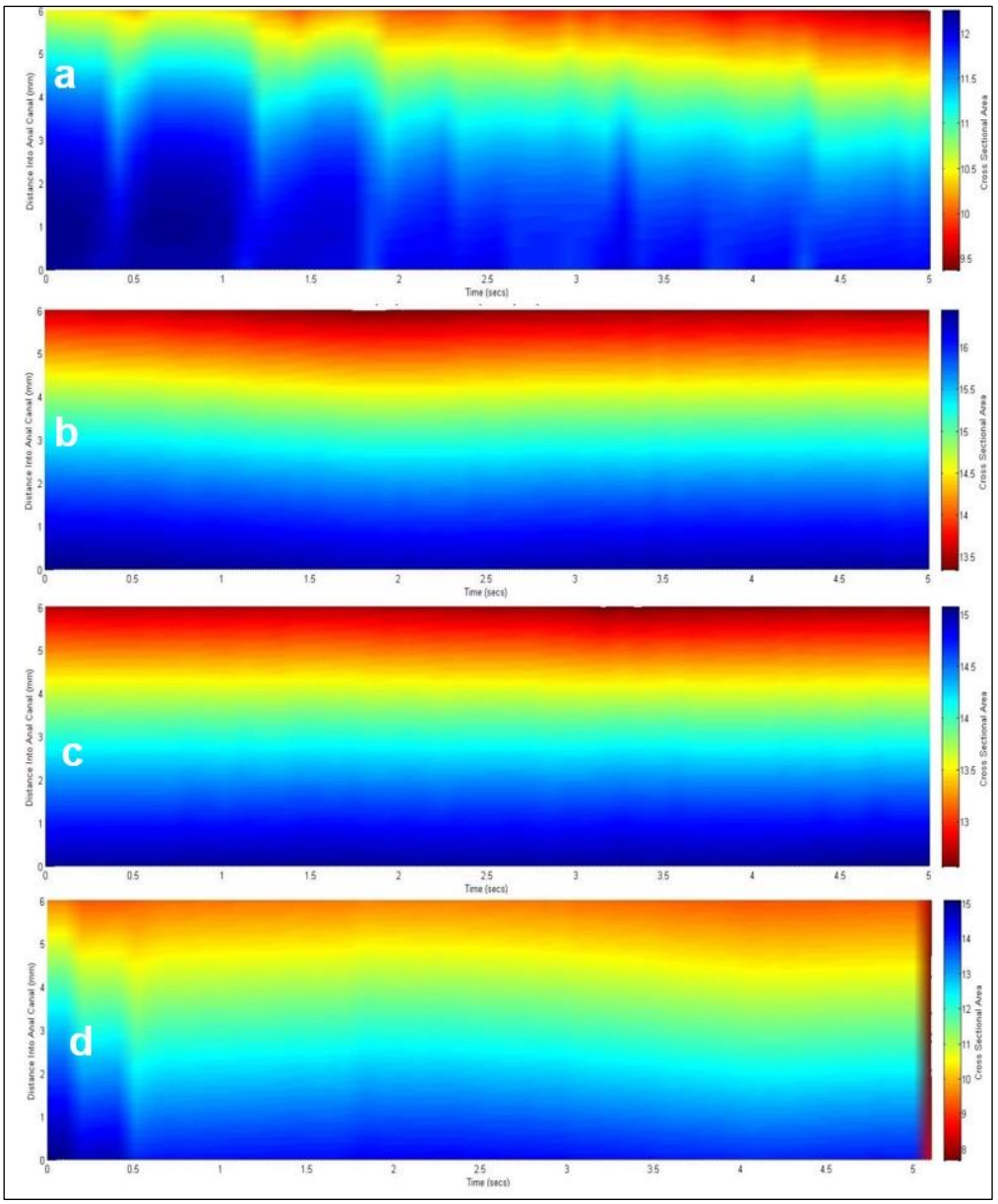


Figure 6.2: Resting phase in a single SSc subject demonstrating an open lower AC segment and closed mid & upper segment which start opening with incremental distension, on moving down from plot a to d; AC: anal canal.

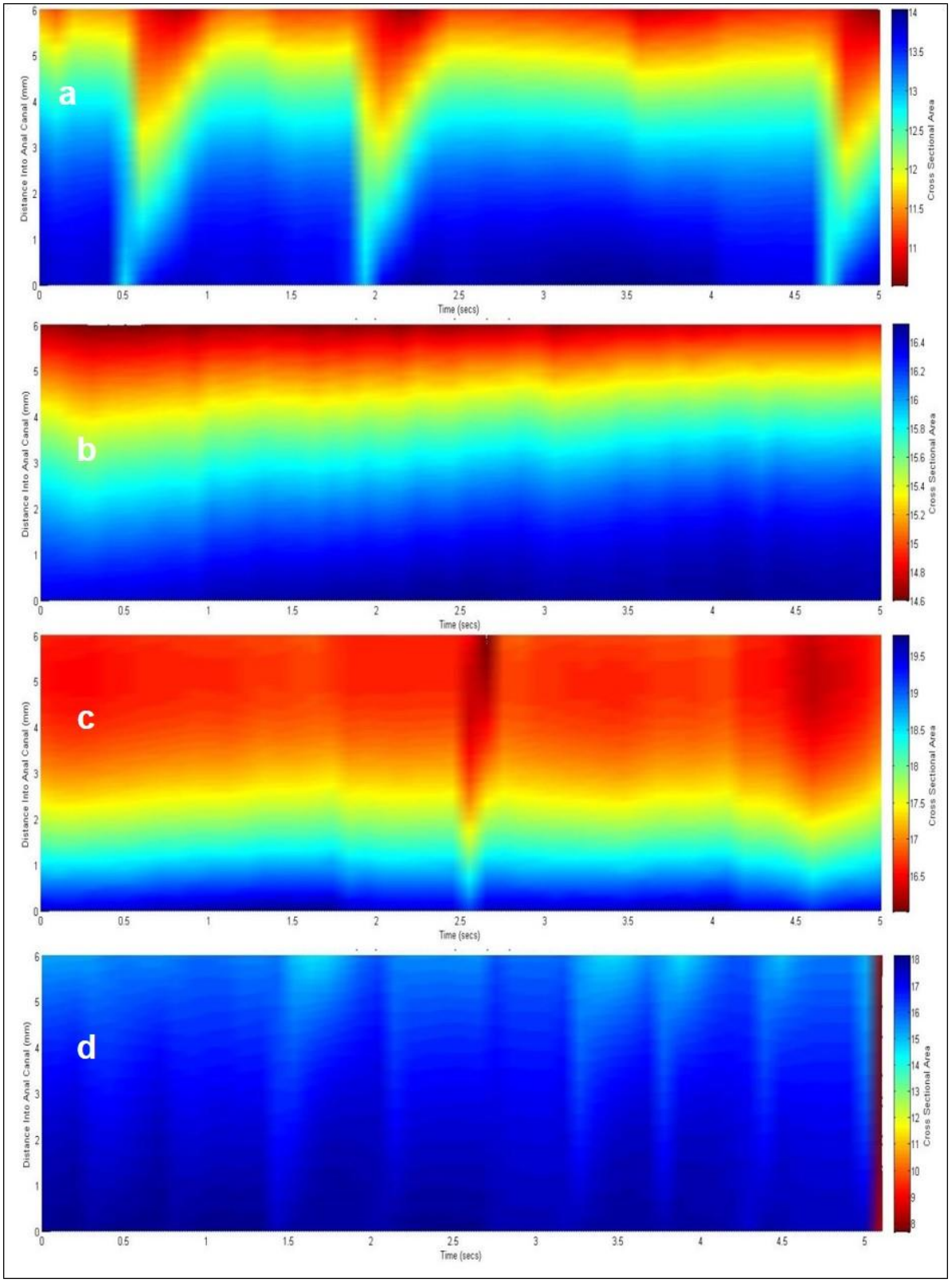
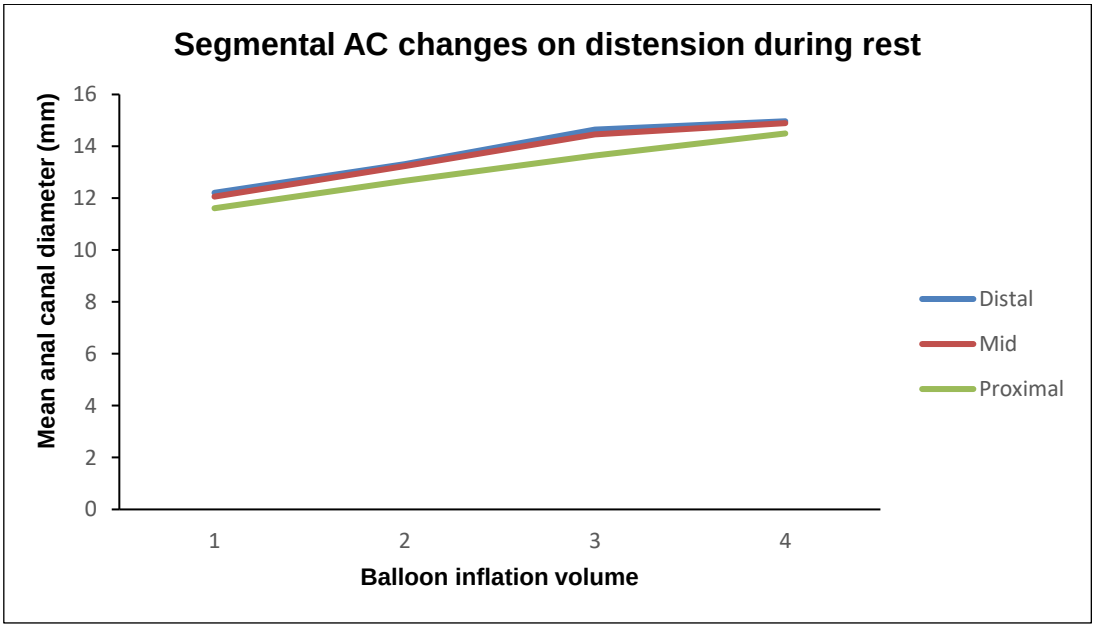


