University of Southampton

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Epidemiology and Treatment of Interstitial Cystitis/Painful Bladder Syndrome and the Potential of Medical Grade Manuka Honey as Therapeutic Strategy

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Abstract

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Interstitial Cystitis/Painful Bladder Syndrome (IC/PBS) is a complex urological disease with no known cure yet. There are over 180 available treatment options, the effectiveness of which are variable. Scarce data exist regarding perception of treatments and Health Related Quality of Life (HRQoL) in this patient population. Herein, an epidemiologic description of the treatments used in IC/PBS and the relationship between illness perception and severity of symptoms was undertaken using an online questionnaire completed by members of the Bladder Health UK (BHUK). The O'Leary/Sant, Pelvic Urgency and Frequency (PUF), Brief Illness Perception and King Health questionnaires were used to collect data from study participants. A key finding of this work shows that irrespective of background treatments, many patients remain symptomatic with a mean ± SD O'Leary/Sant scores of 20.12 ± 9.38. Many participants were not on oral treatment. There were no significant differences between the O'Leary/Sant scores of cohorts currently taking oral medications and those who remain untreated ($p=0.234$). Amitriptyline was the most common medication used either alone or in combination for the treatment of the disease in the cohort. In terms of lifestyle changes, the O'Leary/Sant scores of those drinking alcohol were significantly lower than those not drinking ($p\leq0.05$). There was marked deterioration of HRQoL of the respondents as evaluated by KHQ and BIP-Q. About 25% of the sample believed that IC/PBS had negative consequences for their daily lives while more than 75% could see no end to their symptoms. Most of the participants indicated that their disease made them worry and become emotionally unstable with a resultant decrease in quality of life. The BIP-Q items that were associated with IC/PBS severity were (Adjusted Odd Ratio (AOR) and Confidence Interval (CI)): Consequence 0.09 (0.02-0.38); Treatment control 2.702 (1.25-5.81); Identity 0.141 (0.03-0.60) and Concern 9.36 (1.52-57.63). Overall, this survey identified that over 80% of the participants were still symptomatic despite many being on no specific oral therapy. Participants were using non-guideline recommended treatments such as gabapentenoids, antibiotics, smooth muscle relaxants, and herbal formulae amongst others. This suggests a need to test the hypotheses as to whether such non-guideline treatments could be useful in IC/PBS using appropriately designed experimental studies. Accordingly, the cell culture work was aimed at demonstrating the potentials of medical grade Manuka honey (MH) on IC/PBS cellular model. This was done using β-hexosaminidase release assay, histamine ELISA and SDS PAGE. MH was tolerable at 2% and 4% and significantly inhibited release of beta hexosaminidase in SP, IgE and A23187 models $p\leq0.001$, $p=0.000$ and $p\leq0.001$ respectively. It also inhibited the release of histamine ($p\leq0.05$). Likewise, MH at 4% also inhibited the release of IL-8 ($p\leq0.001$) and GM-CSF ($p=0.000$) following A23187 stimulation and IL-8 ($p\leq0.001$) and GM-CSF ($p=0.000$) after being challenged by Substance P (SP). Furthermore, MH attenuates the expression of Akt ($p\leq0.05$), p38 ($p\leq0.05$) and ERKI ($p\leq0.05$) in SP challenged LAD2 cells. Similarly, MH suppressed the expression of ERK I and ERK II ($p=0.000$) in A23187 and IgE stimulated LAD2 cells. These results suggest that MH could be a novel candidate agent for the treatment for IC/PBS.

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Research Thesis: Declaration of Authorship

Print name: Kamaluddeen Garba

Title of thesis: Epidemiology and Treatments of Interstitial Cystitis/Painful Bladder Syndrome and the Potentials of Medical Grade Manuka Honey as a Therapeutic Strategy

I declare that this thesis and the work presented in it are my own and has been generated by me as the result of my own original research.

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Definitions and Abbreviations

ACTH.....Adrenocorticotrophic Hormone

AOR .....Adjusted Odd Ratio

Ag .....Antigen

APF .....Antiproliferative Factor

AUA .....American Urological Association

A23187 .....Calcium Ionophore

BAUS.....British Association of Urological Surgeons

BHUK .....Bladder Health UK

BIPQ.....Brief Illness Perception Questionnaire

BMCMC .....Bone Marrow Derived Cultured Mast Cells

Ca^{2+}.....Calcium Ion

CCL.....Chemokine

CI .....Confidence Interval

COB.....Cystitis and Overactive Bladder

CRH.....Corticotrophin Releasing Hormone

CS .....Chondroitin Sulphate

CS/HA .....Chondroitin Sulphate/Hyaluronic Acid

CTMCs .....Connective Tissue Mast Cells

CXCL .....Cytokine

CysTLR1 .....Cysteinyl Leukotriene Receptor 1

DAG .....Diacyl Glycerol

DMSO .....Dimethyl Sulphoxide

EAU.....European Association of Urology

ESSIC.....European Society for the Study of Interstitial Cystitis

FIC .....Feline Interstitial Cystitis

FcεRI.....High Affinity Immunoglobin E Receptor

Definitions and Abbreviations

GAG ..... Glycosaminoglycan

GMCSF ..... Granulocyte Monocyte Colony Stimulating Factor

GPCR ..... G-Protein Coupled Receptor

HA ..... Hyaluronic Acid

HPA ..... Hypothalamo-Pituitary Axis

HRQOL ..... Health Related Quality of Life

HSC ..... Haematopoeitic Stem Cell

IC ..... Interstitial Cystitis

IC/PBS ..... Interstitial Cystitis/Painful Bladder Syndrome

ICDB ..... Interstitial Cystitis Database

ICPI ..... O'Leary/Sant Interstitial Cystitis Problem Index

ICS ..... International Continence Society

ICSI ..... O'Leary/Sant Interstitial Cystitis Symptom Index

IgE ..... Immunoglobin E

IL-1 ..... Interleukin 1

IL-1β ..... Interleukin 1 β

IL-4 ..... Interleukin 4

IL-8 ..... Interleukin 8

KHQ ..... King's Health Questionnaire

LAD2 ..... Laboratory of Allergic Disease 2

LDH ..... Lactate Dehydrogenase

LUT ..... Lower Urinary Tract

LPS ..... Lipopolysaccharides

MAPK ..... Mitogen Activated Protein Kinase

mTORC ..... Mammalian Target of Rapamycin

MRGPRX2 ..... Mas Related G-Protein Coupled Receptor X2

mRNA ..... Messenger Ribonucleic Acid

MS ..... Multiple Sclerosis

Na-K-ATPase.....Sodium-Potassium ATPase

NGF.....Nerve Growth Factor

NIDDK.....National Institute of Digestive Diabetes and Kidney Disease

OAB .....Overactive Bladder

PCR .....Polymerase Chain Reaction

PDK1.....Protein Dependent Kinase 1

PGE_2.....Prostaglandin E2

PI3K .....Phosphoinositide

PLC.....Phospholipase C

PPI .....Proton Pump Inhibitors

PRV .....Pseudo Rabies Virus

PUF .....Pelvic Pain and Urgency/Frequency

QOL .....Quality of Life

RCT .....Randomise Control Trial

SCF.....Stem Cell Factor

SP.....Substance P

TCA .....Tricyclic Antidepressant

TEER .....Transepithelial Electrical Resistance

TNF-α.....Tumour Necrosis Factor Alpha

UTIs .....Urinary Tract Infections

VAS .....Visual Analogue Scale

VEGF.....Vascular Endothelial Growth Factor

Chapter 1 Introduction

This chapter will provide a background to the research project. The research questions will be clearly stated and justified in the context of the treatments offered for interstitial cystitis/painful bladder syndrome including but not limited to the aim and objectives of the study.

1.1 Interstitial Cystitis/Painful Bladder Syndrome

Interstitial Cystitis/Painful Bladder Syndrome (IC/PBS) is a urological disorder of uncertain cause, which is difficult to diagnose and hard to treat. Primarily, it is a disorder of the urinary bladder characterised by abdominal pain centred on the bladder together with lower urinary tract symptoms (LUTS) such as urgency, frequency, and difficulty in voiding in the absence of urinary tract infection (UTI). Diagnostic criteria are often variable depending on the guidelines used but, overall, the persistence of these symptoms for over 3 months with a feeling of pain or pressure in the suprapubic region or related to the bladder after excluding infections supports the diagnosis.

It is a chronic inflammatory disease that affects millions of people around the world. It is more prevalent in women (90%) than men (10%) (Morales-Solchaga *et al.*, 2019). Little is known regarding the global epidemiological burden of the disease; however, it is well reported in western countries and some parts of Asia. Accurate figures for prevalence and incidence are difficult to establish due to difficulty in disease diagnosis and LUTS, which overlap with those characteristic of other bladder pathologies. However, conservative estimates shows that disease prevalence in the United States (US) has risen steeply from 21.8/100,000 in 2002 to 40.2/100,000 in 2013. Against this, incidence rates of 21.8/100,000 in 2002 to 21.1/100,000 in 2013 (Lee, Chang and Tsai, 2018) remain relatively stable. The latter being in part due to lifestyle measures and stringent diagnostic criteria. In Korea, the prevalence was estimated at 261/100,000 in 2008 (Choe *et al.*, 2011). In Austria, cumulative prevalence was reported to be 306/100,000 in 2007 (Temml *et al.*, 2007). The true incidence of the disease process is still poorly estimated due symptom overlap with other common bladder problems such as overactive bladder (OAB) and Urinary Tract Infections (UTI) (Marinkovic *et al.*, 2009). The epidemiological cause of IC/PBS is still unknown and that partly justifies the motivation for this research.

IC/PBS is a chronic inflammatory disorder whose precise aetiology remain elusive. But it is believed that the effect of various noxious stimuli on the urinary bladder trigger the inflammatory phase of the disorder that is believed to be important in the disease pathogenesis.

Based on histological characteristics, two types of IC/PBS generally exist. The Hunner (ulcer) type and the non-Hunner (non-ulcer) type. The Hunner variant is distinguished by petechial haemorrhage upon cystoscopic examination following hydrodistension whilst the non-Hunner do not present with this feature (Han, Shin and Choo, 2019). Similarly, Hunner subtype is commoner in the older age, less bladder pain and more nocturia (Van Moh, Vetter and Lai, 2018). It is to be noted that Hunner subtype reflects inflammatory process in the disease which is followed by the release of inflammatory filtrates (Akiyama and Hanno, 2019). In the same vein, Hunner variants are more responsive to bladder-directed therapy like Triamcinolone and fulguration than the non-Hunner type (Van Moh, Vetter and Lai, 2018).

1.2 The bladder

The bladder is a hollow organ in the anterior pelvic area, posterior to the pubic symphysis; that serves as the final storage organ for urine prior to its eventual release achieved by voiding through the urethra. It is connected to the kidney by a narrow, slender, and tubular organ called the ureter (left and right). The latter serves as conduit for urine passage from the kidneys to the bladder. In males, inferior to the bladder is the prostrate. In females there is no prostrate, the bladder resting on the surface of the urogenital diaphragm. The uterus is anatomically posterior to the bladder. Posterior to the bladder is the rectum, which empties via the anus. Inferiorly, the bladder is connected to the urethra, which traverses the penis in men and lies anterior to the vagina in women finally ending at the external urethral meatus. The average urine bladder capacity is 400-600ml; micturition reflexes being activated when the urine volume approaches 300 ml and stretch receptors, located in the bladder wall, conduct afferent nerve signals centrally.

The bladder wall is multi-layered, the most superficial part being the epithelium, which is made up of transitional cells supported by a basement membrane. Underlying it is the lamina propria. Deep to the lamina propria lies the detrusor muscle, which contains three-layers and is responsible for bladder contraction during voiding. The adventitial layer lies outside of the detrusor posteriorly and inferiorly, while the serosa is a layer of visceral peritoneum. The layers of the bladder are as presented in Figure 1-1. External sphincter surrounds the sphincter and is supplied by somatic nerves the former being richly innervated by parasympathetic nerve fibres. The lamina propria houses neuronal and immune cells, the latter being primarily represented by mast cells, which together with noxious stimuli mediate inflammatory events in the bladder.

Superficial to the epithelium is a protective covering consisting of long chain polysaccharide sugars cross-linked by amino sugars and known as the glycosaminoglycan (GAG) layer. It is negatively

charged and hydrophilic in nature, which provides both buffer and impermebiality to the urothelium (Lewis, 2000). The bladder is innervated by the autonomic nervous system (ANS). The parasympathetic fibres controls micturition whilst sympathetic nerves supply the bladder blood vessels (Stevens and Lowe, 2005).

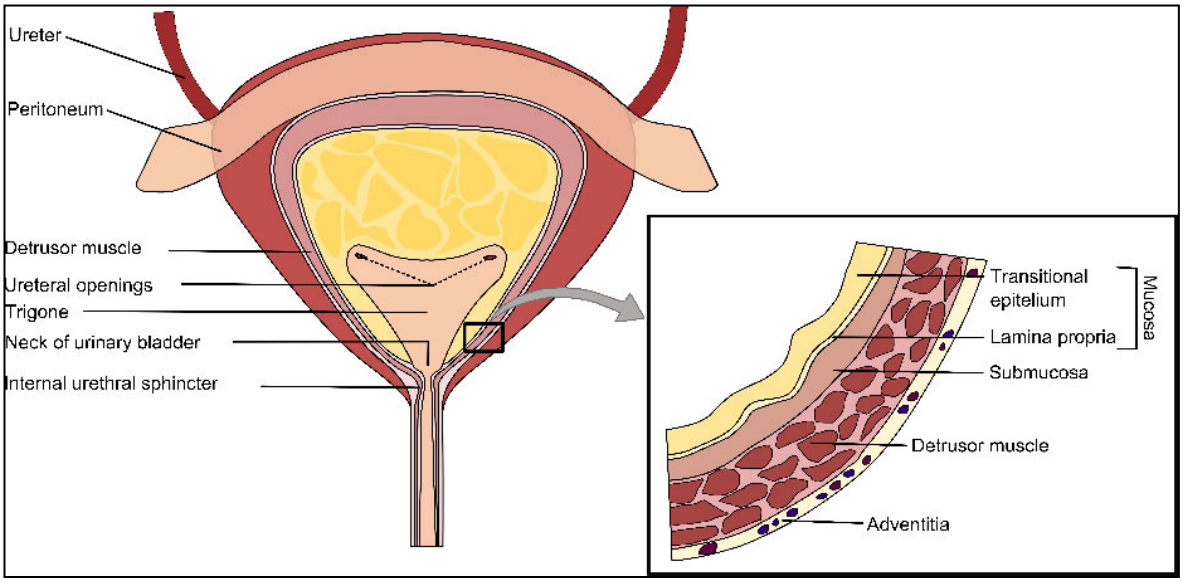


Figure 1-1: Anatomical structure of the human bladder and inner tissues. Reproduced Roccabianca and Bush (2016).

1.3 Pathogenesis of IC/PBS

It is thought that IC/PBS is primarily a pathology of the bladder urothelium. The urothelium serves to protect the bladder tissues from the ingress of toxic urinary contents and uropathogenic bacteria. The precise aetiology of the disease is still poorly understood. However, loss of bladder mucosal integrity through sloughing of the transitional epithelium housing and the glycosaminoglycan layer (GAG), which acts as protective layer and prevent the adherence of micro-organism. This alters the bladder urothelial cytoskeletal cells and starts the pathological cycle. This loss of urothelial cytoskeleton in IC/PBS is supported in part by expression of zonula occludens-1 (ZO-1) and occludins in IC/PBS bladder biopsies compared to healthy controls (Lee and Lee, 2014). The ZO-1 and other occludins are tight junction-resistant proteins that confer mechanical support for the impermebiality of the bladder urothelium.

Consequently, there is influx of potassium ions (K^+) through the membrane, which sensitizes afferent nerve fibres with subsequent depolarisation (Marshall, 2003). This results in IC/PBS symptoms and other accompanying symptoms of such as urgency, spasm of the detrusor muscle, and frequency. Furthermore, the urothelial dysfunction theory is strengthened by the fact that E-cadherin – a transmembrane cell adhesion molecule that maintains urothelial permeability - was poorly expressed in samples of IC/PBS and correlated strongly with visual analogue scores (Shie and Kuo, 2011). In addition, renal outer medullary K^+ channels (ROMK), potassium gated pumps that maintain the ionic balance of K^+ in the bladder urothelium, were recently found to be over-expressed in the apical cells of the bladder urothelium of IC/PBC bladder biopsies compared to healthy controls (Lee *et al.*, 2017). As a result, there is a movement of K^+ along electrical and concentration gradients with nerve fibre depolarisation and afferent nerve fibre stimulation.

1.4 Inflammation in Interstitial Cystitis/Painful Bladder Syndrome

Inflammation describes the body's response to the adverse effects of foreign substances. It is a complex physiological process involving the recruitment of immune cells. The inflammatory event is bi-directional; in other words, it can either be protective or destructive. The process can be acute i.e., when the reaction to the foreign substance (allergen) is immediate and short lived or chronic when the process is persistent.

The acute inflammatory process lasts between seconds, hours, or days and up to years and is subject to feedback to ensure that the process lasts no longer than necessary. Increased blood flow, formation of exudates, increased tissue permeability and the recruitment of neutrophils to the site of reaction characterises acute inflammation. Upon removal of the adverse stimulus from the body, the acute inflammatory phase ceases.

If the body is unable to remove the injurious agent – be this infectious, allergic or both, which results in chronic inflammation, which lasts from days to years. In addition, it is characterised by recruitment of other immune cells such as macrophages and lymphocytes with accompanying fibroblast formation due to unrepaired tissue damage. Chronic inflammation is seen in many disease states and IC/PBS is no exception (Theoharides *et al.*, 2012).

IC/PBS is a chronic inflammation of the urinary bladder (Sonal *et al.*, 2011). The loss of the bladder urothelial integrity via a damaged GAG layer leads to the movement of urinary foreign and noxious agents from the bladder lumen to the interstitium; particularly potassium ions, which subsequently leads to hyper-excitability of C-fibres and release of substance P via TRPA1 channels (transient receptor potentials channel ankiryn 1). The result is the subsequent activation and degranulation of mast cells in the sub-urothelial layer (lamina propria) of the bladder (Lowe *et al.*, 1997; Sant *et al.*, 2007). The release of mast cell cytoplasmic granules via degranulation activates the inflammatory pathway leading to further tissue damage; substance P in particular increases intracellular calcium, activation of phospholipase C (PLC) and Mas Related G-Protein Couple Receptor X2 (MRGPRX2), which in turn releases histamine from the nerve fibres (Fischer *et al.*, 2017) and a vicious cycle is set. Various inflammatory mediators such as histamine and cytokines have been reported in the urine samples of these patients suggesting the strong involvement of inflammation in this disease process (Lamale *et al.*, 2006). Inflammatory filtrates were earlier detected in the IC/PBS diseased bladder (Fall, Johansson and Vahlne, 1985). The fact that mastocytosis was reported in all the bladder layers of IC/PBS is compelling evidence for the involvement of inflammation in the disease pathogenesis (Malik *et al.*, 2018).