Figure 6.3: Complete opening of lower anal canal seen at maximal distension during resting phase in a single SSc subject. The balloon inflation volume increases incrementally on moving down from plot a to d.

Data for segmental changes due to distension during rest

The contour plots revealed an opening of the anal canal with distension. Analysis of the segmental changes confirmed this by demonstrating an increase in mean AC_{Dia} with an increase in distension. An interesting point to note on the segmental analysis was that the mean AC_{Dia} was found to be lowest in the proximal anal canal at all inflation volumes, i.e. the upper-most segment was the most closed part of the anal canal. This is in contrast to the healthy cohort where the lower-most segment was the most closed part (i.e. had the lowest mean AC_{Dia}) even at maximal distension during the resting phase. The observation of lower-most anal canal being the most closed part was also seen in FI patients at minimal distension. However, this changed with distension and at maximal distension the upper most segment was noted to be more closed than the lower-most segment. The graph showing these changes alongwith the raw data is displayed below in Figure 6.4.



Anal Canal segment	Mean AC diameter (mm) at incremental balloon inflation volumes			
	1	2	3	4
Distal	12.21	13.32	14.65	14.97
Mid	12.06	13.24	14.46	14.89
Proximal	11.61	12.67	13.64	14.50

Figure 6.4: Segmental differences in the mean anal canal diameter on subjecting anal canal to incremental distension during resting phase across all the subjects in group 3; AC = anal canal

6.4.2 Squeeze phase

The voluntary contraction of the sphincter muscles revealed varying patterns in the scleroderma patients. In two subjects a common finding, of closure of the mid and the upper anal canal but not the lower-most anal canal, was noted (Figure 6 & 7). This was quite dissimilar to the healthy controls where closure of the lower-most anal canal was seen during the voluntary contraction.

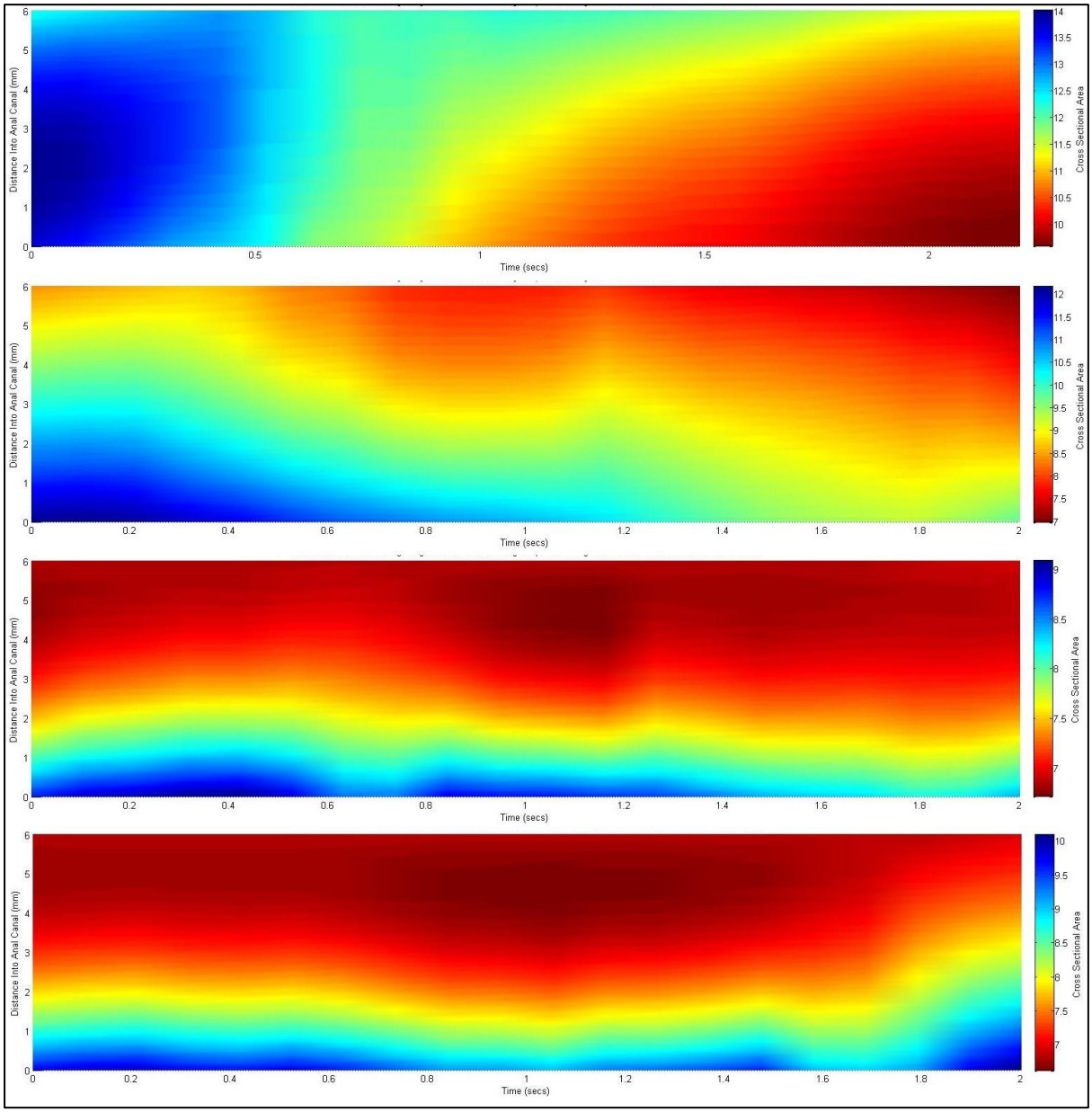


Figure 6.5: Squeeze phase in a single subject across incremental balloon volumes

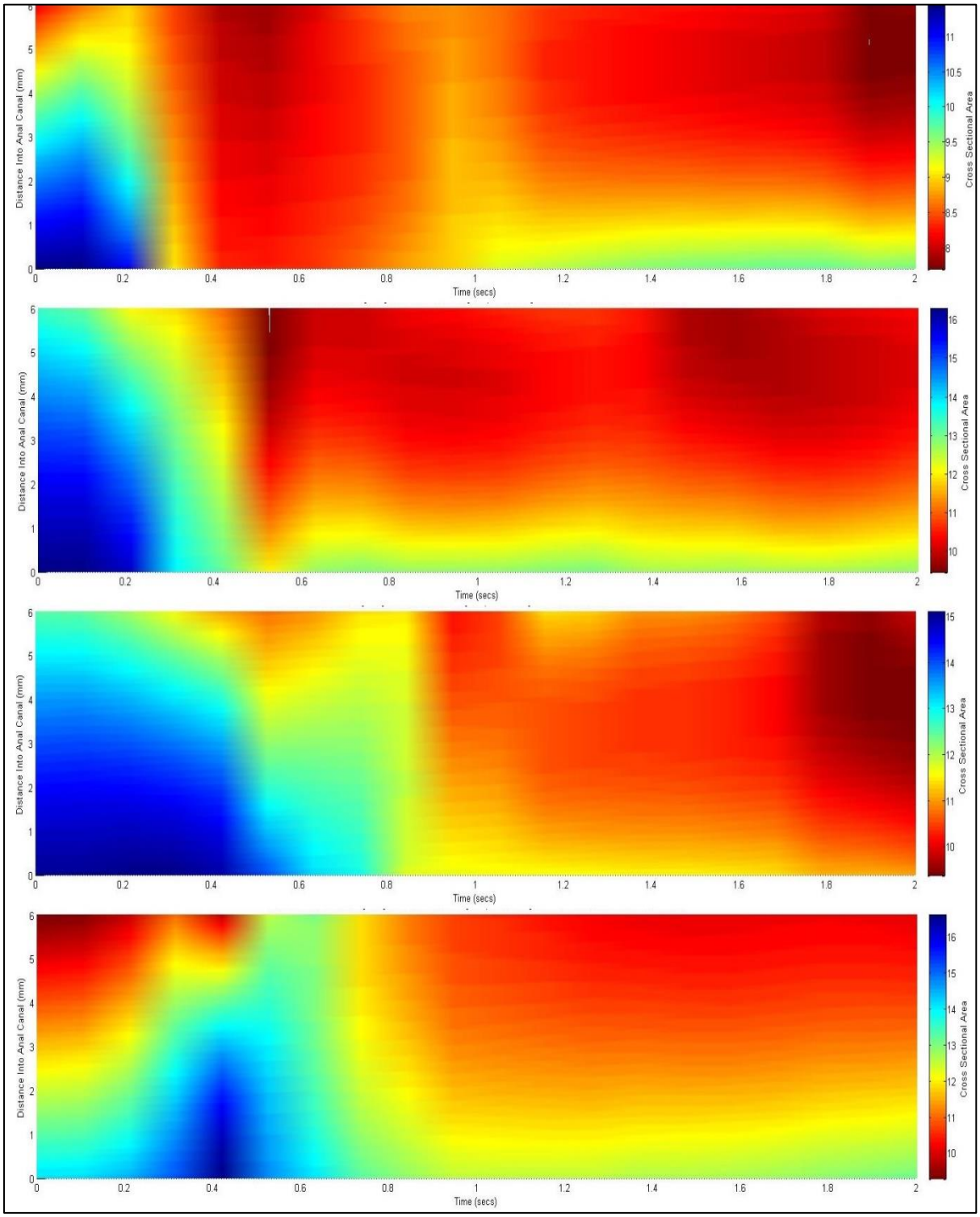
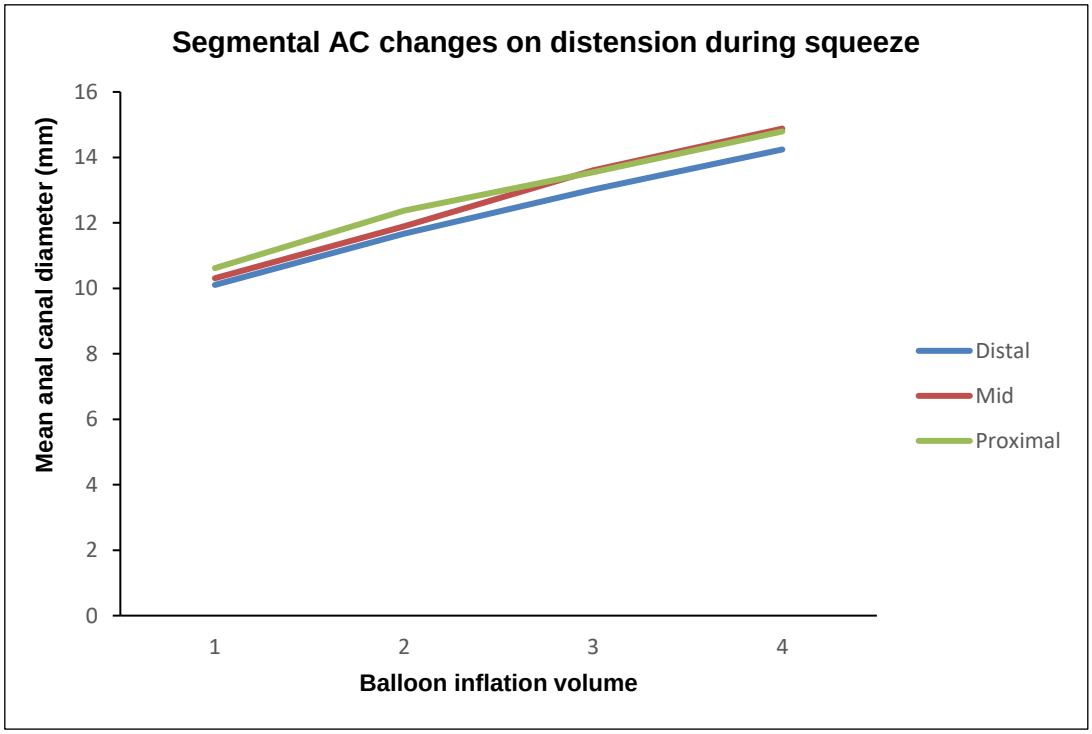


Figure 6.6: Squeeze phase in a single subject across incremental balloon volumes

Data for segmental changes due to distension during squeeze

The segmental analysis of SSc patients during the squeeze phase demonstrated the lower-most segment as having the lowest mean AC_{Dia} at all distension volumes (i.e. it was the most closed part of the anal canal). The upper-most anal canal had the highest mean AC_{Dia} initially (i.e. was the most open part of the anal canal) but this changed with the distension to a mean AC_{Dia} lower than that seen in the mid anal canal at the higher two inflation volumes but still higher than the mean AC_{Dia} of the lower-most anal canal. The data and the graph demonstrating these changes is shown below in Figure 6.7.



Anal Canal segment	Mean AC diameter (mm) at incremental balloon inflation volumes			
	1	2	3	4
Distal	10.10	11.67	13.02	14.24
Mid	10.31	11.89	13.61	14.88
Proximal	10.62	12.38	13.55	14.79

Figure 6.7: Segmental differences in the mean anal canal diameter on subjecting anal canal to incremental distension during squeeze phase across all the subjects in group 3; AC = anal canal

6.4.3 RAIR phase

Similar to the squeeze phase, there was no distinctive pattern observed during the RAIR phase in the scleroderma patients. The lower-most anal canal was noted to be either open at minimal inflation volumes or opened up completely with distension. On the other hand, the upper-most anal canal was found to be closed even at the lowest inflation volume (Figure 6.8 & 6.9).