It is imperative to note that chronic inflammation also leads to the release of catecholamines, which in turn cause contraction of smooth muscles leading to the spasm seen in this condition. Furthermore, Substance P (NK_1) receptors encoding mRNA were conspicuously expressed in IC/PBS bladder biopsies. The specific ligand is a neurotransmitter of the afferent C-fibres that mediates nociception (Marchand, Sant and Kream, 1998), suggesting underlying neurogenic inflammation in this disease condition. Recent evidence suggests that the neurogenic inflammation resulting from substance P stimulation with accompanying mast cell degranulation is in part mediated by the MRGPRX2 receptor (Tatemoto *et al.*, 2018).

Moreover, the pain, oedema and fibrosis characteristic of the disease are a direct consequence of mast cell degranulation through sensitisation of neuronal cells via substance P mediated stimulation (Marinkovic *et al.*, 2009).

1.5 Treatment of Interstitial Cystitis/Painful Bladder Syndrome

IC/PBS is a disease with no known cure. The goal of treatment remains decreasing symptoms and improving quality of life. Whilst the aetiology is poorly understood, treatments are geared toward correcting the underlying proposed pathologic mechanisms. In practice, treatment is broadly divided into conservative, pharmacological and surgical, with simpler non-invasive treatments being tried before more invasive (Vij, Srikrishna and Cardozo, 2012). Conservative treatment encompasses dietary restrictions (Friedlander, Shorter and Moldwin, 2012), stress reduction, lifestyle modification and behavioural modification (Cox *et al.*, 2016).

1.5.1 Conservative treatment

Dietary restriction and elimination have long been considered in the management of patients with IC/PBS and is referenced by several guidelines and bladder related bodies. Of note, this has been strongly advocated as first line treatments by the Royal College of Obstetrics and Gynaecology; the American Urological Association; the European Association of Urology and the Canadian Urological Association amongst others (Cox *et al.*, 2016; Tirlapur and Khan, 2016). This is a fallout of several studies suggesting their benefits. Importantly, Friedlander, Shorter and Moldwin (2012) identified that 90% of IC/PBS patients reported sensitivities to some diets. A strong correlation was found between symptom exacerbations (as measured by elevation of O'Leary/Sant scores) and ingestion of alcoholic beverages, coffee, tea, soda, artificial sweeteners, and hot chillies in 90% of respondents replying to a self-administered questionnaire in the US (Shorter *et al.*, 2014). Although diet sensitivities in IC/PBS are highly individualised there is a pressing need to document this amongst these individuals in the UK. With hundreds of thousands of people living with IC/PBS in the UK, little epidemiological research has been done, which document the dietary habits of this class of patients. This project will in part fill this research niche to try to help these patients.

Patient education and psychological support were hypothesised to have modest benefits in IC/PBS treatments (Bosch, 2014). Patient perception of flare triggers has been used to tailor treatments and provide useful insight into the prognosis of the disease (Lai *et al.*, 2019).

In another vein, patients perception of their illness, as propounded by Leventhal, Phillips and Burns (2016), is critical in providing self-care in disease especially for those with chronic time frame. Given that IC/PBS is a chronic inflammatory disease, patient perception could be used to understand the psychosocial component of the disease with a view of improving Health Related Quality of Life (HRQoL). Central to the patient education approach is the patient perception of the disease. This is important given the subjective nature of pain, the disconnect between physicians' assessment and patients reported symptoms, and the lack of consensus of a diagnostic benchmark for the disease. Patient perception in disease areas like IC/PBS with clinically immeasurable constructs will help incorporate patient's views into the holistic management of the condition. To date there is paucity of data regarding patient's perception of IC/PBS using validated tools and the relationship between perception and severity of symptoms is unclear. In addition, addressing patient perception in this disease area could help in understanding emotional needs and enable the development of valuable coping strategies designed to address the psychological components of IC/PBS behavioural management.

Rothrock *et al.* (2001) demonstrated a correlation between stress levels and symptom severity in IC/PBS patients. Consequently, stress reduction forms a major part of the conservative management of the disease. Stress reduction and patient education in the form of counselling helps patients in identifying specific flare triggers and ways to avoid or mitigate their effects. Stressful anxiety modulates the hypothalamo-pituitary axis (HPA) through corticotrophin mediated degranulation of bladder mast cells via corticotrophin releasing hormone receptor 1 (CRHR1) (Karalis *et al.*, 1991; Theoharides *et al.*, 2012). This together with SP-mediated stimulation, synergizes mast cell degranulation and symptom exacerbations. One strategy for stress reduction involves patient support groups and clubs where counselling services are provided as with Bladder Health UK. In addition, mindfulness-stress based therapy has been shown to significantly reduce both symptoms and problem indices of the O'Leary/Sant scores suggesting the utility of this treatment for IC/PBS patients (Kanter *et al.*, 2016). Thus, it has been instituted as part of the conservative measures in most treatment guidelines for IC/PBS.

1.5.2 Pharmacological treatments

The ineffectiveness of conservative measures in controlling symptoms of IC/PBS prompts intervention with pharmacological agents or in most cases they are instituted simultaneously with conservative measures as a multimodal treatment. The American Urological Association (AUA) guidelines recommend pharmacological agents as second line treatments. Antihistamines, antidepressants, immunosuppressants and GAG replacing agents are pharmacotherapies that are

advocated by the European Association for Urology (EAU) and believed to have significant effects in controlling the symptoms of the disease (Davis, Brady and Creagh, 2014).

They are either administered orally or in some cases as seen with GAG replacing agents (other than pentosan polysulphate) and local anaesthetics being given intravesically.

Pentosan polysulphate (PPS) acts primarily through replenishing the GAG layer and has demonstrated clinical effectiveness in controlling pain, urgency and frequency in patients with IC/PBS compared to controls in a double blind trial (Nickel *et al.*, 2005b). Amitriptyline, an antidepressant, was reported to reduce the pain and urgency in IC/PBS patients compared to control albeit with mild anticholinergic side effects (van Ophoven *et al.*, 2004). The mechanism through which this clinical effect is mediated could be related to smooth muscle relaxant properties, inhibition of mast cell degranulation, neurogenic pain relief and possibly anxiolytic and sedative actions (Gurgel *et al.*, 2013). The antihistamines congeners such as hydroxyzine and cimetidine have modest benefits in symptoms control. They both act through blockade of the histamine receptors H_1 and H_2. However, the strength of evidence favours cimetidine over hydroxyzine in controlling symptoms of IC/PBS both in the EAU and AUA guidelines (Cox *et al.*, 2016). Cimetidine has shown improvement in pain and nocturia amongst 36 IC/PBS patients randomised to either the cimetidine 400 mg twice daily or controls, but with no obvious change in immune cell density (Thilagarajah, Witherow and Walker, 2001). In a non-randomised case report, Theoharides and Sant (1997) reported that hydroxyzine significantly reduced visual analogue pain scores to 40% from base line. Although no significant change was observed when PPS and hydroxyzine were compared head-to-head a slight improvement was observed when hydroxyzine was added to PPS (Sant *et al.*, 2003a). This points to the low strength of either treatment when use alone.

Many pharmacological interventions are used to treat this IC/PBS and this research will, inter alia, seek to find out which oral treatments are being used in the UK currently.

1.5.3 Surgical intervention

Surgical procedures in IC/PBS treatments encompass intravesical botulinum toxin, lesion ablation, ileal conduit diversion, cystoplasty and or cystectomy with diversion or reconstruction. Cystoplasty and cystectomy have been more popular due to the predictable outcomes of these interventions in comparison to urinary diversion alone (Brandt *et al.*, 2019). The main goal of treatment is to treat Hunner lesions as in the case of endoscopic ablation or, for patient's refractory to all conservative and pharmacological treatments, cystectomy may be considered. The indication for surgical intervention is dependent on a poor response to conservative management.

As a rule of thumb, poor bladder functional capacity following cystoscopic examination is an indication that surgical intervention might give better prognosis (Hohenfellner *et al.*, 2000). The place of surgical cystectomy in the treatment of the disease is still not clear considering most studies reporting the benefits of this procedure are underpowered and clear clinical benefit is only observable in select patients (Andersen *et al.*, 2012). For instance the study reported by Mateu Arrom *et al.* (2019) showing better quality of life following surgery had a sample size of 35, with no control arm and a retrospective design. A longitudinal design by Kim *et al.* (2014) showed marked improvement in both the problem and symptom indices of the O'Leary/Sant scale and functional bladder capacity at 6 months compared to the pre-operative period following supratrigonal cystectomy and cystoplasty. However, the low sample size (n=45) precludes firm generalisation of these findings. Overall, little information is available regarding the place of surgery in this patient group.

1.6 Mast cells in IC/PBS

Mast cells were first discovered by Paul Erhlich in 1878 and he named them "mastzellen" meaning well-fed cells, owing to their abundant granular contents (Vyas and Krishnaswamy, 2006). Kitamura and Miyoshi (1978) had shown that they originate from the bone marrow as hematopoietic stem cells (HSC), circulating as immature cellular components in systemic circulation, thereafter, differentiating into mature immune cells in the presence of SCF ligand and the kit receptor (CD117) in the tissues. Immature mast cells from the bone marrow known as mast cell progenitors (MCp) are attracted from the peripheral blood by the chemokines such as monocyte chemoattractant protein-1 (MCP-1) and RANTES (Zsebo *et al.*, 1990; Conti *et al.*, 1997). The immature mast cell completes the differentiation process in peripheral tissues under the influence of stem cell factor (SCF) and nerve growth factor (NGF) (Theoharides *et al.*, 2012). They confer the first line of defence against antigens in the organ where they reside, which is why they are situated in the epithelia and mucosae of tissues with proximity to the outside body or tissues more vulnerable to infectious and traumatic challenge, for instance, skin, GI, and respiratory tracts. Although, they can be found in tissues distant to the external environment as exemplified in the bladder and the dura matter of the meninges in the brain, their densities are far less than in the bladder and brain compared to skin and respiratory tract (Yamada *et al.*, 2000; Varatharaj *et al.*, 2012). Mast cells mediate type-I hypersensitivity reactions, tissue repair and cross talk with B and T lymphocytes by recruiting them to site of injury and or infections (Espinosa and Valitutti, 2018).

Mast cell involvement in the regulation of pathology outside of the conventional allergy paradigm is significantly greater than initially thought. Thus, their roles in chronic inflammatory/non-allergic disorders are increasingly appreciated. Of late, the mast cell occupies a centre-stage in various

chronic inflammatory disorders, the commonest of which include: IC/PBS, chronic prostatitis, neuropathic pain, endometriosis and irritable bowel disease amongst others too numerous to mention (Anupam, Lawrence and Kalpna, 2015).

A sufficient body of evidence suggests that mast cells are implicated in the development of interstitial cystitis/painful bladder syndrome. In animal models of IC/PBS, mast cell numbers both in the bladder wall and urine were reported to be significantly higher than controls (Roth, 2007; Liu *et al.*, 2012). Supporting this Wang *et al.* (2016) reported that mast cell counts were both higher in bladder tissue and urine samples when mast-cell-intact mice (URO-OVA) were compared with transgenic mast-cell-deficient mice (URO-OVA/$Kit^{W\text{-}sh}$) after one week induction of Interstitial cystitis. Interestingly, with respect to clinical correlates, voiding, as assessed by the volume and frequency was significantly higher in the mast-cell-intact mice.

In the same manner, mast cell counts were reported to be higher in mice with conserved mast cell function compared to genetically modified strains with deficient mast cells (URO-OVA/$Kit^{W\text{-}sh}$) after being induced with pseudo-rabies virus (PRV) (Rudick *et al.*, 2008). Importantly, higher mast cell counts and IC/PBS symptoms were noticed when URO-OVA/$Kit^{W\text{-}sh}$ strains were inoculated with fresh bone marrow, suggesting that mast cells were responsible for symptom manifestation in this model of IC/PBS. However, this method ignores the fact that bone marrow contains diverse biologically active substances such as heparin which, could have been responsible for the expression of IC-like symptoms. It would have been more valid, if the mice had been transfused with inoculum containing only mast cells e.g. bone-marrow derived cultured mast cells (BMCMCs). These data have underscored the pivotal role of the mast cell in the development of IC/PBS in animal models and strengthened the proposition that, mast cells are critical in IC/PBS. Thus, understanding mast cell roles and functions is necessary to aid the design of targetted treatments for IC/PBS.

Patients with higher mast cell counts in the detrusor layer of the bladder (often referred to as detrusor mastocytosis) were shown to be associated with increased bladder damage, which positively correlated with symptoms expression (Larsen *et al.*, 2008). Boucher *et al.* (1995) reported significantly higher tryptase levels in urine samples of patients with IC/PBS compared to age and sex matched controls with other lower uriniary tract dysfunction. A similar outcome of mast cell degranulated was observed when bladder biopsies were visualized via immunostaining with toluidine dye.

Destrusor mastocytosis was also observed in the biopsies of the IC/PBS patients (Christmas and Rode, 1991). This data suggests the existence of a positive correlation between IC/PBS and mast cell density.

Overall, the evidence in support of the central roles of mast cells in IC/PBS is strong explaining why the European Society for the Study of Interstitial Cystitis (ESSIC) at its meeting at Copenhagen in 2004, adopted a detrusor mast cell count of ≥28 cells/mm^2 as diagnostic for IC/PBS (Nordling, 2004; van de Merwe *et al.*, 2008).

1.6.1 Mast cells as targets for treatments of IC/PBS

The release of mast cell mediators via degranulation has been demonstrated to have a role in the pathogenesis of IC/PBS as depicted in Figure 1-2 and Figure 1-3. This pathological function is primarily orchestrated by histamine and cytokines released through SP- stimulation. Although, recent evidence suggests involvement of the IgE pathway also. Other secondary drivers of mast cell activation include leukotrienes as represented in Figure 1-2 below.

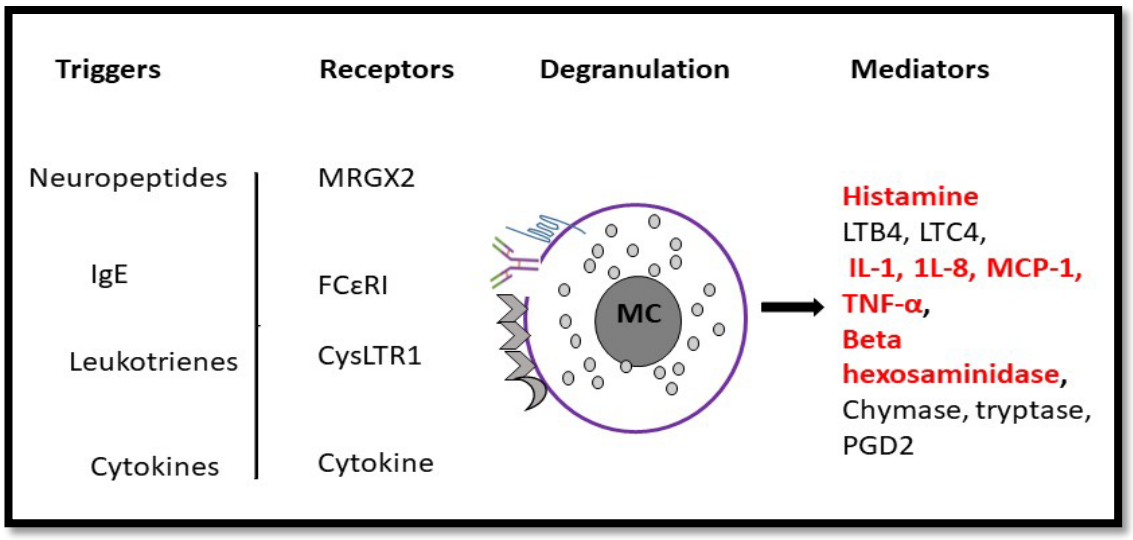


Figure 1-2: Schematic presentation of mast cell degranulation with stimuli, receptors, and mediators. Neuropeptides such as substance P and IgE are the main triggers of degranulation in IC/PBS. The mediators in red represents major drivers of IC/PBS symptoms. Cysteinyl leukotrienes and cytokines act in autocrine pattern whilst SP and IgE paracrine.

Following mast cell activation, mediators such as histamine act on the effector organ receptors to mediate the erythema, vasodilation, and pain typical of inflammatory events in a paracrine pattern. Cytokines on the other hand, acts in an autocrine manner to cause positive feedback, thus, sustaining the mast cell activation process and consequently perpetuating the inflammatory cascade.

Mast cell modulators used in IC/PBS which include, cimetidine, amitriptyline, and hydroxyzine act primarily through competitive antagonism of the receptors mediating the inflammatory response.

This strategy in part explained the mechanism of actions of hydroxyzine and cimetidine acting through blockade of H_1 and H_2 receptors respectively (Simons and Simons, 1994; Brimblecombe *et al.*, 2010). Whilst this seems to have produced modest outcomes, other mediators released such as cytokines and leukotrienes that drive the inflammation cascade are unaffected by these drug actions and this may explain the poorer clinical benefits of this class of pharmacological agents (Shan *et al.*, 2019). Given this, it would seem worthwhile to target the inhibition of the mediator release by stabilising mast cells and preventing degranulation as depicted in Figure 1-3 below.

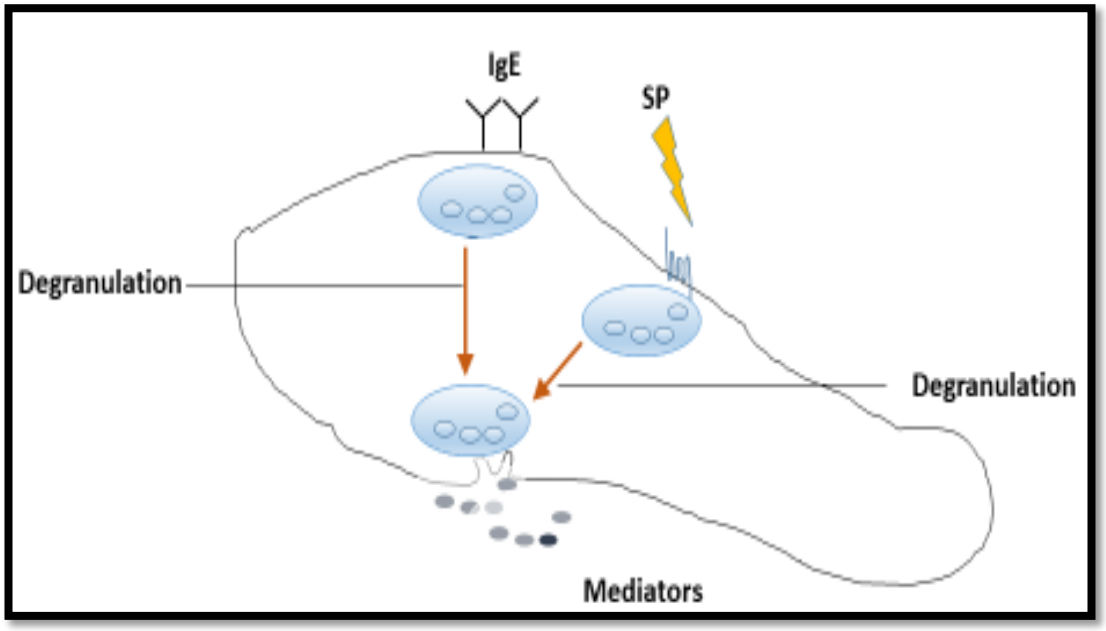


Figure 1-3: Schematic presentation of mast degranulation following Substance P and anti-IgE stimulation. IgE and SP act on FcεRI and MRGPX2 to cause degranulation of cytoplasmic granules leading to mediator release.