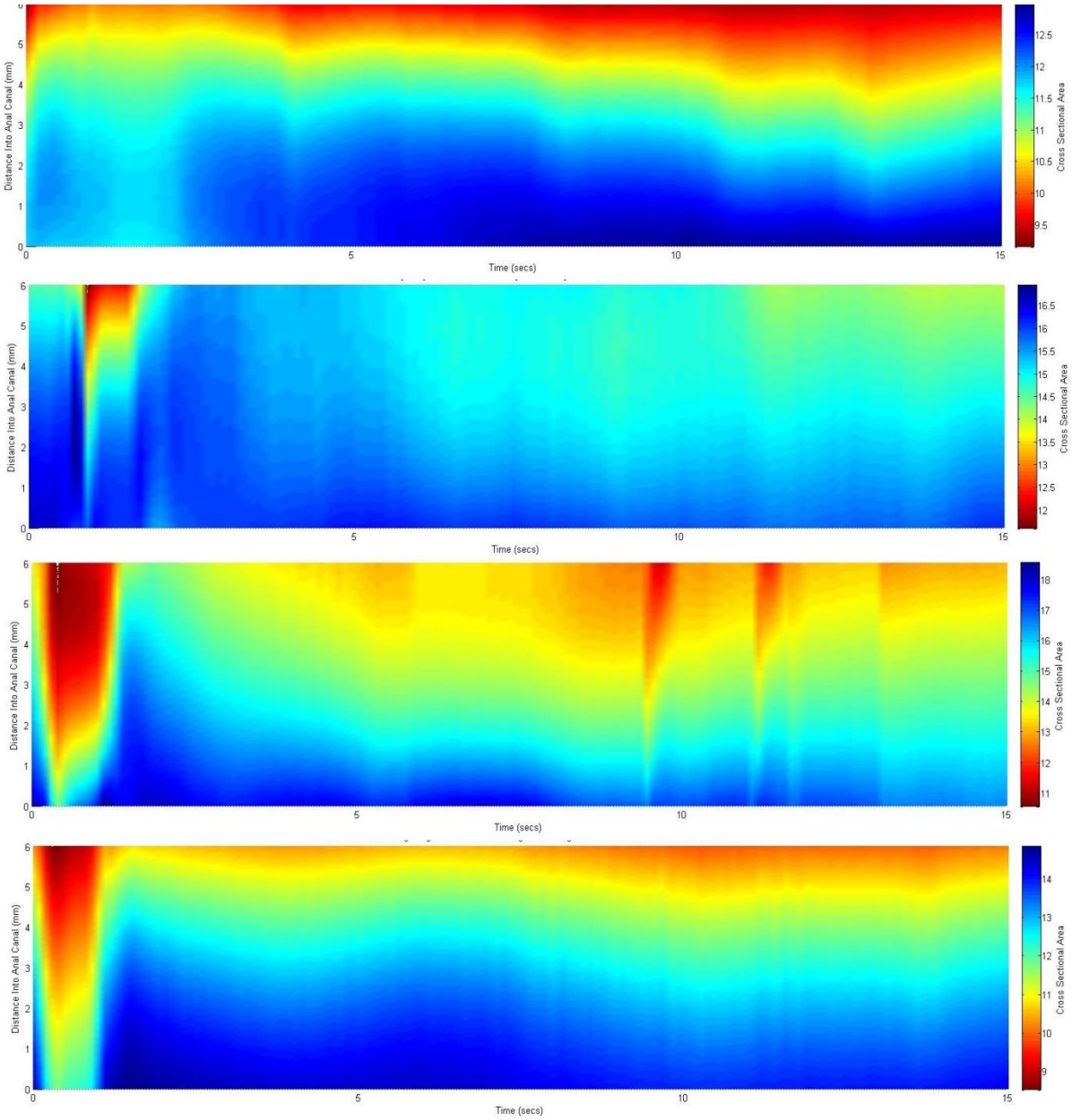


Figure 6.8: RAIR phase in a single subject across incremental balloon volumes

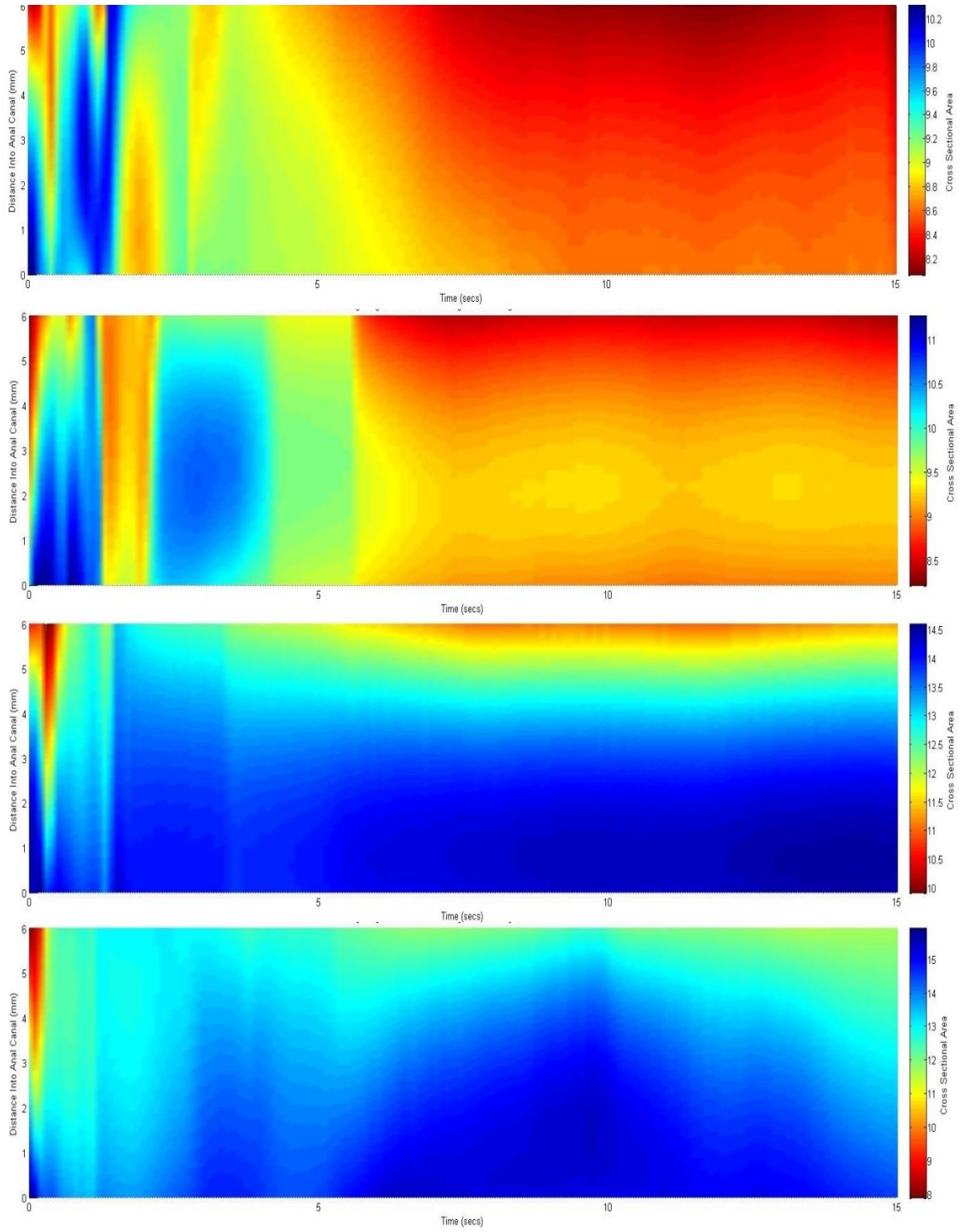
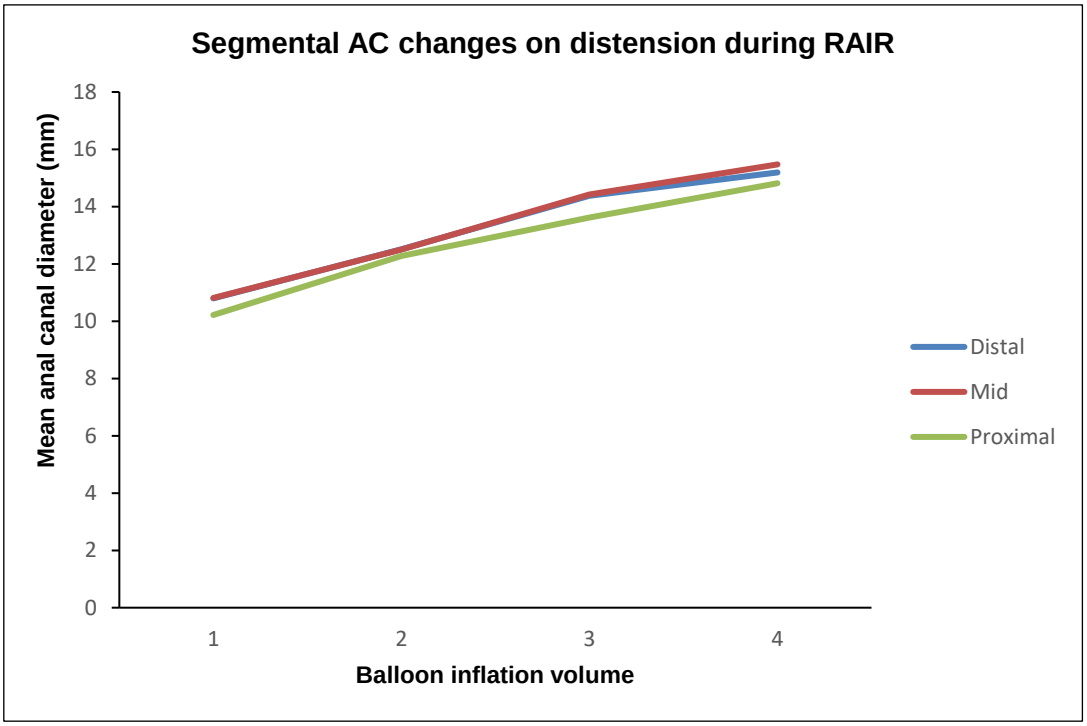


Figure 6.9: RAIR phase in a single subject across incremental balloon volumes

Data for segmental changes due to distension during RAIR

The segmental changes during the RAIR phase mirrored those seen during the resting phase with the upper-most anal canal having the lowest mean AC_{Dia} at all distension volumes (i.e. being the most closed part of the anal canal). The mid and the lower-most segment had almost similar mean AC_{Dia} apart from at maximal distension where the mean AC_{Dia} of the lower most segment reduced slightly below that of the mid anal canal. These findings are displayed below in Figure 6.10.



Anal Canal segment	Mean AC diameter (mm) at incremental balloon inflation volumes			
	1	2	3	4
Distal	10.80	12.52	14.38	15.19
Mid	10.81	12.50	14.43	15.47
Proximal	10.22	12.28	13.62	14.81

Figure 6.10: Segmental differences in the mean anal canal diameter on subjecting anal canal to incremental distension during RAIR phase across all the subjects in group 3; AC = anal canal

6.5 Comparison with healthy cohort

The contour plots of SSc patients have so far revealed variances from the healthy cohort. To check whether these differences were significant, statistical analysis was undertaken. The SSc patient data did not have a normal distribution and as such, Mann-Whitney test was used to compare the data between the scleroderma patients and the healthy subjects. Mann-Whitney test is a non-parametric equivalent of the independent t-test and takes into account the median rather than the mean.

The comparison included all the patients in both the groups across all the balloon inflation volumes. Each of the three phases namely resting, squeeze and RAIR was tested separately to compare the three anal canal segments in between the two groups. The full data from the Mann-Whitney test is presented below in Table 6.1. There was a total of 8 SSc patients and 19 healthy cohort, each of them having test at 4 balloon inflation volumes. Hence the data points being shown as 32 for the SSc group and 76 for the healthy cohort. The plots showing these segmental differences between the SSc patients and the healthy cohorts are presented in Figure 6.11-6.13.

In the resting phase, the median AC_{Dia} was higher (i.e. the anal canal was open) in the lower and mid segments of SSc patients in comparison to the healthy cohort but this did not achieve a statistical significance (14.11 mm vs 11.41 mm, p = 0.10 and 14.13 mm vs 13.84 mm, p = 0.83 respectively). The upper anal canal on the other hand was more closed in the SSC patients (12.15 mm vs 14.88 mm, p = 0.06).

During the squeeze phase, the median AC_{Dia} was lower in the upper anal canal (10.93 mm vs 14.27 mm, p = .03) but not in the lower anal canal where it was more than in the healthy cohort. However, this difference was not found to be significant (11.43 mm vs 10.45 mm, p =0.12).

The comparison of the RAIR phase in two groups revealed similar result, with the median AC_{Dia} of the upper anal canal being lower (i.e. more closed) in the SSc patients than the healthy cohort (11.23 mm vs 14.34 mm, p = 0.04). The lower segment was more open in SSc patients but was not found to be significantly different from the healthy cohort (13.08 mm vs 12.34 mm, p = 0.63).

Group Statistics

Test phase	Subjects	N	Median	IQR	p value
Resting					
Lower anal canal	HC	76	11.41	9.54-13.51	0.10
	SSC	32	14.11	9.02-16.41	
Middle anal canal	HC	76	13.84	11.24-16.48	0.837
	SSC	32	14.13	10.26-16.99	
Upper anal canal	HC	76	14.88	12.01-17.47	0.06
	SSC	32	12.15	8.85-16.39	
Squeeze					
Lower anal canal	HC	76	10.45	8.62-12.34	0.12
	SSC	32	11.43	8.72-14.49	
Middle anal canal	HC	76	13.13	10.25-15.18	0.44
	SSC	32	11.57	8.82-15.80	
Upper anal canal	HC	76	14.27	11.38-16.79	0.03
	SSC	32	10.93	8.13-15.71	
RAIR					
Lower anal canal	HC	76	12.34	9.64-14.70	0.63
	SSC	32	13.08	9.37-15.63	
Middle anal canal	HC	76	14.49	11.31-17.06	0.25
	SSC	32	13.23	9.85-16.81	
Upper anal canal	HC	76	14.34	11.24-17.39	0.04
	SSC	32	11.23	8.80-15.54	

Table 6.1: Group Statistics from Mann-Whitney test
(HC; healthy control, SSc; Scleroderma group, IQR; Interquartile range)

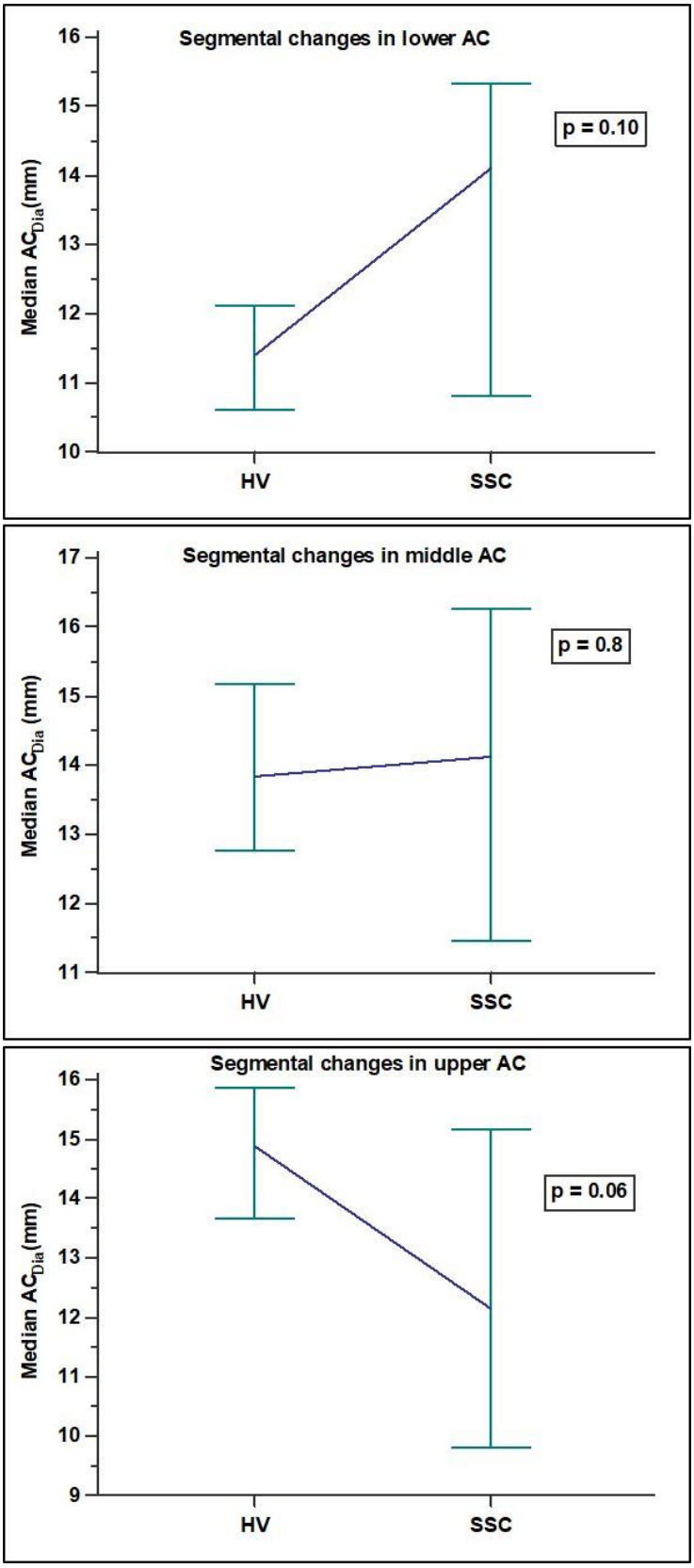


Figure 6.1: Mann Whitney test comparing median AC_{Dia} in HV and SSC during resting phase; HV=healthy volunteers, SSC=Scleroderma patients, AC=Anal canal

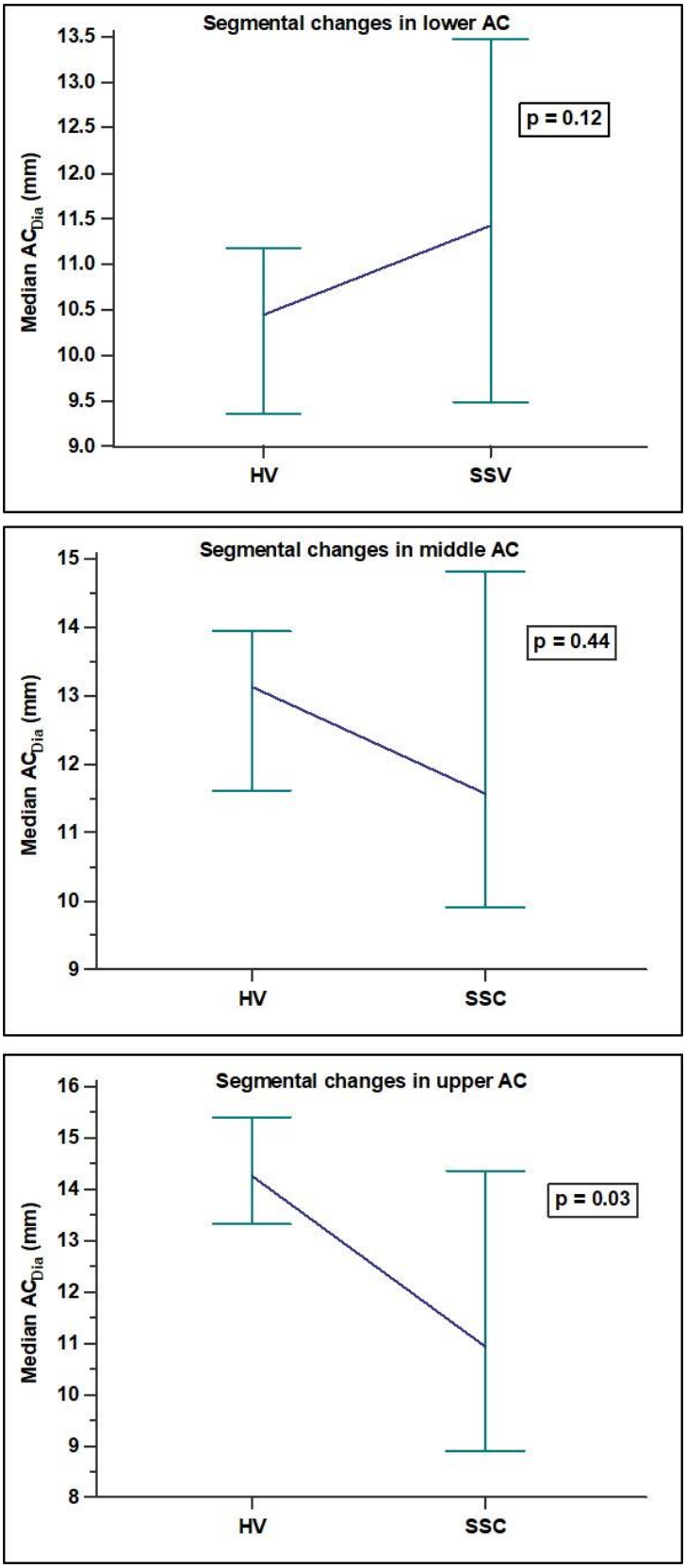


Figure 6.2: Mann Whitney test comparing median AC_{Dia} in HV and SSC during squeeze phase; HV=healthy volunteers, SSC=Scleroderma patients, AC=Anal canal