1.6.1.1 Mast cell signalling

Central to the activation of mast cells by specific ligands culminating in degranulation lies an intermediate route of signal transduction that activates specific second messengers in the form of phosphorylated proteins and Ca^{2+}, which regulate the degranulation events. Communication between cells and the extracellular environment involves both receptor mediated and non-receptor mediated processes. Receptor mediated processes include both immunological activation in the form of IgE and GPCR receptor sup-families activation whilst an ion-gated channel such as voltage gated Ca^{2+} is a classic example of a non-immunological pathway for activation of mast cells.

Several signalling pathways uniquely activate specific pathways that result in the activation of specific intermediaries, which themselves act as second messengers that are critical to cell survival, proliferation, differentiation, and cytokine production. Cross talk exists between these unique pathways, which act simultaneously to orchestrate mediator release via degranulation.

One pathway of signal transduction through IgE involves the tyrosine pathway; where cross linking of the FcεRI (high affinity receptor) initiates specific activation of transmembrane proteins with subsequent activation of Fyn and Lyn kinases, which are Src family members, and finally PLCγ1 hydrolyses the transmembrane lipids phosphadityl inositol 4, 5 biphosphate (P_1P_2) to inositol 3, 4, 5 triphosphate (IP_3) and diacyl glycerol (DAG) (Pribluda and Metzger, 1987; Blank and Rivera, 2004). Alternatively, the non-IgE mediated receptor pathway involves a basic secreatagogue, such as Substance P, which acts on heterotrimeric GPCR to hydrolyse P_1P_2 to IP_3 and DAG via PLCβ. DAG is lipophilic and remains in the cytoplasmic membrane where it activates protein kinase C (PKC) to cause calcium ion release. On the other hand, IP_3, a cytoplasmic product acts on its receptor on the endoplasmic reticulum to cause release of Ca^{2+} leading to stromal interacting molecule 1 (STIM1) that acts as a Ca^{2+} sensor that signals depletion of Ca^{2+} ER concentration. This mediates opening of store operating calcium entry (SOCE) ORAI1 leading to massive extracellular influx of Ca^{2+}, which in turn results in the degranulation of mast cells (Gilfillan and Tkaczyk, 2006).

Alternatively, IgE receptor activation acts on the PI3K pathway, where phosphodityl inositol 3-kinase acts on P_1P_2 to generate P_1P_3. The latter recruit's protein dependent kinase 1 (PDK1) and Akt from the cytoplasm to the cytoplasmic membrane. Both PDKI and Akt possess pleckstrin homology (PH) docking sites where PDKI phosphorylates Akt and the phosphorylated Akt dissociates from the membrane to cytoplasm where it translocates into the nucleus to activate specific targets notably mammalian target of rapamycin 1 (mTORC1) to cause degranulation. It is noteworthy that Akt is an important second messenger in the PI3K-pathway. Equally important in the signal transduction process is the mitogen activated protein kinase (MAPK) pathway. Furthermore, PKC generated secondary to hydrolysis of P_1P_2, activates Ras through Guanine Exchange Factors where resting Ras coupled with guanine is activated to Ras-GTP and activates a three-tier specific protein kinase pathway leading to the production of extracellular receptor kinases 1 and 2 (ERK 1 and 2), p38 MAPK and c-Jun N-terminal kinase (JNK), which translocate into the nucleus causing cytokine production, cell proliferation, degranulation through specific gene transcription. PI3K and MAPK signalling is activated by both IgE and non-IgE dependent mechanisms.

Following Ca^{2+} channel activation of mast cells by phorbol esters and calcium ionophore. Ca^{2+} Ionophore directly activates Ca^{2+} channels and stimulates ER release of Ca^{2+} analogous to IgE and SP ligand stimulation via STIM1 ORAI.

This serves as a convergence in the IgE and non-IgE dependent degranulation. Critical to the ion-gated channel degranulation are specific cellular events involving Soluble N-ethylmaleimide sensitive factor attachment proteins receptors (SNAREs) vesicular and target protein occur as a prelude to degranulation via this pathway. PKC generated from hydrolysis of P_1P_2 directly activates resting Ras to Ras-GTP, which consequently phosphorylates the MAPK cascade leading to degranulation and cytokine production via ERK, JNK and p38 production (Dolmetsch *et al.*, 2001). The Ras-GTP pathway is a common signalling pathway in mast cell degranulation in either route. Furthermore, in the phosphoinositide's (PI3K) pathway – where phosphoinositides-3-kinase acts on P_IP_2 to yield P_IP_3 (Cantley, 2002). Following the P_IP_3 production, PDKI and Akt are recruited to- the membrane, leading to Akt phosphorylation and consequently degranulation. Overall, the route to mast cell signalling and degranulation from transmembrane P_IP_2 bifurcates into the DAG-IP_3 routes or the phosphoinositides-P_IP_3 depending, which catalytic enzyme is involved.

It should be borne in mind that although both IgE and non-IgE mediated degranulation of mast cells induces mediator release, some notable physiological and histological differences exist between the two. IgE degranulation produces large spherical granules with long lasting effect in contrast to Non-IgE release which produces short granules with transient biological actions (Gaudenzio *et al.*, 2016). Serum IgE levels have been shown to be increased in the blood sample of IC/PBS patients compared to controls (Jhang and Kuo, 2015b; Jhang *et al.*, 2016). In addition, anaphylactic degranulation of the bladder mast cells was observed in the bladder biopsies of IC/PBS patients (Gamper *et al.*, 2015), through instantaneous release of mast cell mediators detected via immunohistochemical technique. This underscores the importance of IgE and hypersensitivity in the disease pathogenesis.

Overall, the whole signalling event is interwoven and interrelated with complete cross talk and signal relay. The process is as presented in Figure 1-4 and Figure 1-5 below.

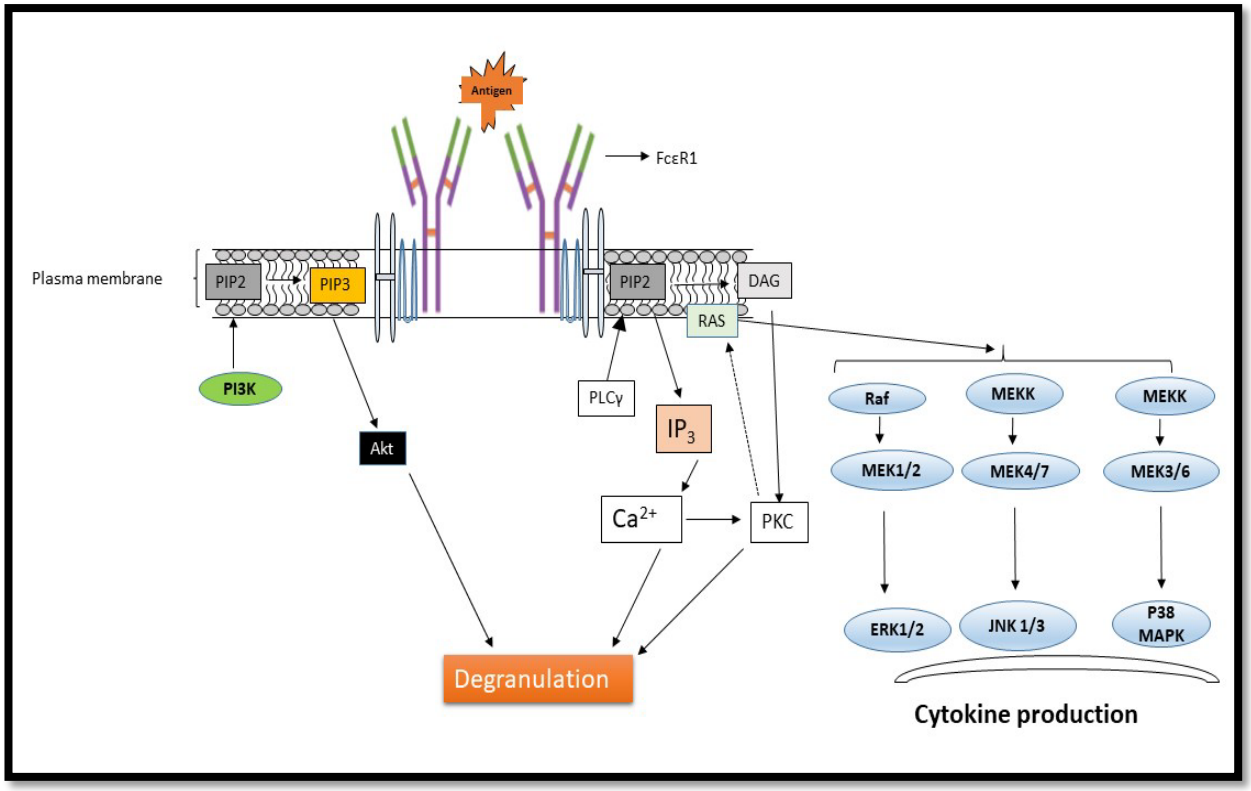


Figure 1-4 Schematic presentation of FcεRI signalling leading to degranulation and cytokine production in mast cells

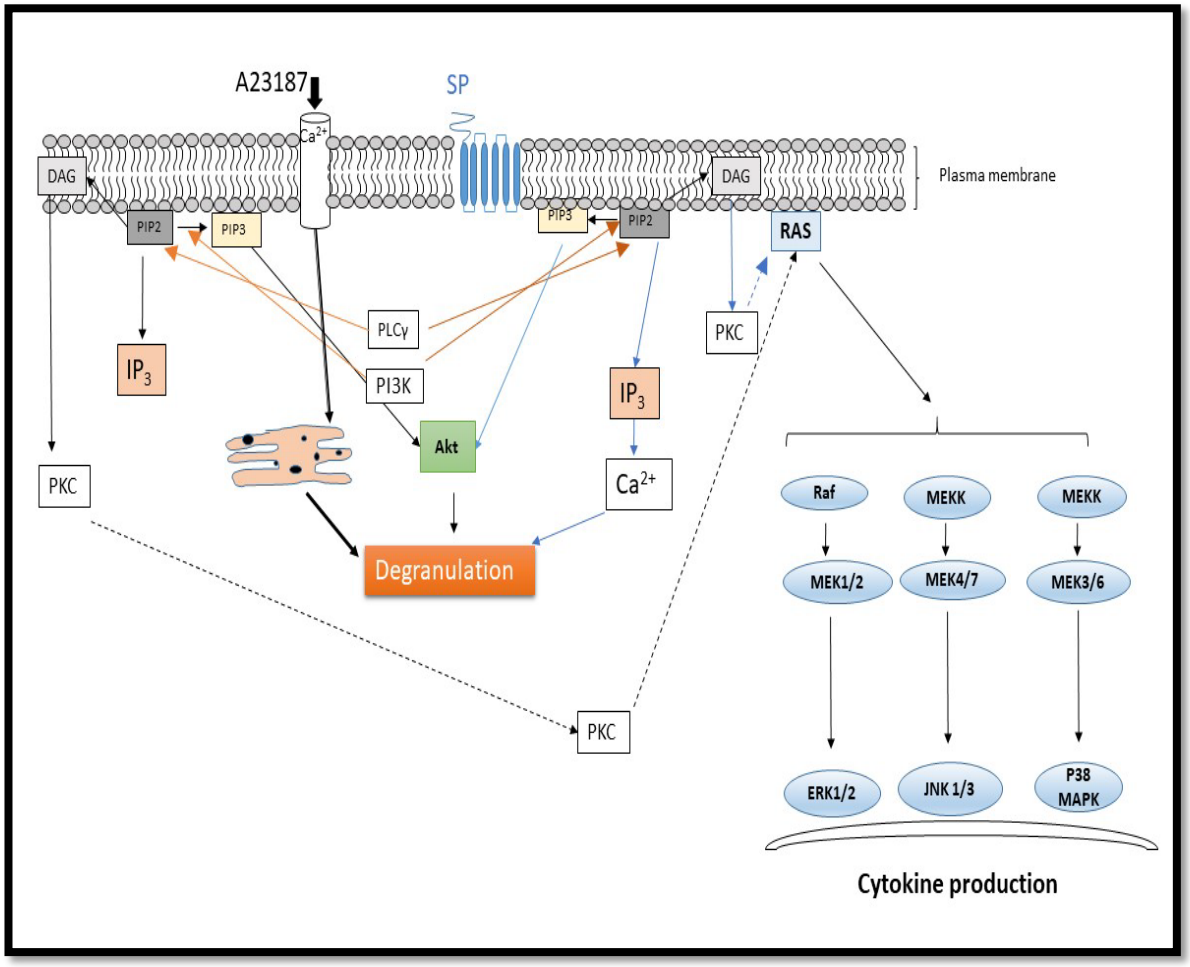


Figure 1-5 Schematic presentation of GPCR and Calcium mediated signalling in mast cells leading to degranulation and cytokine production in mast cells

1.7 Research aims, objectives and questions

This project aimed at answering some pertinent research questions relating to the perception and treatment of interstitial cystitis. The main objective of this research is to describe IC/PBS treatments and test the potential of medical grade manuka honey, its effect on downstream signalling in an in vitro model of the disease. The main research questions are:

1. How do people living with IC/PBS perceive their disease and the available treatments?
2. What is the relationship between Illness perception and treatment to disease severity?
3. How is Health Related Quality of Life (HRQoL) in an IC/PBS cohort?
4. Is there any potential role for medical grade medical grade Manuka honey in the treatment of IC/PBS?
5. What are the possible mechanistic pathways by which medical grade Manuka honey stabilises the mast cell?

Chapter 2 Literature Review

2.1 Literature search methods

The literature search was conducted on the following databases using free text: Medline, CINNAHL, Embase, Scopus, Cochrane and AMED, while subject search was performed on Web of Science and TRIP as shown in Appendix A. The Cochrane database was also searched and reviewed. Medline, Embase, Scopus, AMED and WoS were used to ensure that biomedical and pharmacological evidence relating to the treatment of IC/PBS was retrieved accurately whilst Psychinfo and Cinnahl were used to ensure that qualitative data relating to illness perception and non-pharmacological treatments of the disease were not missed. More importantly, ongoing research in cystitis foundations/groups notably Bladder Health UK and the ICA were carefully studied. Titles and abstracts of papers published in the English language were carefully read and reviewed. Papers were not limited by years and search terms used were encompassing; this made the inclusion criteria wider and thereby pertinent articles were less likely to be missed.

The results of the literature search can be grouped into: Pathophysiology and treatment of IC/PBS. Theories relating to the disease pathogenesis will be discussed. The medical benefits of honey were reviewed in relation to its use as anti-inflammatory agent as a whole and as a mast cell stabiliser.

2.1.1 Pathophysiology of IC/PBS

Several factors have been proposed to explain the causes of interstitial cystitis/painful bladder syndrome but none of these have clearly established the precise aetiology of this disease. The absence of a known cure for this disease makes research in this area important and worthwhile. The evidence so far suggests that the causes of IC/PBS are multifactorial, a finding, which reflects the various clinical presentations of the disease. Some hypotheses have been advanced to explain the possible causes of the disease. For instance, the glycosaminoglycan hypothesis, the urothelial hypothesis, the mast cell activation hypothesis and the neuro-endocrine hypothesis, amongst others, have been proposed. These will be outlined briefly below.

2.1.1.1 Glycosaminoglycan hypothesis

The glycosaminoglycan (GAG) layer comprises an epithelial barrier of long chain polysaccharide sugars cross-linked by amino sugars, negatively charged and hydrophilic in nature which provides both buffer and impermebiality functions to the urothelium (Lewis, 2000).

The hyaluronate, glucoronate, heparin, derman, chondroitin proteoglycans and mucins provide classical examples of GAGs. Compared to other GAGs, hyaluronate is devoid of the sulphate salt and is not linked to a protein core (Gomelsky and Dmochowski, 2012). In addition, chondroitin contributes significantly to the urothelial barrier compared to other GAG subtypes (Janssen *et al.*, 2013). The GAG layer sits on top of three cell layers, the umbrella, intermediate and basal cell layers, which constitute the urothelium. Though devoid of a vascular system and nerve supply, the urothelium is rich in immunocellular and neurocellular components (Hurst *et al.*, 2015).

The GAG layer provides the first line of protection against the noxious effects of urotoxins in the urine (Lewis and Kleine, 2000). Disruption of this mechanical barrier by any cause, allows the passage of the urine and its solutes, particularly potassium to penetrate this layer and cause depolarization of afferent nerves in the lamina propria, which houses mast cells, nerves, and blood vessels. This initiates a painful stimulus – a hallmark of IC/PBS.

Several studies have reported on how the GAG layer protects urothelial integrity. For instance, Hurst *et al.* (2015) established that disruption of the GAG layer by HCl acid, causes the leakage of urinary toxins to the lamina propria where they stimulate sensory nerve endings. In the same manner, digestion of the GAG layer by chondroitinase in both human and porcine urothelium has been shown to increase the permeability of the layer as measured by transepithelial electrical resistance (TEER). This has further confirmed the role of the GAG layer in maintaining urothelial integrity and protecting it from urotoxins (Janssen *et al.*, 2013). Parsons *et al.* (1998) studied 231 patients with IC/PBS and 41 healthy controls. In the study, intravesical administration of potassium was shown to correlate well with pain sensation and urinary frequency in the disease arm as compared with null pain sensation in healthy subjects; a finding consistent with the hypothesis that IC/PBS patients have damaged GAG layer compared to healthy controls. One of the more striking findings of this study was that, of the two cations in the urine, it is potassium not sodium that mediates the painful sensation in about 90% of the patients. Upon replacement of the GAG deficiency by heparin, the dual symptoms of pain and urgency were completely abolished in the IC/PBS group. This further reinforces the argument that the GAG layer in the urothelium provides a physical barrier to the ingress of urinary toxins and its disruption could be linked to the pathogenesis of IC/PBS.