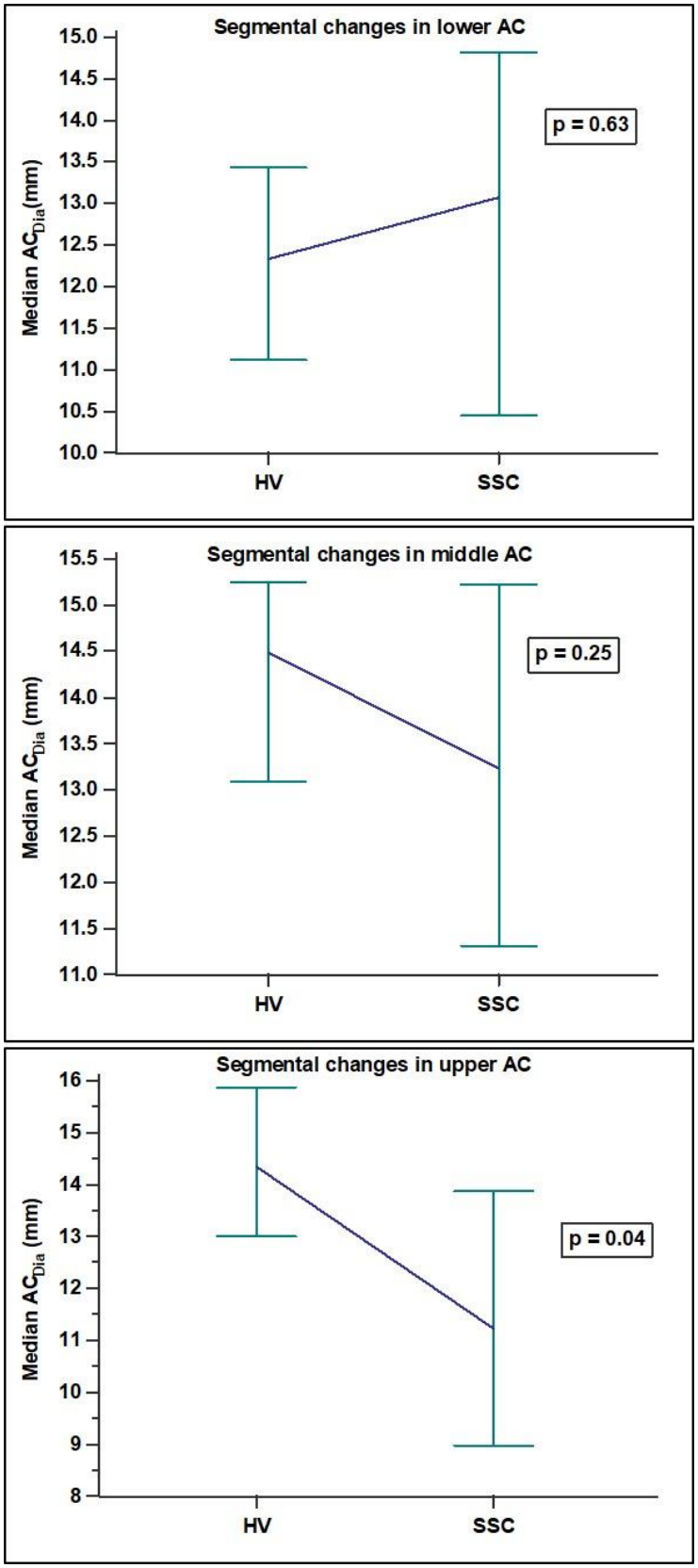


Figure 6.3: Mann Whitney test comparing median AC_{Dia} in HV and SSC during RAIR phase; HV=healthy volunteers, SSC=Scleroderma patients, AC=Anal canal

6.6 Conclusion

The GI involvement in scleroderma patients is well documented. This study helped further explore some of the known physiological features of scleroderma patients using a new modality, the EndoFLIP.

Firstly, the functional anal canal length (or high-pressure zone) was found to be lower in scleroderma patients as compared to the healthy population. About 75% of patients in this group had a functional anal length of 2cm. Functional anal canal length corresponds anatomically to the condensation of the smooth muscle fibres, of the internal anal sphincter, which is affected by the disease in this patient group. The fibrosis in smooth muscles of scleroderma patients is different from the age-related fibrosis of IAS, which results in its thickening. Here, the associated vasculopathy of arterioles leads to ischaemia resulting in atrophy of IAS [121]. Similar results have previously been described in a study of 17 scleroderma patients which found the mean functional length to be about 2.4cm in disease group as compared to 3.7cm in the healthy controls [409]. Moreover, this finding also corroborates with studies which demonstrated IAS atrophy, in scleroderma patients, using diagnostic modalities such as MRI [138] and EAUS [139].

Several studies have demonstrated a low resting pressure in scleroderma patients [409-411] but this is the first study demonstrating the actual functional changes in the anal canal. The EndoFLIP revealed that during resting phase the lower-most anal canal was more open in the scleroderma patients as compared to the healthy subjects. The distal segment has been found to maintain closure, in the healthy cohort, even at the maximal distension and it being open in the SSc patients presumably points towards the pathophysiology of incontinence in these patients. The open distal segment is unable to retain the contents that enter the anal canal during the process of sampling, thereby resulting in incontinence. Despite the p value not reaching the arbitrary number of 0.05 ($p=0.10$), while comparing the lower-most anal canal of SSC and healthy controls, it is very likely that an effect is still present but the small sample size has led to a type II error in this study. A small sample size has a high likelihood of producing a result which is not sufficiently powered and the statistical power is what detects a real difference in between the two groups. By having a small sample size, it is likely that this study was unable to detect the difference thereby providing falsely negative results. Further studies with a bigger sample size may help confirm these functional changes in the SSc patients.

Another significant finding was that during the resting phase, the upper-most anal canal in scleroderma patients was found to be more closed than healthy subjects, $p = 0.06$. SSC

patients have been found to have reduced rectal capacity in previous studies [409,411]. The fibrous connective tissue proliferation in the rectal submucosa and the muscularis mucosae has been postulated to be the cause for reduced rectal capacity in these patients [121]. It is likely that more than usual closure of the upper anal canal in SSC patients is due to IAS fibrosis in this patient group in addition to the changes in the adjacent rectal tissue.

The SSc does not affect the external anal sphincter which is a striated muscle and as such the squeeze pressure is expected to be maintained in these patients. However, this study noted that the squeeze in this patient group was actually affected. In the lower-most anal canal, the median AC_{Dia} was lower in the healthy subjects as compared to the SSc patients, pointing to a better squeeze mechanism in the healthy subjects. This did not reach a statistically significant value ($p=0.12$), which might represent a type II error due to the small sample size, as discussed previously. A similar finding of reduced squeeze pressure has also been reported by another study in the past [255]. In depth analysis with MATLAB revealed the problem to be present only in the lower-most anal canal which did not close during squeeze as opposed to the mid and upper anal canal which did. It is difficult to explain this but the non-compressibility of fibrotic IAS by the EAS might be one of the responsible factors. A certain degree of fibrosis of the IAS is known to be a normal age-related phenomenon. However, in SSc patients there is activation of multiple fibrotic pathways due to immune activation, microvascular damage, and fibroblast transformation into the myofibroblast. This fibrosis leads to an increased stiffness and decreased elasticity of IAS, thereby making it difficult to be compressed by the EAS.

The RAIR has been found to be affected in SSc patients [409-411], a finding which was replicated in this study. During the RAIR phase, most of the patients did not display the typical RAIR pattern that was seen in the healthy controls, where an initial reduction in AC_{Dia} was followed by relaxation and returning of anal canal to its original state. Instead, the RAIR was either completely absent or led to a complete opening of the lower and mid anal canal. This absence of RAIR seen on the contour plots of the SSc patients has previously been reported by several studies [217,409,412]. Thoua et al. reported absence of RAIR in 46% of SSc patients with incontinence symptoms whereas Heyt et al. reported this figure to be as high as 86% in their study on SSc patients with incontinence. Jaffin et al. found 50% of SSc patients in their study did not have a RAIR on anorectal manometry [135]. The absence of RAIR in SSc patients is postulated to be due to not only myogenic but also neurogenic causes. The neurogenic component in SSc is supported by histological studies. Malandrini et al. obtained rectal biopsies from SSc patients and showed structurally normal enteric neurons, but abnormal axon terminals, with axoplasm devoid of cytoskeletal elements, suggestive of degeneration and depletion of neurotransmitters [413]. Other studies showing depletion of the

neuropeptides NY and YY and higher anal sensory threshold in SSc patients also support the neuropathic grounds of the disease [208].

In conclusion, EndoFLIP seems to be a promising technology which has helped visualise the functional changes that take place in the anal canal of scleroderma patients. By confirming the previously known features of anal physiology in SSC patients EndoFLIP has proven its validity. Furthermore, it has demonstrated functional changes during the resting, squeeze and RAIR phase which helps explain the pathophysiology of incontinence in these patients. However, future studies with large sample size are still required to get better conclusive data and to establish the clinical utility of EndoFLIP.

Chapter 7

Conclusions & Future work

7.1 Main findings

My work is mainly concerned with demonstrating the functionality of the anal canal with the help of custom-made probes for the EndoFLIP device. I have looked at the healthy cohort to validate the EndoFLIP for use in anorectal studies. This was followed by establishing clinical utility of EndoFLIP by studying the idiopathic faecal incontinence patients and scleroderma patients. The key findings are presented below.

7.1.1 In vitro testing

I believe that in vitro study, done using a pig anorectum was a significant first step. I established the repeatability of the custom-made anorectal probes with this study. Furthermore, this also provided a rough guide to the inflation volumes to be used for the anal canal balloon during the human tests.

7.1.2 Healthy cohort

EndoFLIP is a relatively new diagnostic modality in the field of anorectal physiology. The aim of this study was to investigate the repeatability of EndoFLIP in healthy cohort and to check the agreement between the existing knowledge and EndoFLIP results.

7.1.2.1 I used custom-made probes of three separate lengths (2, 3 & 4 cm) corresponding to the shorter functional length of the anal canal. Although, the repeatability of the custom-made probes was proven in the in-vitro settings, this being a pilot study I believed it was essential to check the repeatability in humans as well. I confirmed the repeatability with the help of statistical testing.

7.1.2.2 In this study, I found significant segmental changes during the voluntary contraction which were not demonstrated in the two previous studies from different centres which used EndoFLIP to study the anorectal physiology. I believe the likely reason for this is the use of longer probes (which sat in the rectum proximally and outside the anal verge distally) in those two studies. This highlights the importance of using shorter probes which are better suited to the anorectum and allow us to obtain more accurate results and overcome the shortcomings of the previous studies.

7.1.2.3 My study on healthy cohort has confirmed the heterogeneity of the anal canal on dynamic testing with the EndoFLIP. EndoFLIP revealed that the distal anal canal segment was the most closed part of the anal canal followed by mid and proximal anal canal segments

during the resting phase. Applying the Laplace's law, this would imply different pressure is generated in different anal canal segments. This highlights the key difference between EndoFLIP and anorectal manometry which assumes anal canal to have a static functionality and provides only one pressure reading for whole of the anal canal.

7.1.2.4 With the EndoFLIP testing, I found the distal anal canal segment to be most resilient to change even on maximal distension during resting and squeeze phase. This finding highlights the importance of the distal anal canal in maintaining continence and is a new addition to our existing physiological knowledge of the anal canal.

7.1.2.5 I have also confirmed the role of puborectalis muscle in the anal canal closure during voluntary contraction. Furthermore, during the RAIR phase, EndoFLIP helped me demonstrate a brief closure of distal anal canal segment (on rectal distension with the balloon) followed by opening of mid and upper segment. These findings correspond well to our existing knowledge of anal canal functioning.

By demonstrating the existing physiological knowledge of anal canal in real-time with EndoFLIP during voluntary contraction and the RAIR phase I have validated the use of EndoFLIP in further anorectal studies.

7.1.3 Faecal incontinence patients

The EndoFLIP testing helped gain further insight into biomechanical properties and segmental variations of the anal canal in the healthy cohort. My rationale behind studying the faecal incontinent patients was to investigate the clinical utility of EndoFLIP. The key findings in this group and how they differ from the healthy cohort are discussed below.

7.1.3.1 The FI patients did not demonstrate the homogeneity that was the hallmark of the healthy cohort. This was quite obvious during the voluntary contraction and the RAIR phase, where the contour plots revealed various disorganised attempts at closing the anal canal emphasising the pathophysiological mechanism of incontinence in these patients and highlighting the potential of EndoFLIP as an investigating modality for anorectal disorders.

7.1.3.2 I found that a consistent finding in FI patients was a minimal or non-closure of the distal anal canal during the voluntary contraction. This segmental variation was confirmed to be significantly different from the healthy cohort. This finding has not been demonstrated in previous studies and as such adds to our knowledge of the true pathophysiological changes in the anal canal which lead to the urge incontinence experienced by these patients. This

finding also provides further strength to the observation made in healthy cohort about the distal anal canal playing a significant role in maintaining continence.

Furthermore, this finding differs from the Danish study which used a longer EndoFLIP probe to evaluate the FI patients and concluded that the FI patients were able to keep significant part of the anal canal closed even at higher distension volumes. This further highlights the importance of using shorter probe to evaluate anal canal more accurately.

7.1.3.3 This study revealed that the mid anal canal was open during voluntary contraction in FI patients. This segmental variation was confirmed to be statistically significant on comparison with the healthy cohort. This finding authenticates the results from the only other study which observed similar findings using EndoFLIP to evaluate the FI patients. This adds to our existing knowledge of pathophysiology in FI patients.

7.1.3.4 Another significant finding in my study was the opening of distal anal canal segment with minimal distension during the RAIR phase. This abnormality was picked up despite the pressure graph demonstrating a response which would be classed as normal during anorectal manometry. This finding demonstrates the edge EndoFLIP provides over anorectal manometry in evaluating anal canal physiology. It further sheds light on the pathophysiology of passive incontinence in these patients by demonstrating the inability of anal canal to maintain closure during the process of sampling, which occurs several times a day.

7.1.3.5 I observed that the anal canal in the FI patients had a generally poor closure in comparison to the healthy cohort. Furthermore, a complete opening of the distal segment with distension was also noted during the resting phase. This segmental difference from the healthy cohort was found to be statistically significant and explains the pathophysiology of passive incontinence in this group, thereby proving the clinical utility of EndoFLIP.

7.1.3.6 This study investigated the distensibility of anal canal in FI patients and found it to be higher than in the healthy cohort, implying that the anal canal opens up at much lower pressure in the FI patients as compared to the healthy cohort. This finding highlights the clinical utility of EndoFLIP in discriminating FI patients from the healthy.

7.1.3.7 The results from FI patients confirmed the puborectalis muscle function in the proximal anal canal segment, similar to that noted in the healthy cohort. Confirming the existing physiological knowledge helps validate the EndoFLIP for use in the anorectal studies.

7.1.4 Scleroderma patients

The GI involvement in scleroderma patients is well documented. This study helped further explore pathophysiology of incontinence in scleroderma patients and also establish clinical utility of the EndoFLIP.

7.1.4.1 This study demonstrated a lower functional anal canal length in the scleroderma patients and authenticates the result from a previous study with similar findings. Moreover, this finding also corroborates with studies which demonstrated IAS atrophy, in scleroderma patients, using diagnostic modalities such as MRI [138] and EAUS [139].

7.1.4.2 Several studies have demonstrated a low resting pressure in scleroderma patients, but this is one of the few studies demonstrating the actual functional changes in the anal canal. The EndoFLIP revealed that during resting phase the lower-most anal canal was more open in the scleroderma patients as compared to the healthy subjects. Although this segmental variation did not achieve the arbitrary p value of 0.05 or less, possibly due to type II error, it is very likely that this still demonstrates the pathophysiology of passive incontinence in these patients.

7.1.4.3 An interesting find in my study was impairment of the squeeze mechanism in SSc patients. Although the SSc does not affect the external anal sphincter which is a striated muscle, I found that the distal anal canal did not close as much as in the healthy cohort. This might be due to the non-compressibility of fibrotic IAS by the EAS. This did not reach a statistical significance ($p=0.12$) which might be a type II error due to small sample size or an actual chance finding. Further studies might help clarify if this finding demonstrates the pathophysiology of incontinence in the SSc patients.