Antiproliferative factor (APF) is a glycoprotein that decreases trans-epithelial resistance, increasing the leakage of the urinary contents via the epithelium. This was shown to be elevated in the urine samples of patients with IC/PBS suggesting that the GAG layer is compromised in these patients and could explain the mechanism of the disease process (Keay *et al.*, 2001).

In a related finding, denudation of the epithelium is well reported among patients with the ulcer phenotype of IC/PBS in contrast to healthy controls (Erickson *et al.*, 2008; Lee, Yang and Lee, 2016).

It is worth mentioning that whilst disruption of the GAG layer has been proposed as one possible pathogenesis underlying IC/PBS, a growing body of evidence suggests that this layer could play a role in other disease areas of the bladder (Madersbacher, van Ophoven and van Kerrebroeck, 2013). The utility of GAG replacement therapy for patients with recurrent Urinary Tract Infections (UTIs) could suggest that the GAG layer is also compromised in this condition (Gomelsky and Dmochowski, 2012).

2.1.1.2 Urothelial hypothesis

The urothelium in conjunction with the GAG serves as an impermeable layer between the urinary contents of the bladder and the tissue beneath it. An enzyme localized on the basolateral membranes, Na-K ATPase, transports 3-sodium ions into the cell for every 2-potassium ions expelled outside the cell to maintain a negative intracellular balance. A recent study by Lee, Yang and Lee (2016) demonstrated down regulation of this enzyme in the bladder biopsies of IC/PBS patients suggesting that it is a consequence of urothelial denudation. This in part explains the urothelial dysfunction seen in these patients.

The bladder is a storage/transit organ for the urine formed via the secretive and absorptive processes in the nephrons of the kidneys. It contains several cations, which are maintained in a physiological balance. Argade *et al.* (2016) found an increase in the urinay excretion of these cations in IC/PBS male patients compared to healthy controls. This could stem from the fact that a disrupted GAG layer in particular and the urothelium in general could be responsible for the outward movement of these cations from the bladder tissue layers to the urine where they are subsequently voided with the urinary contents. Although this study has further supported the leaky urothelium concept in the pathogenesis of IC/PBS, the sex of the participants in this study does not reflect the general demography of the IC/PBS, as the disease is far more common in women than men. Therefore, the relevance and applicability of this finding is rather low and more studies need to be performed in females to increase the validity of this outcome.

2.1.1.3 Mast cell activation hypothesis

Mast cells are tissue-resident neuro-immune cellular components that mediate a plethora of effects within the body. They originate from haematopoietic stem cells and differentiate/or mature in the tissue in which they reside. Mast cells are commonly found in vascularised tissues, airways, gastrointestinal tract (GIT) or areas vulnerable to pathogenic allergic infiltration. Murine mast cells have been classified based on their anatomical habitat as either connective tissue-based mast cells (CTMCs) or mucosal based mast cells (MMCs). However, in humans, two distinct phenotypes have emerged: a mast cell containing tryptase (MC_T) and a mast cell containing tryptase and chymase (MC_{TC}) (Vyas and Krishnaswamy, 2006).

Activation of mast cells is usually a physiologic process to perceive or detect threats by allergens or invading pathogens (Akin, 2017). However, an imbalance in the activation cascade either due to mastocytosis, secondary to neoplastic expression of the protein-receptors and/or ligand over-production produces pathologic effect commonly observed in chronic inflammatory diseases including IC/PBS (Hundley *et al.*, 2004). There is a growing body of evidence to suggest that mast cell activation plays a vital role in the pathogenesis of IC/PBS (Newsome, 2003). Bjorling *et al.* (1999) demonstrated that mast cells are activated by substance P (SP) and lipopolysaccharide (LPS) in normal rats with IC/PBS with intact mast cells compared to an absence of cystitis seen in genetically modified rats deficient in mast cells. This study established the crucial role of mast cell activation in a rodent model of IC/PBS. Furthermore, Peeker *et al.* (2000) compared mast cell distributions in IC/PBS patient biopsies (classic and non-classic) with healthy controls and discovered an almost a ten-fold increase in mast cell numbers in the ulcer IC/PBS group and a two-three-fold increase in the non-ulcer group compared to the controls.

In related findings, mast cell degranulation products have been found in the urine of IC/PBS patients, particularly the autacoid histamine and its metabolite methyl histamine. Other products found in urine are prostaglandin E2 (PGE2), IL-8 and eosinophils, which correlate with the mast cell numbers seen on bladder biopsy (Lamale *et al.*, 2006). These and other research findings clearly corroborate the earlier held concept that mast cell activation significantly contributes to the pathophysiology of IC/PBS. Overall, there is an ample evidence to suggest that mast cell activation and a defective urothelium may work in concert to explain the entire symptom complex seen in IC/PBS, although the sequence of the processes remains unclear i.e.; whether a leaky urothelium causes mast cell activation or vice-versa. These are questions, which further research will seek to answer.

2.1.1.4 Neuro-endocrine hypothesis

The involvement of hormones and immune-cellular components has been suggested in the aetiology of IC/PBS. Stress is reported to increase Substance P in various sections of the brain which consequently activates the hypothalamo pituitary axis (HPA) axis (Black, 2002). The activated HPA then stimulates the release of adrenocorticotrophic hormone (ACTH), which consequently stimulates the adrenal medulla to release glucocorticoids that have anti-inflammatory properties. Paradoxically, the activation of corticotrophin releasing hormone (CRH) outside the CNS mediates pro-inflammatory effects (Karalis *et al.*, 1991). This may explain the reason why stress could exacerbate the symptoms of IC/PBS.

Acute stress mice models demonstrate increased mast cells and an increase in the number of SP-rich fibres (Peters *et al.*, 2005). A similar result was obtained in a one-week stress exposure to mice, where the number of degranulated cells was significantly ($p \leq 0.01$) higher than seen in the controls (Arck *et al.*, 2001). Furthermore, corticotrophin releasing hormone receptor-1 (CRHR-1) is fully expressed on mast cells, which when activated, cause degranulation and release of pro-inflammatory mediators specifically VEGF (Cao *et al.*, 2005). More importantly, Substance P upregulates the expression of CRHR-1 on short term exposure whilst chronic administration downregulates it (Asadi *et al.*, 2012). In the same manner, CRH was shown to stimulate selective mast cell release in several tissues including the bladder, which could explain its effect in IC/PBS (Theoharides *et al.*, 2012). It can be argued that greater activity of the HPA axis underlies the cause of IC/PBS. Analogous to human IC/PBS in veterinary practice there is syndrome feline IC (FIC), seen exclusively in cats with similar clinical manifestations to those in humans (Westropp and Buffington, 2002). On the contrary, significantly ($p \leq 0.005$) lower levels of ACTH were observed in cats with feline interstitial cystitis (FIC) compared to healthy controls in addition to decreased ($p \leq 0.05$) weight of adrenal glands. This can be attributed to the fact that corticotrophin releasing hormone (CRH) effect in IC/PBS is localised to the bladder. Thus, plasma concentration of ACTH does not correlate with clinical manifestation of disease.

Additionally, enhanced sympathetic tone in the central nervous system has been postulated as a possible mechanism for the initiation and exacerbation of IC/PBS. Buffington and Pacak (2001) investigated the daytime changes in cortisol levels between cats with and without feline IC where eight cats with feline IC and eight healthy cats were anaesthetized and blood samples assayed by HPLC for catecholamine's and their metabolites. Plasma norepinephrine values were significantly higher ($p \leq 0.05$) in the feline IC cats than in healthy cats, suggesting an increase in the sympathetic drive and central nervous system involvement in the pathogenesis of feline IC.

Following this theme, nerve growth factor (NGF) has emerged as neurotrophin regulating vagal and inflammatory nociception in the urinary bladder through receptor interactions (Steers *et al.*, 1991; Allen and Dawbarn, 2006; Schnegelsberg *et al.*, 2009). This in part leads to an increase in mast cells and sensory nerve fibres densities in the detrusor muscles and the submucosa, thus decreasing the pain threshold via neuronal hyperalgesia. The role of NGF as a useful pain biomarker has been investigated in cyclophosphamide induced cystitis in mice (Fujita *et al.*, 2016). An increased level of this nociceptive biomarker in the bladder homogenates of the mice was reported. Similar results were obtained in a transgenic mouse model over-expressing NGF where elevations of bladder mast cell numbers and both sympathetic and sensory nerve fibres were noted (Schnegelsberg *et al.*, 2009). Importantly, there was an increase in somatic hypersensitivity as assessed by von Frey filament testing in the abdominal and plantar dermatomes of the mice. Consistent with this finding, is the increased painful sensation in the bladder, which is characteristic of the clinical presentation of this disorder.

Even though elevated levels of NGF in IC/PBS are reported in other diseases of the lower urinary tract, such as chronic cystitis and idiopathic sensory urgency, this finding underscores a possible pivotal role for neuronal and inflammatory involvement in both the pathology and the disease progression seen in IC/PBS (Lowe *et al.*, 1997).

In general, NGF causes sensory nerve innervation, which consequently increases neuropeptide and Substance P release which in turn causes mast cell degranulation and the start of self-sustaining vicious cycle of inflammations and pain.

In summary, a leaky urothelium results in the depolarisation of sensory nerve endings in the bladder wall which in turn activates mast cells in the lamina propria and the detrusor muscles to liberate inflammatory mediators including Substance P and catecholamines. The catecholamines act on the sympathetic innervation of sensory nerve endings resulting in the release of Substance P, which is a stimulus for mast cell degranulation continuing the vicious circle. Similarly, increased activity of the HPA may activate the release of cortisol from the adrenal cortex, which in turn activates bladder mast cells helping to sustain the cycle. This is illustrated in Figure 2-1.

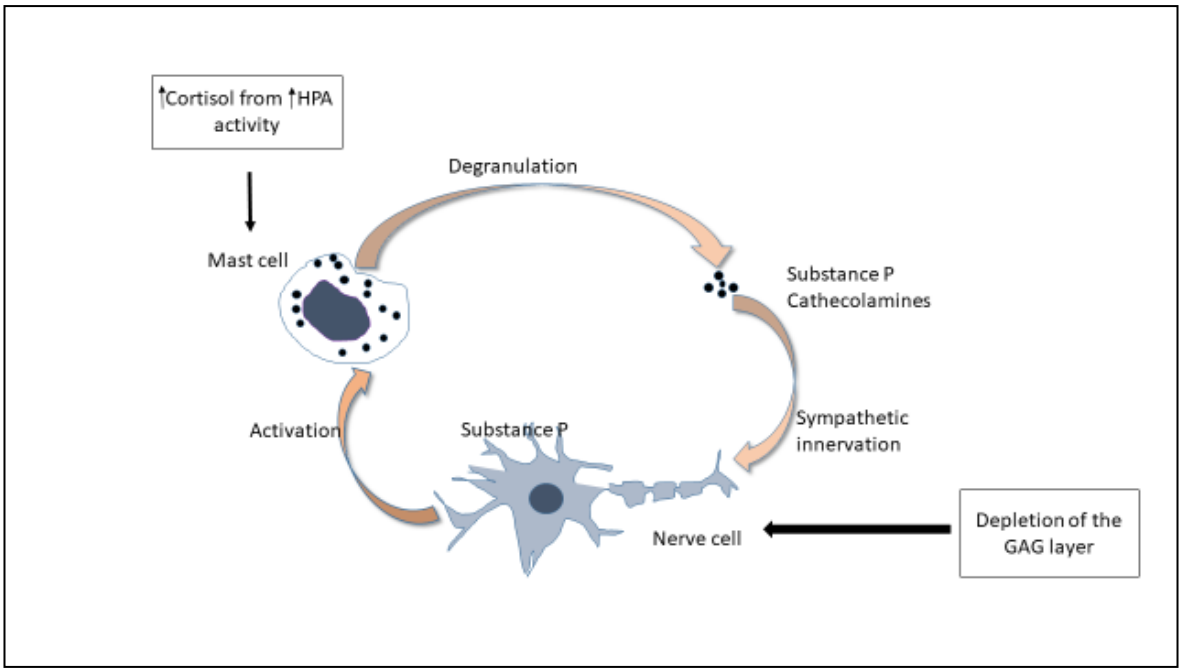


Figure 2-1 Schematic relationship of the etiologic causes of IC/PBS. Loss of the bladder GAG layer causes depolarisation of nerve cells, activation of substance P which activate mast cells in the bladder causing degranulation and release of catecholamines that further deplete the GAG layer and stimulation of afferent nerve fibres.

2.1.2 Treatment of IC/PBS

IC/PBS is a disorder with no known cure; thus, treatment is aimed at reducing the symptoms of pain, urinary frequency and improving quality of life associated with the disease. Consequently, most guidelines suggests starting behavioural management, which encompasses dietary modification and stress reduction amongst other strategies. Unsatisfactory responses to conservative management prompts pharmacological interventions, which include both oral formulations and intravesical instillations. Treatment is geared towards addressing the multifactorial pathologies associated with the disease. Some of these treatment approaches are briefly outlined below.

2.1.2.1 Dietary management

Sensitivities to dietary components are well documented in about 90% of patients living with IC/PBS (Friedlander, Shorter and Moldwin, 2012). Shorter *et al.* (2014) found a strong correlation between symptom exacerbations and the ingestion of coffee, tea, soda, alcoholic beverages, citrus fruits and juices, artificial sweeteners, and hot chillies in over 90% of respondents in a self-administered questionnaire. Both problem and symptom domains of the O`Leary/Sant questionnaire were found to be significantly higher ($p \leq 0.05$) in responders taking the offending foods. As a result, nutritional

management in the form of dietary manipulation, dietary elimination and dietary changes have been developed and have a prominent role in the management of IC/PBS through symptoms relief (Gordon *et al.*, 2015).

Hanley *et al.* (2009) reported a significant reduction in two components of the O' Leary/Sant Questionnaire in the dietary component of multimodal therapy after 10-months of follow up. Consequently, dietary management is recommended in the following guidelines: Royal College of Obstetrics and Gynaecology (RCOG); European Association of Urology (EAU); Canadian Urological Association (CUA) and the American Urological Association (AUA) (Tirlapur and Khan, 2014; Cox *et al.*, 2016; Cashman and Biers, 2018).

Whilst strict dietary modification should be approached with caution, as excessive elimination of diets with essential nutrients could result in adverse effects, in the same vein, limiting fluid intake could increase the concentration of soluble metabolites in the urinary bladder, thus, which might act adversly with the urothelium. On the contrary, excessive fluid intake increases the frequency of voiding which further aggravates patient symptoms (Friedlander, Shorter and Moldwin, 2012). Overall, it is important to strike a balance between patient convenience and improvement in quality of life. Notwithstanding, dietary management is hypothesized to have profound effects on the quality of life of patients living with IC/PBS (Bade, Peeters and Mensink, 1997). Consequently, descriptive accounts of diets in these patients could add substantially to the existing literature and improve quality of care.

2.1.2.2 GAG replacement therapy

The significance of the GAG layer in maintaining the urothelial integrity of the bladder was elaborated in 1.5.2. Restoration of the damaged GAG layer is the basis of treatment for most chronic pathologies of the bladder, most notably IC/PBS but also including recurrent UTIs and chemical or radiation cystitis (Costantini, Lazzeri and Porena, 2013). Agents that repair the damaged GAG layer by coating the urothelium are available as oral formulations and intravesical instillations. The aim is to reduce the passage of noxious urinary constituents that cause mast cell activation, mediator release, and C-fibre activation culminating in symptom improvement (Bassi *et al.*, 2011). They have been reported to be useful in the treatment of IC/PBS. Pentosan polysulphate (PPS), chondroitin sulphate, hyaluronic acid and heparin are representative of the GAG replacement agents with hyaluronic acid being the most cost-effective (Belknap, Blalock and Erickson, 2015; Barua *et al.*, 2016). Whilst they are becoming increasingly popular as treatment options, studies supporting their benefits are at best under-powered, few and suffer from lack of proper design (Chintea and Belal, 2013). Some of the agents that act by repairing/replenishing the GAG layer will be discussed below.

2.1.2.2.1 Pentosan polysulphate

Pentosan polysulphate (PPS) is available as oral and (rarely used) intravesical formulations. It is the most highly studied GAG with relatively robust clinical evidence for its use (Dimitrakov *et al.*, 2007). It is composed of penstoses as shown in Figure 2-2. Santos *et al.* (2017) established that PPS provides the highest clinical benefits in IC/PBS patients compared to available pharmacological interventions based on RCT data. Parsons and Mulholland (1987) conducted the first RCT with PPS.

A total of 75 patients were randomized to treatment with oral PPS 100 mg (thrice daily) or 200 mg (twice daily) and a placebo over a 3-month study period. Poor response to treament was a requirement for switch over from placebo-treatment or vice-versa for an additional 3-months. Of the 62 patients that succesfully completed the study, patient reported improvement of symptoms as assessed by pain, urgency and frequency favoured the PPS arm.

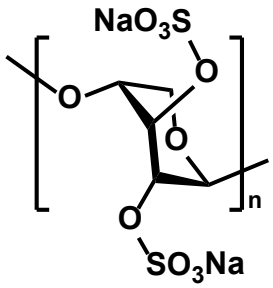
In a related study, PPS was reported to be superior to placebo in a randomised-double blind-multicentre-placebo control study (Mulholland *et al.*, 1990). A total of 100-patients were randomly assigned to either PPS or placebo (1:1) over a 3-month study period. Patient reported improvement in symptoms of pain and urgency was in favour of the treatment group albeit not statistically significant. The mean volume of voids and and the total volume of urine passed did not differ in the two arms of the study.

Both oral and intravesical PPS were shown to produce an improvement of the various domains of the symptom scores of patients in a study by Davis *et al.* (2008), Forty one (41) female patients were randomised to either PPS or placebo groups in a double blind trial. Patients received PPS instillations or placebo for 6-weeks then subsequently oral PPS for 12-weeks. The side effect profile was comparable between the two groups. The 6-week initial PPS intravesical administration did not take into account that IC/PBS is a chronic disorder and the clinical benefits of treatment observed mostly after 12-weeks of therapy. The outcome might have been more convincing if the initial 6-weeks bladder instillation had been for longer period.

In a further study; Nickel *et al.* (2005a) conducted a double blind randomised control study to determine the recommended oral dose of PPS producing maximal clinical benefits in 230 patients on daily regimens of 300 mg, 600 mg and 900 mg in a trial lasting 32-weeks. There were significant improvement in the patient-reported outcome measures on the Interstitial Cystitis Symptom Index (ICSI) and PORIS components of the questionnaire, however, these were not dose-dependent.

Although, 2% of the patients on 900 mg PPS reported diarrhea which was significant ($p \leq 0.05$) overrall side effects were generally mild as was previously reported for the drug across the three dosages used.

More recently, a concise review by National Institute for Health and Care Excellence (NICE) approved oral PPS for oral treatment of Hunner ulcers of IC/PBS patients refractory to second line oral treatments (NICE, 2021). However, it notes that the treatment be undertaken in a secondary health care centre and shouldn't be used concurrently with any other bladder instillations.



Pentosan polysulfate

Figure 2-2 The chemical structure of pentosan polysulphate.

2.1.2.2.2 Hyaluronic acid (HA)

Hyaluronic acid is non-sulphated GAG, also called hyaluran, is a synthetic component of the GAG layer that is employed to replenish the deficient urothelial GAG barrier. It consists of a repeating two glucoside units as shown in Figure 2-3. Urinary hyaluronic acid was found to be higher in IC/PBS patients compared with age-matched healthy controls (Erickson *et al.*, 1998), reflecting its loss as a component of the GAG layer.

A study involving 50-patients refractory to other GAG replacement therapy was conducted by Morales *et al.* (1996) in which patients were administered intravesical hyaluronic acid (40mg/50ml NS) weekly for two weeks and then monthly for one year. A dramatic treatment response was observed at weeks 4, 12 and 20 as assessed by symptom scores, voiding diary and (Visual Analogue Scale) VAS. However, response to treatment reached a near plateau at week 24 and beyond. Conspicously, this study suffered from lack of randomisation and absence of a control arm both which are potential sources of bias. More importantly small sample size limits firm translation of research findings to a larger patient population.

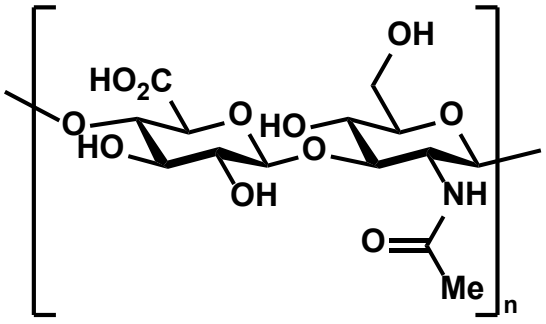
A more sound clinical study on the use of Hyaluronic acid (HA) in IC/PBS individual is provided by the work of Lai, Kuo and Kuo (2013). An equal number of 30-patients were allocated to hyaluronic acid-9-group and hyaluronic acid-12 group each in a prospective randomised design. The Hyaluronic acid-9 group were given 9-instillations in total, while the Hyaluronic acid-12-group were administered 12-instillations altogether in the study period. In the hyaluronic acid-9-group, 40 mg HA was administered intravesically four times weekly followed by the same dose in a five-monthly dosing schedule. While the hyaluronic acid-12-group were administered 40 mg HA fortnightly every two weeks for a total of 12-weeks. The Interstitial Cystitis Symptom Index (ICSI) and Interstitial

Cystitis Problem Index (ICPI); Visual analogue scale (VAS); and functional bladder capacity among others were used to evaluate treatment efficacy. Significant improvement in all evaluation parameters was recorded in both arms of the study after 6-months. Urinary frequency and volume were significantly improved in the hyaluronic acid-12-arm. Conversly, the hyaluronic acid-9-group showed marked improvement after one month of treatment.

This could be due to the initial frequent dosing schedule in that arm of the study. The dosing schedules of intravesical HA do not seem to have a profound effect on treatment outcomes in IC/PBS patients.

More recently a combination of hyaluronic acid (HA) and chondroitin sulphate (CS) is becoming increasingly popular as an instillation for the treatment of IC/PBS. A multi-centre, open label head to head trial was designed to compare efficacy of of HA/CS: DMSO in a 2:1 allocation ratio, among 110 patients over a six month period (Cervigni *et al.*, 2017). Efficacy as assessed by VAS and side effects were the primary endpoints while quality of life and health care cost were secondary endpoints. Data were collected longitudinally at months 0, 3 and 6 of the study period. At 6-months the reduction in pain from baseline was significantly higher ($p \leq 0.05$) in the HC/CS arm than in the DMSO group. Response to treatment-predefined as a 50% reduction in VAS in patients-favoured the HC/CS arm at both 3-months and 6-months (70.3% vs 55.6% and 63.5% vs 55.6% respectively). The range of untoward effects were comparable in both arms of the study. However, the incidence of adverse events related to treatment were higher in the DMSO arm (22.2%) compared to the HC/CS (1.35%) group ($P \leq 0.001$).

Direct cost - defined as cost of medication, hospital visits and instrumentation was in favour of DMSO. Similarly DMSO as indicated by Quality Adjusted Life Years (QALY) was better value for money ($P \leq 0.05$). Having said this, economic evaluation of the treatment can be challenged as the study duration of 6-months likely is too short to make any meaningful conclusions.



Hyalouronic acid

Figure 2-3 The chemical structure of hyaluronic acid.

2.1.2.2.3 Chondroitin sulphate (CS)

Chondroitin is available as a sulphated sodium salt at either position 4 or position 6 as shown in Figure 2-4. It is the treatment most widely used for IC/PBS. Like other GAG analogues, it acts by replacing the damaged or lost GAG proteoglycan component in the GAG layer. A comprehensive review of available clinical evidence relating to HA and CS have reported good outcomes with either the CS or CS/HA formulations in the treatment of IC/PBS (Jung-Soo and Won Jin, 2016).

A 12-week double blind randomised control trial (32 treatment and 33 control) spread across 12 sites in Canada to assess the efficacy of chondroitin sulphate over an inactive control was conducted by Nickel *et al.* (2010). The study consist of six-weeks of treatment and another six-weeks of follow-up in a pilot pattern. Efficacy as evaluated by GRA (Global Response Assessment) and O'Leary/Sant Score (ICSI and ICPI) were the primary and secondary outcomes respectively. At the end of six-weeks, responders-defined as patients who indicated moderate or marked improvement of symptoms on the GRA scale were 22.6% and 39.4% of the control and active groups respectively (p=0.15).

After the 6-week follow-up period the secondary outcomes as measured by ICSI (p=0.138), ICPI (p=0.707) and mean urinary frequency (p=0.877) were not statistically significantly different. Similarly, there were no statistically significant changes in both primary and secondary endpoints at the end of week 12. These findings can be challenged as no justification was made for the power calculation. Some of the weaknessess of this study have already been acknowledged by the authors.

In a study of small sample size involving 9-female and 1-male patient unresponsive to oral treatments participants were treated with a 2% CS instillation 6-times in 10-weeks and then 4-treatments for the remaining 14-weeks during the 24-weeks study (Downey *et al.*, 2015). Treatment efficacy was evaluated as a change from the baseline of the GRA likert scale score and the O'Leary Sant ICSI score. At week 10, the Global Response to treatment was 100%; while by the end of week 24, average pain score, urgency score, symptom score and problem score had all decreased dramatically by 76.6%, 74.2%, 48.9% and 60.0% respectively. However, the reliability of this study is questionable due to low sample size, lack of randomisation and absence of a control arm. However, the outcome points to the potential of CS instillation as a useful therapeutic option.

Rcently, Tutolo *et al.* (2017) conducted a head-to-head comparison of CS (2%) and DMSO (50%) in 36 patients in a randomised multi-centre trial to evaluate treatment efficacy over 18-weeks. The dosing schedule for both treatments was once weekly for 6-weeks, followed by one every month for 4-months and finally once every two months for the remaining two months. Change in GRA was the primary endpoint; the proportions of patients who achieved GRA scores of 6 or 7 was used to

compare the two groups. A total of 8/14 (57%) compared to 6/22 (27%) of patients quit the study in the DMSO and CS groups respectively due to drug adverse effects ranging from pain during instillation and garlic odour in the DMSO group and lack of efficacy in the CS arm. A total of 14.0% and 72.7% achieved the GRA scores of 6 or 7 in the DMSO and Chondroitin sulphate groups respectively. The VAS and O'Leary/Sant scores were significantly ($p \leq 0.05$) in the CS group in comparison to the DMSO arm. This data established the superiority of CS over DMSO at the stated concentrations in the treatment of IC/PBS. The fact that a higher drop-out rate was noticeable in the DMSO demonstrates that it is less well tolerated than CS. Conspicously, small sample size is a major weakness of this study. Altogether, this trial has demonstrated the efficacy of CS over DMSO. The result could be related to the fact that chondroitin sulphate is the most abundant GAG lying on the surface of the mucosal urothelium and that this accounts for its profound protective function (Janssen *et al.*, 2013).

In another study, the effectiveness of CS was compared with HA among 42 female patients showing a positive result on the potassium sensitivity test (PST) (Gulpinar *et al.*, 2018). A total of 21 patients each were randomly assigned to the two treatment groups.

VAS, ICSI and ICSP were recorded at baseline and upon completion of the study at month-6. Variables in the two groups were similar at baseline. VAS pain score, ICSI and ICPI were all significantly lower from the baseline in the two treatment groups. However, inter-group variation appeared to be the same except in 24-hour frequency which was significantly higher in the CS group. Although the clinical trial did not have a control arm the outcome still demonstrates a potential role for CS (and HA) in IC/PBS.

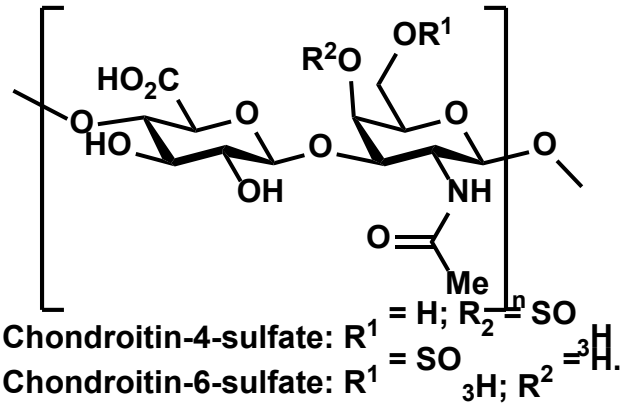


Figure 2-4 The chemical structure of chondroitin sulphate with the sulphate group at either position 4 or 6.

2.1.2.3 Mast cell modulators

The central role of the mast cell in IC/PBS makes it an important target for pharmacological manipulation. Several agents that modify the functions of the mast cell have been found clinically useful in the treatment of the disease. Notable members of this class include cimetidine, amitriptylline and, hydroxyzine amongst others. The first two are licensed in the UK.

2.1.2.3.1 Cimetidine

Cimetidine belongs to the class of drug defined as H_2 receptor antagonists, most commonly indicated for the treatment of gastro-oesophageal reflux disease (GORD), peptic ulcer disease and other gastric acid secretory disorders. Meares (1987) and Lewi (1995) serendipitously reported symptom reduction in IC/PBS patients following oral cimetidine therapy. The limitation of these observations being a lack of randomisation and a control arm.

A more convincing study by Thilagarajah, O'N Witherow and Walker (2001) randomised 36 patients into oral cimetidine (400 mg twice daily) or placebo arms over a three month period in a double blind pattern. Overall the symptom score fell significantly ($p \leq 0.05$) by 19.7% in the cimetidine arm. Both pain and nocturia were significantly lowered in the cimetidine group. Cystoscopic examination and bladder biopsy of the two arms before and after treatment did not show any significant variation. The major pitfall of this study, is that estimation of sample size was not explained and the study duration was short considering the chronicity of this disorder. A study of at least 6 months would likely be required to make any meaningful generalisation in real life.

Additionally, Dasgupta *et al.* (2001) showed an improvement in bladder pain scores and number of voids in 8 out of 14 IC/PBS patients on oral cimetidine (200 mg thrice daily). The study however, fell short of addressing the proposed mechanism by which cimetidine might exert its beneficial effect in IC/PBS as the authors concluded that effect on the bladder is not via blockade of histaminergic receptors (Dasgupta *et al.*, 2001). The basis for this conclusion is flawed, majorly because histamine and gastrin are not physiologically synonymous as the authors alluded. Gastrin, rather than histamine, antibody was used for the histological studies, making the conclusion both problematic and contradictory.

Overall, limited data is available on the clinical benefits of cimetidine in IC/PBS patient and justifies the use of an online survey to collate more data (Fall, Oberpenning and Peeker, 2008).

2.1.2.3.2 Amitriptyline

Amitriptyline belongs to the broad class of tricyclic antidepressants (TCA) that are indicated for use in depression, neuropathic pain and anxiety disorders. Whilst depression is another troublesome

complaint seen in cohorts of IC/PBS patients, the mechanism through which amitriptyline mitigates symptoms in these patients is unrelated to its antidepressant action. This is because the doses used for IC/PBS are much lower than those indicated in depression. Serendipitously, amitriptyline decreases symptoms of IC/PBS after depression has been treated. This led Hanno and Wein (1987) to conduct the first clinical study on the usefulness of amitriptyline in IC/PBS, where 12 IC/PBS patients were treated using titrating doses of 25 mg, 50 mg and 75 mg weekly. A marked improvement in the symptoms of IC/PBS patients was observed in 66% of the patients following a 3-week study. The weakness of this study were that the trial design was neither randomised nor controlled. Similarly, few subjects were enrolled in the study making any clinical inferences problematic.

However, van Ophoven *et al.* (2004) conducted an open-label randomised control trial involving 50-patients (NIDDK met criteria) into amitriptyline and placebo groups over a 4-month study period. One patient from each of the two groups dropped-out, leaving 48-patients for evaluation at the end of the study.

Statistically significant changes for the dual symptoms of pain and urgency were recorded in favour of the amitriptyline group ($p \leq 0.001$). A decrease in the frequency of voiding from baseline was seen in the amitriptyline arm albeit not statistically different from the placebo group ($p=0.063$). Typical of any of the TCAs, the antimuscarinic side effect of dry mouth was higher in the amitriptyline group. This is beneficial considering the antimuscarininc action reduces detrusor contraction and thereby frequency and urgency. The drop out rate was 2% from each arm of the study mainly due to side effects. The short duration of the study is the major weakness of this trial, in addition to troublesome side effects, which could account for the attrition rate in the amitriptyline arm.

In a related study, to evaluate long term tolerability of amitriptyline in IC/PBS patients. Ninety-four patients were enrolled into an open-label prospective placebo control study over a 6-month trial period. Long term amitriptyline therapy appeared to be tolerable at a mean daily dose of 55mg per day (van Ophoven and Hertle, 2005). As expected, side effects accounted for 25% of the patient drop-out in the amitriptyline group. Absence of randomisation is a major weakness of this study, which is a potential source of bias.

A large multi-centre randomised controlled trial was conducted to demonstrate the effectiveness of amitriptyline in a double blind pattern over a 12-week period in patients unresponsive to other treatments (Foster Jr *et al.*, 2010). A total of 271 patients were randomly allocated to either placebo or amitriptyline. The latter being used in escalating doses of 10 mg, 25 mg and 50 mg during the first 3-weeks, based on clinical response and tolerabilty to the stated doses. Subjects were assesed after the first 3-weeks and the drug dose escalated to a maximum of 75 mg based on tolerability

until the 12-week study period end. The primary end point was the GRA score, while O'Leary symptoms and problem indices; Wisconsin IC Symptom Inventory; Health Status Questionnaire for Quality of Life, Hospital Anxiety and Depression Index and Female Sexual Function Index were the secondary endpoints. Notably, pain score, urgency score, ICSI, ICSPI and University of Wisconsin Symptom score fell from baseline by 55.1%, 53.1%, 59.3% and 53.1% in the amitriptylline group while it was lowered from the baseline by 61.6%, 60.9%, 70.5%, 65.1% and 65.1% in the control group (which performed better than amitriptylline) respectively. The changes in the scores between the two groups were not statistically significant. The outcome of this study is persuasive and shows that evidence supporting the use of amitriptylline in IC/PBS is mixed. Despite some modest benefits in some cohorts of patients, there still exists a need to further explore the benefits or otherwise of amitriptyline in this group of patients.

Additionally, data from RCTs for treatment often only provide efficacy information whilst failing to record the side effects seen with these treatments (Ioannidis and Contopoulos-Ioannidis, 1998). In the same vein, clinicians rarely acknowledge the harmful effects of treatment medications filed by patients in the course of clinical visits (Golomb *et al.*, 2007).

Most side effects of medications are often reported during the post marketing surveillance. Although this approach has been useful; the value of this data collection could be enhanced if it were to be performed in a carefully designed disease area such as IC/PBS (Wysowski and Swartz, 2005). Patient reporting of side effects is a valuable tool in assessing the harmful effects of potential treatments particularly in diseases with a chronic course such as IC/PBS (Foster Jr *et al.*, 2010). This would capture valuable information hitherto unreported in the randomised control trials (RCTs) of treatments. In addition, it could guide clinicians in the proper counselling of patients so as to minimize patient discontinuation.

2.1.2.3.3 Hydroxyzine

Hydroxyzine is a selective H_1 receptor antagonist that has been used to treat allergies. This agent was trialled in a study involving 140 patients with IC/PBS who met National Institute of Health Diagnostic Criteria (Theoharides and Sant, 1997). A Visual Analogue Scale was used to score symptom (frequency, nocturia, painful intercourse) improvement before and after intervention. After 3-months treatment, a 40% and 55% improvement in symptoms was observed in IC/PBS patients without and with prior allergies respectively. More importantly, the effect of hydroxyzine was more dramatic in premenopausal women and those with allergies. However, in a head-to head RCT between hydroxyzine and pentosan polysulphate as evaluated by Global Response Assessment (GRA) endpoint at week 24, hydroxyzine did not show any significant (p=0.26) change from base line compared to pentosan polysulphate (p=0.064) (Sant *et al.*, 2003a). The outcome of this study

seems to reveal the weak effect of hydroxyzine in IC/PBS. However, it should be noted, not all IC/PBS patients present with allergies or detrusor mastocytosis where the optimal effect of hydroxyzine has been demonstrated. Overall, the evidence in support of hydroxyzine for IC/PBS is weak due to lack of a sound RCT with proper design. Consequently, this agent is not licensed in the UK.

2.1.2.4 Manuka honey as a new treatment option

The use of honey for medical purposes among various civilisations in the world goes back to ancient times. The Abrahamic scriptures (The Koran and The New and Old Testament) are full of examples of the medical utility of honey. In spite of its outstanding medical heritage, it was only in the 20th century that folkloric claim was scientifically validated (Noori, Khelod and Ahmad, 2011). Clinical evidence in the form of RCTs have shown the anti-inflammatory, anti-oxidant and antimicrobial effects of honey (Yaghoobi, Kazerouni and Kazerouni, 2013). Oryan, Alemzadeh and Moshiri (2016) in a concise review reported that honey has antimicrobial, anti-inflammatory, antioxidant, immuno-stimulatory and tissue remodelling actions. In addition, honey has occupied a central position in the treatment of ulcers and non-healing wounds and burns (Alvarez-Suarez *et al.*, 2014). The biological actions of honey are thought to be due to its hygroscopic, acidic, hypertonic and hydrogen-peroxide producing properties (Noori, Khelod and Ahmad, 2011).