7.1.4.4 I found the upper-most anal canal in scleroderma patients to be significantly more closed than the healthy subjects. I believe this to be due to the fibrotic changes in the adjacent rectal tissue and the IAS. This segment has previously been shown to be the least distensible in a separate EndoFLIP study. My results support the findings of the other study and advances our understanding of the functional changes in the SSc patients.

7.1.4.5 This study confirmed that the RAIR is affected in SSc patients, a finding which has been demonstrated in other studies previously. This absence of RAIR in SSc patients is postulated to be have a myogenic and neurogenic basis. By confirming the existing knowledge EndoFLIP has shown its potential for use in further clinical studies

7.2 Limitations

7.2.1 The healthy cohort and the FI group were not age-matched in this study, with a mean age of 34 (20-75) for the healthy cohort as compared to the mean age of 67 (54-87 for the FI group. Although this might have contributed to some of the differences seen in these two groups, based on our physiological knowledge of the anorectum, I do not believe that an age-matched study would demonstrate significantly different results from this study.

7.2.2 The obstetric history about episiotomy was not specifically checked in the FI group. Although all the subjects in this group had an EAUS and were eligible for inclusion only if there was no demonstrable morphological abnormality, I now am aware of the studies that have demonstrated pudendal nerve damage secondary to episiotomy. There is a possibility that this neurogenic dysfunction contributed to faecal incontinence symptoms in the FI group.

7.2.3 The small number of patients in the SSc group was a particular limitation which might have led to a type II error in this group. Moreover, the parity of the patients in this group was not recorded. Although the patients had EAUS to confirm that there was no morphological abnormality, the possibility of neurogenic injury during delivery contributing to the symptoms of FI cannot be completely ruled out.

7.2.4 Although the use of Imodium was recorded in group 2 and group 3 subjects, a detailed drug history was not taken. I now understand that commonly used anti-hypertensive medications such as calcium channel blockers can lead to a reduced pressure in the anorectal region. Another medication that has been shown to cause relaxation of IAS is sildenafil which is also commonly used in this age group.

7.2.5 Using the pig anorectum to test for repeatability had the limitation of lacking the sphincter tone offered by a live specimen. The elasticity and compliance would also have been altered due to lack of physiological control which is present in live controls. However, despite these limitations, this model was chosen for its similarities to the human morphology and providing better elasticity properties than the other available option of using a rigid tube.

7.2.6 Another limitation of the study was that the recorded data did not represent the whole of the anal canal due to limitations of impedance planimetry technique. To allow the electric field to reach up to the balloon diameter the probe was required to have a gap between the first and last pair of electrodes where no data was recorded. This meant that the data was recorded only for part of the anal canal. This was most noticeable in the smallest 2 cm anal canal balloon where only 3 CSA points were recorded representing slightly less than 50% of the functional anal canal length. As the length of the anal canal balloon increased so did the

area of recordable data, increasing to 80% in the 4 cm long anal canal balloon. In our study, we had only one subject that required 2 cm long anal balloon with the rest divided equally into 3 & 4 cm long balloons. However, it should be noted that despite this limitation, the study still proved to be valuable as it provided new information with regard to anorectal physiology in addition to allowing for an easy distinction between the healthy controls and the FI patients.

7.2.7 A potential drawback of impedance planimetry technique, used by EndoFLIP, is that the movement of the probe within the lumen can result in spurious measurements. However, good repeatability was confirmed both in in-vitro and human studies proving that this was not a real problem in this study.

7.2.8 Finally, multiple uses of the EndoFLIP probes lead to failure of the solid-state pressure transducer in the catheter resulting in some inaccuracies, evident at looking at raw data. However, these were not seen in all the patients and more importantly despite these inaccuracies there was still a significant difference in distensibility index between controls and patients. It is very likely that this difference would have been even more significant were it not for these inaccuracies.

7.2.9 The test was not much different from anorectal manometry and was well tolerated. However, repeated measurements at different inflation volumes did mean that it took longer to perform.

7.2.10 I did not perform any comparison of EndoFLIP data with the anorectal manometry results. This might have helped provide further evidence for use of EndoFLIP in clinical settings in addition to validating the EndoFLIP.

7.3 Future Work

This study has undoubtedly proven the utility of EndoFLIP in anorectal studies. However, further work needs to be done before EndoFLIP can be deployed as a mainstream investigative modality. Future studies with large sample size looking at different subsets of patients will be valuable to understand and develop this diagnostic modality further.

7.3.1 Neuromodulation

EndoFLIP has the potential of enhancing our existing clinical knowledge. For instance, neuromodulation with SNS and PTNS is commonly used to provide symptom relief in FI patients. Although our understanding of the mechanism of actions of these neuromodulation techniques has improved greatly, the fact remains that it is still poorly understood. EndoFLIP can potentially demonstrate the physiological changes that take place in the anorectum of patients receiving such treatment. Moreover, it can help study the effects of different parameters such as frequency and pulse width on anal canal physiology and allow to fine tune these to improve symptom relief.

7.3.2 Intraoperative uses

Another potential use of EndoFLIP is in the field of therapeutics especially in patients undergoing surgery for faecal incontinence. A study designed to look at pre-, intra- and post-op segmental and functional anal canal changes in patients undergoing a procedure such as sphincter augmentation with injectable material, muscle transposition or the experimental treatment modalities such as stem cell injections can provide valuable information which can help refine the technique and improve the functional results.

7.3.3 Scleroderma

I propose a further study with EndoFLIP looking at the SSc patients with and without incontinence symptoms. Previous studies with anorectal manometry have already demonstrated a difference between squeeze pressure, anal sensory threshold and RAIR in between symptomatic and asymptomatic SSc patients. EndoFLIP might help develop further insight into pathophysiology of incontinence in this patient group.

7.3.4 Comparison with ARM

I feel that further research is required comparing the EndoFLIP with conventional anorectal manometry and high-resolution anorectal manometry. This would not only allow us to find the

level of agreement between these different investigation modalities but also help develop clinical utility of EndoFLIP.

7.3.5 Pelvic radiotherapy

I propose a study of patients undergoing pelvic radiotherapy with EndoFLIP to understand the functional changes that take place in these patients. It would be interesting to see

7.3.6 Comparison between age-matched groups

In this study the healthy cohort and FI group were not age-matched. It would be interesting to see if the segmental differences noted in the younger cohort are replicated in the older healthy population. Furthermore, this would also be a chance to ensure that the segmental differences noted in the healthy cohort and the FI group in this study, stand true when age-matched groups are compared.

7.3.7 Comparison with long probes

I have introduced the shorter EndoFLIP probes to study the anorectum and have demonstrated significant differences in the results obtained from them as compared to the results from the longer probes used in other studies. A further study of healthy cohort and FI patients comparing the two probes would provide insight into whether use of these shorter probes actually offers any clinical benefits.

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Appendix 1

Wexner Incontinence Questionnaire

The Wexner score

	Frequency				
Type of incontinence	*Never*	*Rarely*	*Sometimes*	*Usually*	*Always*
Solid	0	1	2	3	4
Liquid	0	1	2	3	4
Gas	0	1	2	3	4
Wears pad	0	1	2	3	4
Lifestyle alteration	0	1	2	3	4

Never, 0; rarely, <1/month; sometimes, <1/week, ⩾1/month; usually, <1/day, ⩾1/week; always, ⩾1/day.

0, perfect; 20, complete incontinence.

Appendix 2

Wexner Constipation Score

Please Answer the Following questions that relate to the emptying of your bowels. Circle the most appropriate number that applies to you.

1.	Typically how often do you empty your bowels?	Score
	1-2 times per 1-2 days	0
	2 times per week	1
	Once per week	2
	Less than once per week	3
	Less than once per month	4
2.	How often do you have to strain to empty your bowels?	Score
	Never	0
	Rarely	1
	Sometimes	2
	Usually	3
	Always	4
3.	How often do you feel you have not fully evacuated your rectum when you empty your bowels?	Score
	Never (always feel empty)	0
	Rarely	1
	Sometimes	2
	Usually	3
	Always (never feel empty)	4
4.	How often do you suffer with abdominal pain due to your bowel evacuation problem?	Score
	Never	0
	Rarely	1
	Sometimes	2
	Usually	3
	Always	4

5.	Typically how long do you spend in the lavatory per attempt?	Score
	Less than 5 mins	0
	5 – 10 minutes	1
	10 – 20 minutes	2
	20 – 30 minutes	3
	More than 30 minutes	4

6.	Which of the following do you need, to help with the emptying of your bowels?	Score
	No help	0
	Laxatives	1
	Digital assistance, suppositories, enema	2

7.	Typically how often do you attempt to empty your bowels **WITHOUT** a result in a 24hr period?	Score
	Never	0
	1 - 3	1
	3 - 6	2
	6 - 9	3
	More than 9	4

8.	How long have you had these bowel symptoms?	Score
	0 years	0
	1 – 5 years	1
	5 – 10 years	2
	10 – 20 years	3
	More than 20 years	4

Total Score: ……..