Manuka honey has attracted considerable attention due to its unique biological action and other pleiotropic effects. It is obtained from the indigenous New Zealand plant, *Leptospermum scoparium* in the Manuka bush (Burns *et al.*, 2018). Manuka honey is rich in sugars (glucose, sucrose, maltose, fructose), phenols, minerals and methyl-glyoxal (MGO); which are thought to account for its biological actions (Deng *et al.*, 2018). Mast cells have been found to be higher in the bladder biopsies of the IC/PBS patients and may account for the inflammatory phase of the disorder via degranulation (Malik *et al.*, 2018). Additionally, in cell culture studies using the LAD2 mast cell line, pre-treatment with honey and its artificial analogues was shown to significantly reduce the release of histamine in a dose-dependent manner (Birch *et al.*, 2011b).

Chapter 3 Methods and Methodology in Exploring IC/PBS Perception

3.1 Research Philosophy

The quest for any scientific enquiry must be founded on valid philosophical assumptions. More often, research methods answer research questions only. However, this approach only addresses the tip of the iceberg in any knowledge enquiry. The answer to research questions must always conform to the general, systematic, and sequential process of knowledge search, which is always enveloped in the research philosophy. In this chapter, the PhD research questions will be supported by the core tenets of philosophy of science. An attempt will be made to justify the choice of these philosophies as it relates to this work. This chapter will focus on research belief, research reasoning, research strategy and research time horizon. It will conclude by outlining the research methods/procedures. In addition, a note on sample size justification will be made.

3.1.1 Research belief

Viewing research questions through a philosophical lens provides the basis for stepwise and methodological paths in unravelling the research answers (Winit-Watjana, 2016). In the philosophical realm, research answers can be broadly grouped into two categories: natural entity and human entity. In seeking to uncover knowledge, the knower (researcher) is expected to place the researchable item into these classes and that will tailor the type of assumption to be followed in systemically addressing the research problem. The natural answer is hard fact, measurable, objectively ascertained by the human senses and subject to law-like generalization, and thus gleaned via the positivist prism (Rodrigues, 2011). This contrasts with the human entity which is amenable to cultural, psychological, and ethnic influences and human interpretation; it is subjective and deeper meanings could be unveiled. Thus, human reality could only be obtained through post-positivist assumptions (Winit-Watjana, 2016).

The above description of philosophical paradigm is rather simplistic. A more detailed account of where a research problem fits into the philosophical realm will place the problem at hand into the ontological, axiological, epistemological sieves through, which the finer particles of the research problem can be picked, identified, and addressed. Most research in health, biomedicine and medicine is anchored on positivist ideology.

However, most research in nursing, physiotherapy and midwifery sub-specialties is well rooted in the post-positivist philosophy (Bunniss and Kelly, 2010).

From an ontological perspective, the proposed research aims at describing IC/PBS treatments. Numerical data will be used to evaluate treatment effectiveness in these patients generating unique (naïve realism) and measurable data that could be transmitted in mathematical language (Ponterotto, 2005). The results will be used to make plausible universal generalisations. Premised on this, unique data is expected from the outcome of this study that is universal and devoid of the human bias arising from subject interpretation. The research problem can be conceptualized from the positivist ideology in stark contrast to the post-positivist tradition where multiple realities are realisable due to subject variation, multiple interpretations, and lack of universality.

From the epistemological standpoint, knowledge is a sum total of relationship between the research item and the researcher (Ponterotto, 2005). Objectivism, subjectivism, and dualism are terms associated with research answers and form the basis for understanding the research problem(s). The concept of objectivism, which may exist with dualism assumes that the researcher and the research item are different entities. The researcher accumulates data by being detached or impartial from the research process to minimize bias and this forms the bedrock of the positivist philosophy. On the other hand, the post-positivists contend that knowledge is an interplay between the researcher and the research item and that the research answer could only be reliably and accurately obtained when the researcher is submerged into the research process as seen in the constructivist-interpretivist research ideology (Crossan, 2003; Winit-Watjana, 2016); wholly by being a research participant or partially by influencing research outcomes.

In the light of the research problem that this work aims to address, an online questionnaire will be completed by research participants and the researcher is not in any way involved in how participants answer their question options. This, as seen from the above, makes the work dualistic due to there being a clear demarcation between the researcher and the research participant, and objective due to non-interference of the researcher. This fits into the positivist research paradigm. To summarise, the researcher has no influence on the outcome of the research, this being purely an observational study and as such fulfils the epistemological requirement for positivism.

The axiological leg of the research belief emphasizes the role of values in research. Following this theme, two extremes of research are popularized viz: value-less or value-prone (Winit-Watjana, 2016). In between these divergent axiological research poles there exists the middle way, where research is value prone and biases arising from this position are fully accounted for and controlled.

Consistent with the positivist belief, the value-less approach is well entrenched in experimental or semi-experimental inquiry where absolute impartiality is a prerequisite for reliable, consistent data, subject to law-like generalisation (Ponterotto, 2005). In other words, the predetermined research instrument – in this study the questionnaire – although designed by the researcher is neither completed by the researcher nor does the researcher have any role in determining the choice the participants will make. In practice, there is a form of bias especially in using non-standardised instruments designed by the researcher. Emotions, empathy and leading questions, gestures most typical of post-positivism research philosophy are grossly absent. These components are all lacking in the methods addressing the research question in this study and this makes it compliant with a positivistic stance.

3.1.2 Research reasoning

Research reasoning describes how theory is developed around the research problem. Initially two opposing systems of logic dominated most scientific inquiry, deductive and inductive reasoning (Rodrigues, 2011). In deductive reasoning hypotheses are tested based on pre-set premises; if all the conditions are met then the conclusion or inferences are valid. Logical reasoning using the deductive approach involves testing a theory and it usually involves quantitative variables that have natural components, all gained through wider readings of academic resources, hence fulfilling positivist criteria. On the other hand, in inductive research reasoning, observations are made, or data collected on a human participant, a pattern or tentative hypothesis or theory or phenomenon is developed or the phenomenon explored based on the processes followed. The fact that themes, interpretations and meanings are construed from the inductive research process which is nominal, narrative, context specific and subject to research subject variation makes this type of reasoning conform to the post-positivist research tradition (Crossan, 2003). Another research logic has emerged which is a blend of the two, i.e., Abduction. In this research reasoning, data is collected or observed, which could be hard facts that are measurable and quantified or narratives or themes that can be qualified; a theory or hypothesis is developed and tested using either strictly inductive or deductive reasoning (Saunders, Lewis and Thornhill, 2015). In essence, data collection in abductive research reasoning is not aimed at answering a question, but rather developing a theory or theme that could be answered later with either inductive or deductive research logic. Deductive reasoning can be used to refute (Saunders, Lewis and Thornhill, 2015) or support a theory, whilst inductive reasoning generates a hypothesis.

Conceptualising the current research problem where data is going to be collected through a survey and analysed quantitatively to describe proportions of participants, a theory is going to be developed and treatments will further be tested using a hypothetical question, which aims to see

how any of the treatments could be optimised in the management of IC/PBS. This fits squarely into an abductive research reasoning, as employed in this study.

The clear advantage of abductive logic is that the researcher could switch between induction and deduction based on the outcome of the data collection/observation by developing a clear theme and appropriate hypotheses that adequately address the research questions.

3.1.3 Methodological Choice

The methodological choice is developed after the logic of enquiry is agreed and justified. Similarly, it is a prelude to the research techniques and procedure. It describes whether qualitative or quantitative methods are to be employed or both (Saunders, Lewis and Thornhill, 2015). As described above, the proposed research is built on abductive reasoning; hence, a multi-method research approach is the methodology of choice. This is because data is to be collected at two stages. In these two phases numerical variables are to be collected and analysed. As such, the methodology aligns with the multi-method quantitative model described previously.

3.1.4 Techniques and Procedures

The first phase of this research is aimed at describing the population of patients living with IC/PBS, to describe their treatments and establish baseline data for quantitative experimental study. This requires a naturalistic non-experimental procedure (Polgar and Thomas, 2008). Naturalistic because the participants are studied in their normal surroundings and non-experimental as no control group is required to achieve the research objectives. Thus, a strict experimental design would be highly inappropriate for this work because the variables at stake cannot be controlled nor modified. These objectives fit into the classical model of a survey study.

However, the second phase of the study will require comparative study of variables chosen from phase one as testing variables with positive and negative outcomes will be set up. The fact that the study utilises hypotheses, interventions and group differentiation make this inquiry purely experimental

3.1.4.1 The survey

Having established previously that survey is an appropriate research method to describe the cohorts of IC/PBS from the Bladder Health UK sample, it is worthwhile identifying which of the survey types and delivery modes are the most appropriate for achieving the research objectives.

Surveys are generally aimed at: describing a population, assessing the effectiveness of treatments and evaluation of the cohorts needs (Marion and Lindsey, 2009), these all align with the research objectives. In the same vein Jacobsen (2012) identified two broad categorizations of surveys: interview and self-administered questionnaire.

In the interview survey, verbal questions are asked by trained interviewers and participants responses are recorded in the form of words, narration, themes and concepts (Forister and Blessing, 2016). The fact that narrations and wordings are collected and analysed makes the interview qualitative in nature; and so, aligned with a post positivist research philosophy. On the other hand, the self-administered questionnaire describes a method where pre-set questions with a set of predetermined factual responses are provided to study; participants choose from these responses in a way which can be analysed quantitatively (Marion and Lindsey, 2009; Jacobsen, 2012). Such a research paradigm conforms to the positivist philosophy. Based on the research belief most suitable to answer this research question as highlighted previously, the most appropriate survey type for the proposed study would be the self-administered questionnaire.

Jacobsen (2012) stated that the self-administered questionnaire has three forms of delivery: On spot completion survey, postal survey, and the internet-based survey. On spot completion survey, as the name implies, describes a setting where a member of the research team presents the questionnaire to one or all the participants, which is completed and returned. This may have the advantage of increasing both response rate and time, together with a concurrent decrease in study time. However, these advantages are offset by bias arising from the presence of the research team and participants are less likely to give true responses. This may have the disadvantage of impacting on both the reproducibility and the reliability of the data (Forister and Blessing, 2016). In addition, for a survey representative of IC/PBS cohorts, it is difficult to get all the study participants at one time and complete the survey simultaneously. Thus, on the spot completion may not be an adequate delivery mode for the research question.

Another form of delivery is the postal survey. This method of delivery is hampered by cost which requires a stamped questionnaire to be sent from and returned to the research team and is characterized by a long study time. Moreover, poor response rates and lag time in response is a major drawback of this method. Considering the limited available time for a research degree, this may not be an appropriate form of delivery for a PhD project, which is time dependent.

In the internet mode of delivery response rate is usually lower than 20-25%, and participants are limited to those with internet access. However, it is the fastest, requires little cost in both logistics and personnel, data is not subject to the bias of on spot completion and there is limited investigator bias (Forister and Blessing, 2016).

Furthermore, the internet form of delivery is more appropriate for individuals with no identifiable address or those that are geographically dispersed (Marion and Lindsey, 2009), which in part describes the cohorts at Bladder Health UK. Moreover, Seybert (2011) reported a steady rise in internet and broadband usage in the UK. This pattern is seen in other industrialised countries particularly the US (Dillman, Smyth and Christian, 2014). In view of the above, the internet form of delivery would be most appropriate for this study. In brief, the type and form of delivery of a typical survey can be illustrated in Figure 3-1.

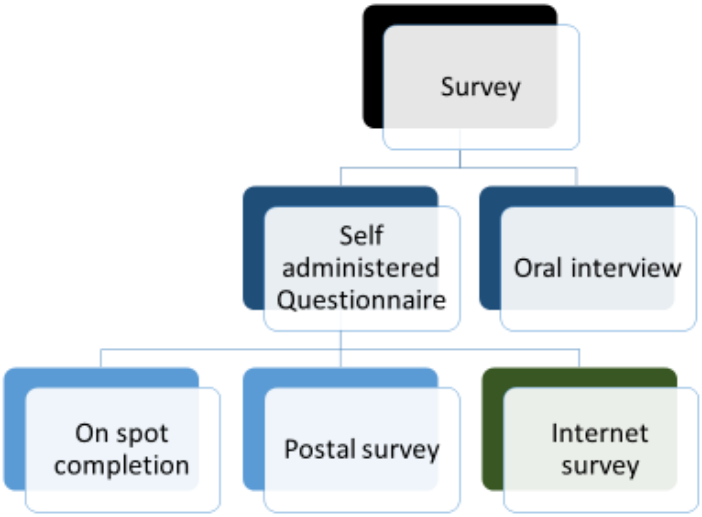


Figure 3-1 various types and forms of surveys

3.1.4.2 Time horizon

After the strategy for the research is decided, the next phase of the research process is the period in which the research is to be performed. By convention, research can either be longitudinal where data can be collected at multiple time points or cross sectional where data is collected at a single point in time (Jacobsen, 2012). Experimental studies like Randomised Control Trials with intervention and control arms represent another type of longitudinal study where data of interest are collected at more than one point.

However, it has been highlighted previously in this text that the study is a snapshot and signposting data will be collected at a single point period. Thus, this study satisfies the criteria for a cross sectional study.

3.1.5 Sample Size Justification

Bladder Health UK (BHUK) has a registered list of 700 members with a previous diagnosis of IC/PBS. Online surveys have a response rate of 20-25% (Jacobsen, 2012).

In view of this, an email was sent to all members to try and achieve a response rate of 20% of the members, which would be equivalent 140 participants.

Thus, the initial projection was to obtain responses from 140 members. However, the actual sample size calculation yielded a value of 171, based on a Confidence Interval (CI) width of 0.15, Confidence Interval of 0.5% and planning proportion of 0.5. A sample size calculator developed by Naing, Winn and Rusli (2006) was used to arrive at the sample size. Thus, a sample size of 171 was aimed at for use in this study. Since 171 is 20% of 855 and 25% of 684, then the charity (with 700 members) was a convenient place to recruit from. Participants identifying information were not sought and incentives were not given for study participation.

3.1.6 Questionnaire Development

The rationale behind the use of the questionnaire will be highlighted including but not limited to the psychometric properties of each of the validated instruments. A total of four validated tools were used for this survey:

- The O' Leary/Sant Interstitial Cystitis Symptom/Problem Index (ICSI/ICPI)
- The Pelvic Pain and Urgency/Frequency questionnaire (PUF)
- The Kings Health Questionnaire (KHQ)
- The Brief Illness Perception Questionnaire (BIPQ)

In addition, the author-developed questionnaire covering diagnosis, sociodemographic indices and past and current treatments supplemented the validated tools to answer the research problem more completely.

3.1.6.1 O'Leary/Sant Symptom and Problem Questionnaire

The O'Leary/Sant questionnaire was designed to monitor disease progression and symptomatic response in IC/PBS. It consists of two broad domains, the symptom and problem indices, all structured as Likert scales (O'Leary *et al.*, 1997). Pain, frequency, urgency, and intensity are captured in the symptom index while the magnitude and impact of the disease dealt with in the problem domain. Each of the domain has four items. The utility of this instruments lies in the use of shorter relevant questionnaire items which makes it easier to complete with better face validity and thereby being less likely to completion failure. Recently, the questionnaire was shown to be a valid tool in IC/PBS treatment outcome (Huang *et al.*, 2018).

The psychometric properties of the questionnaire measured by Cronbach's alpha show test and re-test reliability to be good.

Cronbach's alpha for the symptom and problem domains are >0.85 and 0.92 respectively (O'Leary *et al.*, 1997). This signifies little chance of variance due to error, making the tool to be valid for this measurement. Similarly, test re-test outcomes show correlation coefficients of 0.9 and 0.91 in both symptom and problem indices, respectively, almost a perfect agreement and, thus, highly reproducible.

The greater reliability of this result could be accounted for by the high number of response categories in the two domains of the questionnaire. For instance the symptom index has up to 7-categories whereas the problem index has a maximum of 6-categories, which has been shown to increase the reliability of the outcome (Bowling, 2009).

The overall psychometric properties of this tool are excellent. However, Kushner and Moldwin (2006) posited that the O'Leary/Sant instrument is an inappropriate diagnostic tool because it cannot differentiate IC/PBS from other bladder LUTS pathologies. Thus, it should only be used as a supplementary diagnostic tool or in research settings where the diagnosis of disease has already been established. This fits squarely into the framework of this work, this is because participant's have had their IC/PBS diagnosis confirmed by their caregivers, the tool is used to offer additional layer of diagnostic validity. In addition, participants in the study have a physician-based diagnosis of the disease. Similarly, participants data of whom primary diagnosis weren't IC/PBS were excluded during data cleaning prior to analysis. In addition, Lubeck *et al.* (2001) established the prognostic stability of the instrument in IC/PBS patients, who were on Pentosan polysulphate (300, 600 and 900 mg daily doses) in a double blind randomized controlled trial. All the psychometric properties were good including responsiveness to dose escalation. Similarly, construct validity which measured the relationship between ICSI, and other instruments was good as indicated by strong correlations ($p<0.001$) with all the other comparative tools. This result clearly demonstrates that ICSI is a valid and reliable tool for treatment evaluation in prospective cross-sectional studies.

3.1.6.2 Pelvic Pain Urgency/Frequency (PUF) Questionnaire

Whilst the O'Leary/Sant questionnaire addresses symptom severity and magnitude of pain, the assessment of pain is restricted to the bladder. This bladder-centric restriction of pain in the tool does not reflect the manifestation of symptoms in this patient group who often presents with general body pain in addition to urologic pain (Fall, Logadottir and Peeker, 2014). This shortcoming is addressed by the PUF questionnaire which in addition to assessing bladder pain, also considers non-bladder related pain particularly pelvic pain and dyspareunia in IC/PBS patients. Like the O'Leary/Sant questionnaire it is composed of symptom and problem scores using Likert scales.

The instrument was designed on the hypothesis that a high PUF score would predict the outcome of the potassium sensitivity test (PST) in IC/PBS and patients presenting with gynaecologic pain and dyspareunia (Parsons *et al.*, 2002). A total of 382 women were studied: 42 controls, 103 with gynaecologic pelvic pain and 213 with IC/PBS. Similarly, other PST studies have shown strong correlations between patient symptoms and potassium diffusion into the urothelium. However, Brewer *et al.* (2007) reported poor predictive outcome of PUF in the patients that underwent cystoscopy and hydrodistension but the small sample size (n=97) of the study makes the outcome problematic.

Furthermore, the PUF questionnaire showed good reliability when used to evaluate chronic pelvic pain alongside other standardized instruments (Quaghebeur and Wyndaele, 2013). Taken together, the PUF questionnaire is a valid and reliable tool in the assessment of disease progression and treatment response in cohorts of IC/PBS patients and can be used to evaluate the patient group in the proposed study.

3.1.6.3 Kings Health Questionnaire

The overall impact of IC/PBS in relation to social, sexual, emotional, and daily tasks has not been pooled in a single instrument. For instance, the female sexual function index (FSFI), a generic tool only assesses sexual function. Thus, it is valuable to have a questionnaire that covers other broadly important Health related QoL (HRQoL) measures and the KHQ was designed to address this gap. It is a generic tool designed to assess quality of life in women with different types of urinary incontinence. This makes it somewhat less specific in urologic diseases that affects both sexes such as IC/PBS. However, IC/PBS prevalence is higher in women and 87% of IC/PBS patients are women (Nickel *et al.*, 2005b). Furthermore, it has been used effectively in treatment evaluation for IC/PBS (Ramsay, Small and Conn, 2007; Vasudevan and Moldwin, 2017) and has also been adjudged as good by patients (Vij *et al.*, 2014). Therefore, it has a role in IC/PBS progression and treatment response.

The construct validity of KHQ measured against the Medical Outcome Study Short Form 36 item health survey showed strong correlations between common items on the two questionnaires. Internal consistency and test-retest reliability indicated by Cronbach's alpha of all the domains have a range of 0.725 and 0.892. Similarly test and retest scores measured by Rho have values ranging from 0.80 and 0.96 (Kelleher *et al.*, 1997). Overall, the psychometric properties of the KHQ are good and justify its use for description of treatments in this sample.

3.1.6.4 Brief Illness Perception Questionnaire (B-IPQ)

The Brief Illness Perception Questionnaire (B-PIQ) consists of 8-items structured on a 0-10 numeric scale (Broadbent *et al.*, 2006). The questionnaire is an adaptation of the Illness Perception Questionnaire-Revised (IPQ-R), the differences being that responses are evaluated on a Likert scale rather than a continuous numerical scale as in the B-IPQ. The psychometric evaluation of IPQ-R has already been established (Moss-Morris *et al.*, 2002). Consequently, construct validity between B-IPQ and IPQ-R found significant correlations between the two items for more than 70% of the items on the two instruments. Similarly, reliability measured by test-retest in renal patients showed significant correlations ($p<0.01$) in all the items (Broadbent *et al.*, 2006).

3.1.6.5 Researcher Developed Questionnaire

A set of predetermined questions with possible responses were developed to allow for exploration of participant's opinion, the items covered were sociodemographic indices, diagnosis, medications, and lifestyle see sections 1, 2 and 8 of the questionnaires in appendix B. The questionnaire was assessed for content validity by a member of the urology unit of University Hospital Southampton to ensure the items were relevant to the research questions. Meanwhile, the questions were piloted with a few members of BHUK for face validity. Useful and appropriate feedbacks following such Public Patient Involvement was integrated into and informed the final design of the main instruments (Bagley *et al.*, 2016).

Chapter 4 Results and Discussion of IC/PBS Perception

4.1 Data Collection and Analysis

4.1.1 Data cleaning

The survey hyperlink was sent to the administrator of BHUK for onward transmission to members. By the end of the one-month study period, 671 members had been approached to participate in the study. A total of 201 completed the survey. Of the 201 that completed the survey, 181 met the study inclusion criteria. A total of 28 were excluded due to missing pertinent data for instance age, recurrent UTIs being the primary complaint amongst others. In other words, primary complain wasn't IC/PBS.

4.1.2 Data analysis

Data was collected using isurvey software and result transformed into IBM SPSS statistics for windows version 26.0 (IBM Corp., Armonk, N.Y., USA) for analysis. Categorical variables were presented as proportions whereas continuous variables were presented as mean and standard deviations (SD) or box plots. Multivariate logistic regression was used to test for the association between Illness perceptions as risk factors of Illness severity. Free text entries were group based on authors preference as appropriate.

4.2 Sociodemographic Description of the Sample

The sample population had a mean age of 56.13 years and SD 15.39 years. The minimum and maximum age of the participants were 21 years and 79 years respectively. More than 90% of the participants have been living with their condition for more than 2 years and had no family history of the disease (see Table 4-1). In terms of care providers, 59% indicated they were being managed by a urologist while only 5.8% were being seen by a gynaecologist (Table 4-1). 20.2% of the participants identified themselves as having the ulcer phenotype, 43.9% as having the non-ulcer phenotype whilst 35.8% did not know their disease type (Table 4-1). Almost half of the participants reported a history of allergy although specific allergens were not recorded. There was no significant difference between the O'Leary/Sant scores of those with allergy 19.41 (SD 10.02) and those without 20.76 (SD 8.76) (See Table 4-1).

Regarding beverages, 54.3, 38.7% and 39.9% consumed tea, coffee, and alcohol respectively. Only 4.6% and 1.7% smoked cigarettes or vaped e-cigarette respectively.

There were no significant differences between the O'Leary/Sant scores of those drinking 18.78 (SD 10.02) and not drinking 20.97 (SD 8.89) coffee; and of those drinking 19.24 (SD 9.46) and not drinking 21.16 (SD 9.23) tea. However, there were significant differences in the mean O'Leary/Sant scores of cohorts smoking 27.13 (SD 9.28) and not smoking cigarettes 19.78 (SD 9.28); and those drinking 17.17 (SD 8.98) and not drinking alcohol 22.13 (SD 9.15) (See Table 4-1).

Table 4-1: Sociodemographic and lifestyle description of the sample (n=173)

Variable		N (%)	Mean (SD)
Age			56.13 (15.39)
Gender	Female	161 (93.1)	
	Male	12 (6.9)	
IC/PBS	Ulcer	35 (20.2)	
	Non-ulcer	76 (43.9)	
	Don't know	62 (35.8)	
O'Leary Sant score			20.12 (9.38)
Period since diagnosis of IC/PBS	>6months<1year	3 (1.7)	
	>1year<2years	14 (8.1)	
	>2years<5years	38 (22.0)	
	>5years	118 (68.2)	
Healthcare professional managing IC/PBS	Urologist	102 (59.0)	
	Gynaecologist	10 (5.8)	
	Nurse	17 (9.8)	
	Don't know	23 (13.2)	
	*Others	21 (12.1)	
Family members with IC/PBS	Father/Mother	4 (2.3)	
	Grandfather/Grandmother	1 (0.6)	
	Brother(s)/Sister(s)	0 (0.0)	
	Children/Grandchildren	3 (1.7)	
	Uncle(s)/aunt(ies)	1 (0.6)	

Variable		N (%)	Mean (SD)
	**Cumulative family members with IC/BPS*	9 (5.2)	
Allergy		82 (47.4)	
Cups of coffee drunk in a day	None	106 (61.3)	
	1-2	57 (32.9)	
	3-4	9 (5.2)	
	5-6	1 (0.6)	
Cups of tea drink in a day	None	79 (45.7)	
	1-2	47 (27.2)	
	3-4	30 (17.3)	
	5-6	17 (9.8)	
Units*** of alcohol consume in a week	None	104 (60.1)	
	1-5	48 (27.7)	
	5-10	16 (9.2)	
	> 10	5 (2.9)	
Number of cigarettes smoked in a day	None	165 (95.4)	
	1-5	1 (0.6)	
	5-10	3 (1.7)	
	> 10	4 (2.3)	
Vape e-cigarettes		3 (1.7)	

*Include pain specialist, nutritionist, and non-response by participants,

**Represent total family members with the disease

***unit of alcohol=1 small glass of wine; or=1 shot of spirit; or=half a pint of beer or lager

4.3 Diagnostic Description of the Sample

Only 3 of the participants did not know which diagnostic procedure they had undergone to make their IC/PBS diagnosis. Cystoscopy under anaesthesia appeared to be the most common procedure either alone or in combination with other procedures (See Table 4-2). Furthermore, 70 (40.4%) of the sample indicated they had previously undergone surgery in the pelvis or abdomen.

Regarding comorbid diseases, endometriosis appeared to be the most common in the sample whilst systemic lupus erythematosus was the least reported amongst cohort (See Table 4-3). The relationship between presence/absence of specific comorbid condition and O'Leary/Sant scores was run using a student's t test. Only vulvodynia and chronic fatigue syndrome showed a significant difference (Table 4-5). This means the two comorbid conditions are associated with IC/BPS symptoms. The mean (SD) O'Leary/Sant cores of those having and not having vulvodynia were 24.09 (10.24) and 19.51 (9.12), p=0.029 respectively. Similarly, the scores for those having and not having chronic fatigue syndrome were 26.79 (5.32) and 19.53 (9.44), p=0.05, respectively. In the sample, 7 participants (9.8%) reported bladder surgery, 23 (13.3%) gynaecological operations on the womb/uterus or ovaries, 5 (2.9%) intestinal operations and 25 (14.5%) surgery at other unspecified locations in the abdomen (See Table 4-4).

Table 4-2: Diagnostic procedures underwent by participants for IC/PBS diagnosis by multiple response analysis (n=173)

Diagnostic procedure	Frequency	% responses	% of cases
Cystoscopy under GA*	114	35.6	65.9
Cystoscopy awake	47	14.7	27.2
Hydrodistension	60	18.8	34.7
Bladder biopsy	52	16.3	30.1
Clinical history	44	13.8	25.4
I don't know	3	0.9	1.7
Total	320	100	185.0

*General Anaesthesia

Table 4-3: Distribution of comorbidity in the sample using multiple response analysis (n=173)

Comorbid disease	Frequency	% responses	% of cases
Vulvodynia	23	20.0	30.7
Endometriosis	25	21.7	33.3
Asthma	22	19.1	29.3
Fibromyalgia	21	18.3	28.0
Chronic Fatigue Syndrome	14	12.2	18.7
Systemic Lupus Erythematosus	3	2.6	4.0
Sjogren's syndrome	7	6.1	9.3
Total	115	100.0	153.3

Table 4-4: Showing surgical operations history of participants (n=173)

Body parts	N (%)
Bladder	17 (9.8)
Womb/uterus/ovaries	23 (13.3)
Bowel/intestine	5 (2.9)
Abdomen/pelvis	25 (14.5)
Cumulative surgical operations in the sample	70 (40.4)

Table 4-5: Showing the relationship between each of the comorbidities of IC/PBS and O'Leary/Sant scores of the participants (n=173)

Comorbidity	Status	O'Leary/Sant score-Mean (SD)	P value
Vulvodynia	Yes	24.09 (10.24)	P= 0.029
	No	19.51 (9.12)	
Endometriosis	Yes	22.16 (9.23)	P= 0.241
	No	19.78 (9.39)	
Asthma	Yes	21.23 (10.99)	P= 0.555
	No	19.96 (9.15)	
Fibromyalgia	Yes	23.86 (9.90)	P= 0.51
	No	19.61 (9.22)	
Chronic Fatigue Syndrome	Yes	26.79 (5.32)	P= 0.05
	No	19.53 (9.44)	
Systemic Lupus Erythematosus	Yes	18.6 (2.30)	P= 0.787
	No	20.1 (9.45)	
Sjogren's syndrome	Yes	20.00 (8.52)	P= 0.972
	No	20.13 (9.43)	

Perception of the disease by the respondents

A total of 159 respondents completed the BIP-Q. As illustrated in Table 4-6 and Figure 4-1, more than three-quarters of the respondents believed their IC/PBS could or would persist indefinitely. One-quarter of the respondents were extremely concerned about their bladder condition (See Figure 4-1). Only one-quarter of the sample believed they understood their urologic problem. More than three-quarters of the participants indicated that their symptoms would proceed indefinitely with the 25th, 50th and 75th centiles being 10 as shown in Figure 4-1.

Respondents identified various factors perceived to be the main causes of their bladder condition as shown Table 4-7 below. These fell into the following domains: Infection and inflammation (UTIs, tonsillitis, cystitis, viral infection, auto-immune disease, and pain) and Lifestyle (diet, stress, anxiety, emotions, bereavement, holding on urine for too long, obesity, dehydration, and heatwave) accounted for more than half of all causes. Others included Pelvic surgery/procedures (hysterectomy, surgery around the pelvis, caesarean section, and childbirth); hormonal dysregulation (changes in hormones because of menopause); and an anatomical defect (poor bladder lining).

Other ancillary causes included: Fibroids, constipation, Irritable bowel syndrome, chronic fatigue syndrome and ketamine abuse

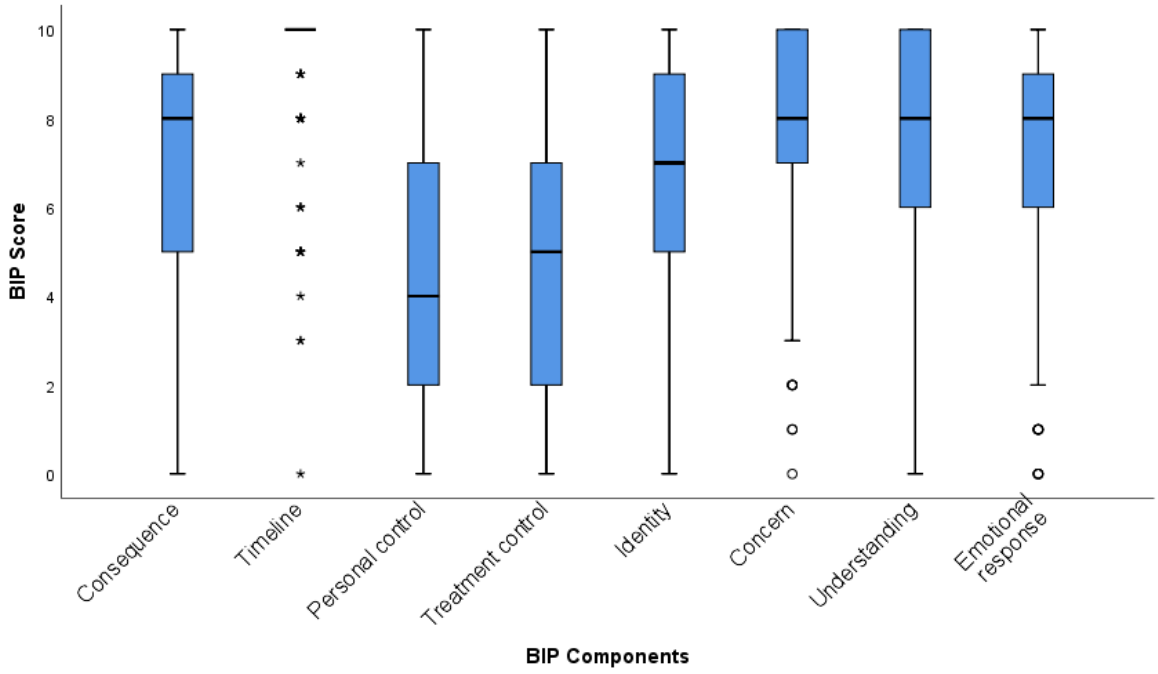


Figure 4-1: A 10-point rating of IC/PBS by Brief Illness Perception (BIP) score (n=159). The box blots show upper and lower values (BIP score) with 75th, 50th and 25th per centiles. BIP scores are positively skewed in consequence, treatment control and emotional response, whilst negatively skewed in personal control concern; symmetrical in identity and understanding. The box blot for timeline indicates upper, 75th, 50th and 25th per centiles to be the same with few outliers represented as *.

Table 4-6: Means (SD) of IC/PBS of perception from BIP-Q scale (n=159)

Item	Mean (SD)	Scale (min-max)	Higher score means
Consequence	7.03 (2.64)	0-10	Greater perceived impacts of IC/PBS
Timeline	9.31 (1.67)	0-10	Stronger perception of chronicity of IC/PBS
Personal control	4.25 (2.96)	0-10	Greater personal control of IC/PBS
Treatment control	4.71 (3.17)	0-10	Stronger belief in the role of treatments in IC/PBS
Identity	7.04 (2.38)	0-10	Increased association of symptoms with IC/PBS
Concern	7.69 (2.48)	0-10	More concerned emotionally by IC/PBS
Understanding	7.68 (2.58)	0-10	Better understanding of IC/PBS
Emotional response	6.99 (2.79)	0-10	Highly affected emotionally by IC/PBS

Table 4-7 Perceived causes of IC/PBS in the sample (n=175)

Cause	N (%)
Infection and inflammation	51 (29.1)
Lifestyle	39 (22.3)
No idea	34 (19.4)
Pelvic surgery/procedure	11 (6.3)

Cause	**N (%)**
Diet	8 (4.6)
Medication side effects	8 (4.6)
Childbirth	4 (2.3)
Autoimmune disease	3 (1.7)
Endometriosis	3 (1.7)
Genetics	3 (1.7)
Sjogren's syndrome	3 (1.7)
Neurological	2 (1.1)
Menopause or cancer	2 (1.1)
Miscellaneous	2 (1.1)
Sexual activity	1 (0.6)
Fibromyalgia	1 (0.6)

4.3.1 Association between Illness Perception and Severity of Symptoms

An O'Leary/Sant cumulative score of 12 and above has a predictive value that is both positive and specific. Thus, respondents were dichotomised 0-18 (mild to moderate IC/PBS symptoms) and 19-36 (moderate to severe IC/PBS symptoms). Similarly, the 10-point scale of the BIP-Q was stratified into lower level (0-5) and higher level >5; based on the covariates tested. The covariates were: Consequence, timeline, personal control, treatment control, identity, concern, understanding and emotional response. Multivariable logistic regression was used to test for BIP-Q items as risk factors for IC/PBS severity. The result of the univariable logistic regressions is presented as crude OR, 95% CI and their corresponding p values (See Table 4-8), while that of the multivariable logistic regression is presented as adjusted OR, 95% CI and their corresponding p values (See Table 4-9). P values less than 0.05 were considered statistically significant. Goodness-of-fit model assumptions were checked using Hosmer-Lemeshow and Omnibus tests ($p \leq 0.05$). Multicollinearity (to ensure that independent variables were not correlated) was checked by standard error of mean of the B coefficients.

The logistic regression model is statistically significant χ^2 (4) = 51.317, p= 0.000 and explained 37% (Nagelkerke R^2) of the variance of IC/PBS severity and correctly classified 74.2% of the cases. Of the 8 BIP-Q items, only one item (understanding) was excluded in the multivariate analysis (; $p>0.1$).

Of those included, consequence (expected effects and outcome of the illness), treatment control (extent to which the patient believes that treatment can help recovery from or control of the illness), Identity (the label the person uses to describe the illness and the symptoms they view as being part of the disease) and concern (anxiety about the disease) had odds of 0.094, 2.702, 0.141 and 9.363 for predicting the severity of IC/PBS, respectively (Table 4-9). This translates to a 0.094- and 0.141-fold reduction in the odds of predicting disease severity for the consequence (disease effects and outcome) and Identity (labelling) items of the BIP-Q. Conversely, there was an increase in the odds for predicting disease severity by 2.702 and 9.363 times respectively for the treatment control and concern items

Table 4-8: A univariable logistic regression analysis of Illness perception items as predictors of IC/PBS severity (n=159)

		95% CI for OR[a]		
Variable	**OR[a]**	**Lower**	**Higher**	**P value**
Consequence	0.099	0.042	0.234	0.000
Timeline	0.270	0.069	1.060	0.061
Personal control	2.040	1.059	3.93	0.033*
Treatment control	3.41	1.761	6.62	0.000
Identity	0.126	0.055	0.290	0.000
Concern	0.364	0.164	0.805	0.013*
Understanding	1.266	0.551	2.91	0.578
Emotional response	0.308	0.143	0.662	0.003*

OR[a]=Crude odds ratio, * p≤0.05

Table 4-9 A multivariate logistic regression analysis of illness perception items as predictors of IC/PBS severity (n=159).

		95% CI for OR		
Variable	**OR**	**Lower**	**Higher**	**P value**
Consequence	0.094	0.023	0.386	0.001*
Treatment control	2.702	1.26	5.81	0.011*
Identity	0.141	0.033	0.600	0.008*
Concern	9.363	1.52	57.63	0.016*

OR=Adjusted odds ratio, p≤0.05

4.4 Treatment Description of the Sample

Of those that completed the treatment domain of the questionnaire, Amitriptyline was the most common medication that had been used previously either alone or in combination with other drugs whilst cyclosporine was the least reported (See Table 4-10). A total of 47.2% reported a history of allergy (Table 4-1).

Similarly, in respect to the current medications being taken for IC/PBS by participants, amitriptyline was the most used either alone or in combination, whilst L-arginine was the least used (See Table 4-11). A total of 63.8% and 46.0% did not drink coffee and tea respectively (Table 4-1).

The Mean (SD) O'Leary/Sant scores of those who did or did not have a history of allergy were 18.16 (SD 9.58) and 19.04 (SD 9.01) respectively (p=0.065). In the same vein, The O'Leary Sant scores of those consuming and not consuming coffees were 18.75 ± 9.27 and 20.39 ± 8.86 (p=0.689); whilst those drinking and not drinking tea have O'Leary Sant scores of 17.61 ± 9.62 and 20.58 ± 8.64 (p=0.175) respectively (Table 4-12).

Table 4-10: Proportions of previous medications consumed by participants using multiple response analysis (n=173)

Medication status	Frequency	% responses	% of cases
Pentosan polysulphate	33	9.0	19.4
Amitriptyline	100	27.4	58.8
Cimetidine	57	15.6	33.5
L-arginine	10	2.7	5.9
Cyclosporine	9	2.5	5.3
Hydroxyzine	34	9.3	20.0
Prednisolone	15	4.1	8.8
None	22	6.0	12.9
*Others	75	20.5	44.1
I don't know	10	2.7	5.9
Total	365	100.0	214.7

*Other drugs used previously include nortriptyline, solifenacin, mirabregon, duloxetine, tamsulosin, diclofenac, gabapentin, pregabalin, paracetamol, naproxen, uribel.

From table 2, (sample size is 173), the frequency and per cent cases is 214.3% because some participants had taken more than one drug in the past and could have multiple responses to include both past and current drug usage, A fifth of participants reported being on no treatment at the time of the survey.

Table 4-11: Proportions of current medications used by the participants using multiple response analysis (n=173)

Medication status	Frequency	% responses	% of cases
Pentosan polysulphate	16	7.3	9.4
Amitriptyline	45	20.5	26.5
Cimetidine	11	5.0	6.5
L-arginine	2	0.9	1.2
Hydroxyzine	13	5.9	7.6
Prednisolone	3	1.4	1.8
None	62	28.3	36.5
*Others	63	28.8	37.1
I don't know	4	1.8	2.4
Total	219	100.0	128.8

*Others currently in use viz: Antibiotics (cefradine, flucloxacillin, cephalexin, nitrofurantoin); Smooth antispasmodics (solifenacin, oxybutynin, tamsulosin); Proton pump inhibitors (ranitidine, lansoprazole); Antidepressants (nortriptyline, imipramine, fluoxetine, duloxetine); Opioids (morphine, paracetamol and codeine, phenazopyridine, naltrexone, oxycodone, hydromorphone, tramadol); NSAIDs (celecoxib) Gabapentenoids (pregabalin, gabapentin); heparin hexamethylenetetramine, herbal tea and cannabis oil.

As with Table 4-10, Table 4-11 has a frequency lower than the sample size indicating that the number of participants that had chosen single medication status were 128 out of 163 surveyed. Of note amitriptyline was a widely used medication either alone or in combination while L-arginine was the least used.

Participants not currently on any medication and those on medication had mean O'Leary Scores of 20.06 (SD 9.06) and 18.55 ± 9.15 (p=0.940) respectively.

Table 4-12: The relationship between medications, beverages intake and O'Leary Sant Scores of Participants (n=163)

Variable	Status	O'Leary Sant Score (Mean ± SD)	P value
Medications	Yes	18.55 ± 9.15	0.940
	No	20.06 ± 9.06	
Tea	Yes	17.61 ± 9.62	0.175
	No	20.58 ± 8.64	
Coffee	Yes	18.75 ± 9.27	0.689
	No	20.39 ± 8.86	
Allergy	Yes	18.16 ± 9.58	0.065
	No	19.04 ± 9.01	

4.5 Description of Sample by PUF Scores

The sample had a mean PUF (0-36) score of 21.42 (SD of 4.53), the minimum and maximum PUF scores recorded being 8 and 30 respectively. The 25th, 50th and 75th percentiles of PUF scores are as shown in Figure 4-3.

A Spearman's rank correlation was performed to assess the relationship between age of the respondents and their PUF scores. Thirty-six (36) respondents completed the PUF questionnaire and supplied their ages. Preliminary inspection suggests the relationship to be monotonic by visual inspection of the scatter plot see Figure 4-3. There was a statistically insignificant negative weak correlation between the age of the respondents and their PUF scores, $\rho\ (36) = -0.279$, $p \geq 0.01$. The R^2=0.037 suggesting the relationship between age and PUF scores explained by only 3.7% of the variation seen in the cohort (Figure 4-3).

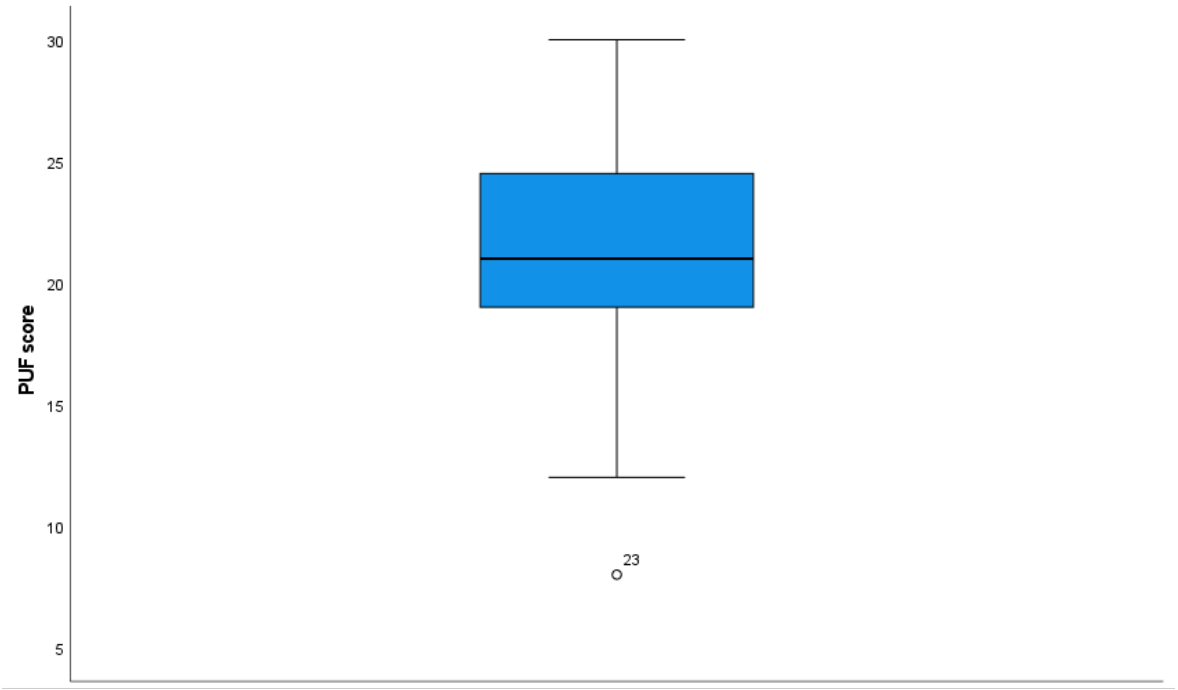


Figure 4-2 A box plot showing 25th (19), 50th (21) and 75th (25) percentiles of the PUF scores in the sample (n=36). The PUF scores is negatively skewed. Precisely 75% of the cohorts have PUF scores of 19 and above and half of them have PUF scores of 21. Entry 23 is an outlier in the dataset.

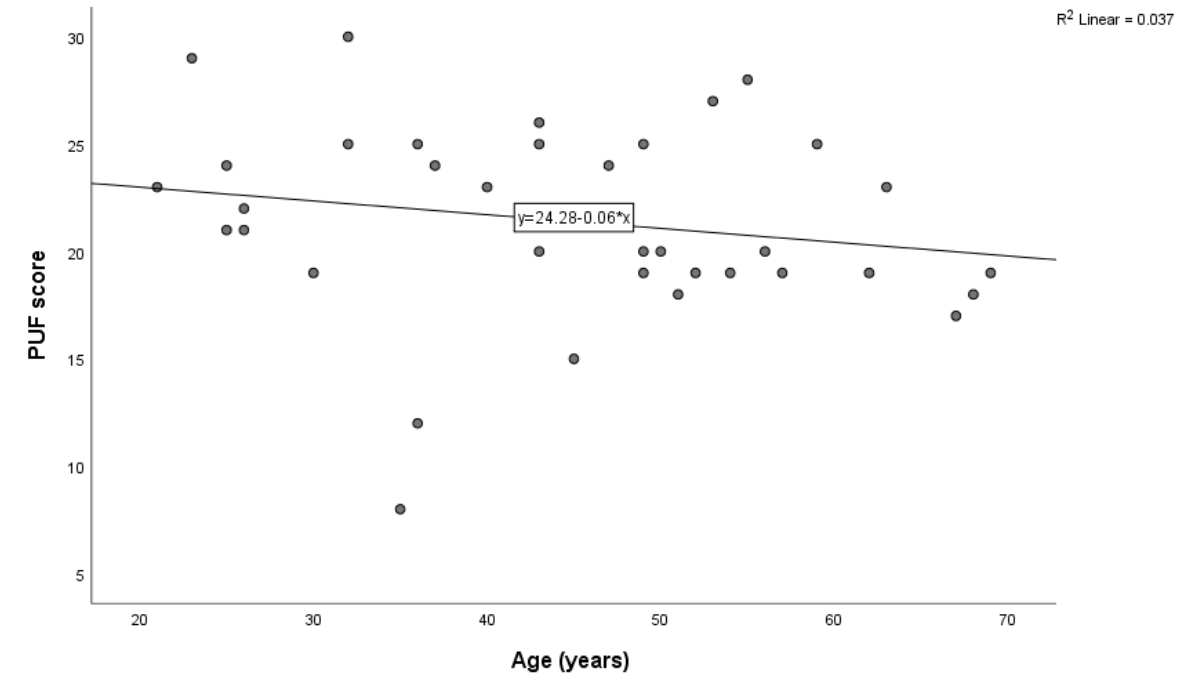


Figure 4-3 A scatter plot of age and PUF scores in the sample (n=36)

4.6 Description of the Sample by the O'Leary/Sant Score

The mean O'Leary Sant score (0-37) of the sample was 20.12 with a SD of 9.38. The minimum and maximum O'Leary scores were 0 and 37 respectively. The 25th, 50th and 75th percentiles of the O'Leary Sant are as shown in Figure 4-4.

A Spearman's rank correlation was run to assess the relationship between age of the respondents and their O'Leary/Sant scores. One hundred and seventy-three (173) respondents completed the O'Leary/Sant questionnaire and supplied their ages. Preliminary inspection suggests the relationship to be monotonic by visual inspection of the scatter plot see Figure 4-5. There was statistically significant negative weak correlation between the age of the respondents and their O'Leary/Sant scores, ρ (173) = -0.199, p ≤ 0.01. The R^2=0.042 suggesting a relationship between age and O'Leary/Sant sore only explained 4.2% of the variation seen in the sample (Figure 4-5).

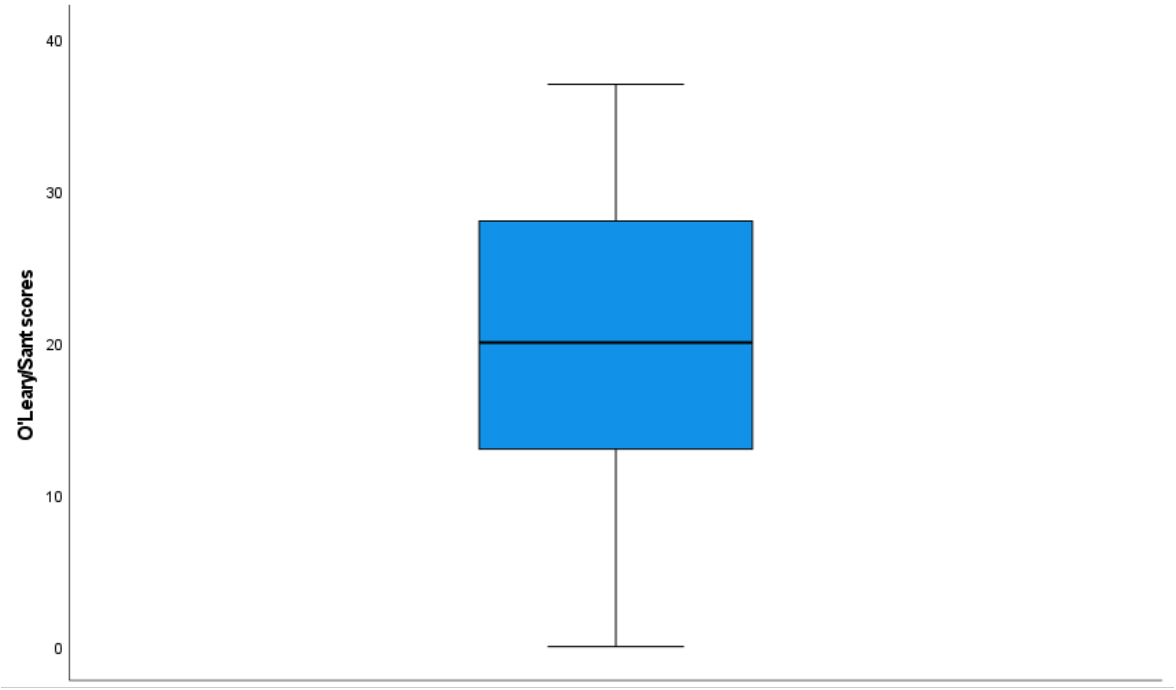


Figure 4-4: A box plot showing 25th (12.5), 50th (20) and 75th (28) percentiles of O'Leary/Sant scores in the sample (n=173). The O'Leary scores are symmetrical. Exactly half of the cohorts have O'Leary score is 20 and at least 75% of the respondents have O'Leary score scores of 12.5.

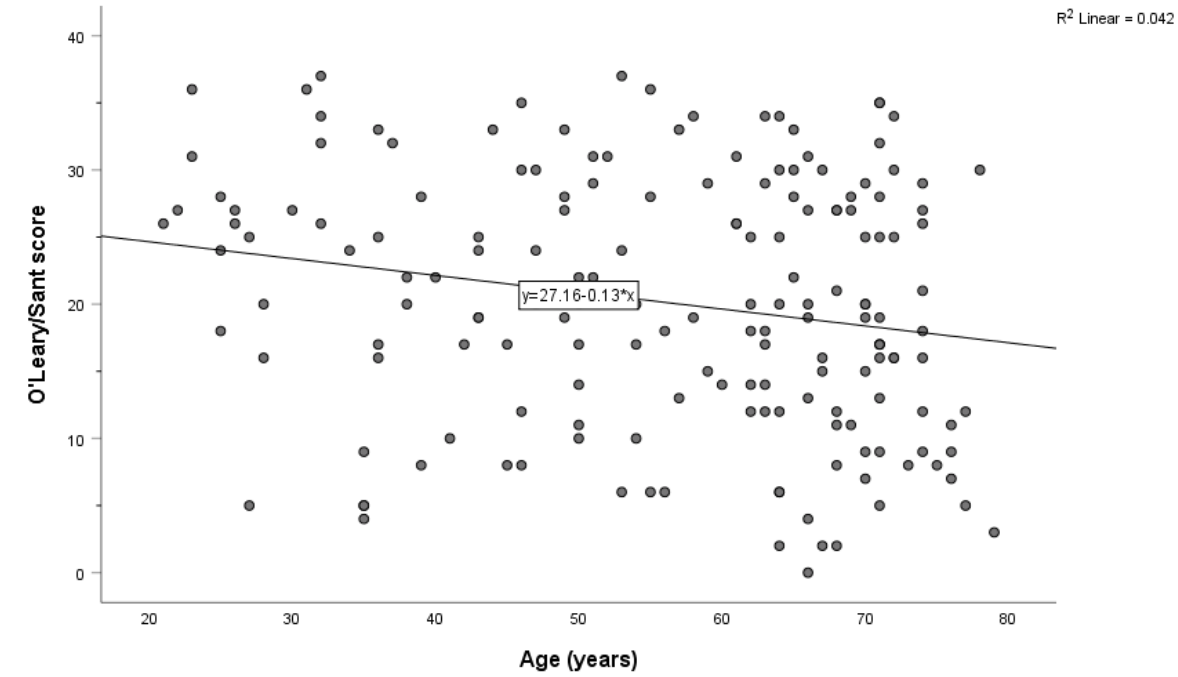


Figure 4-5: A scatter plot of age and O'Leary Sant scores in the sample (n=173)

4.7 Description of the Sample by Kings Health Questionnaire

From Table 4-13, about 88.4% of the participants perceived their general health as not very good (See Table 4-13). In terms of limitations: household tasks, jobs, sports, and travels were restricted in 72.8%, 87.2%, 87.2%, and 94.6% of participants, respectively (Table 4-13).

Regarding social life: social life, visiting friends, keeping a partner, sexual life and family life were limited in 82.3%, 74.8%, 76.9%, 83% and 76.9% of participants respectively (Table 4-13).

The cohort reported a poor quality of life in measures in all the domains as shown in Table 4-13. Severity measures and General Health Perceptions are below average score (Table 4-13). Only, 11% of respondents identified themselves as having poor health perception (Table 4-14). Sleep/energy had the highest score followed by incontinence impact (Table 4-13). Similarly, only 4.8% of the respondents reported that their sleep had not been affected by their bladder condition (Table 4-13).

Table 4-13: A Health-Related Quality of Life Estimate of the Participants by The King's Health Questionnaire (n=147)

Domain	Mean ±SD scores	Score
General Health Perception	40.64 (27.16)	0-100
Incontinence impact	68.02 (34.20)	0-100
Role limitations	54.76 (33.39)	0-100
Physical limitations	66.66 (30.34)	0-100
Social limitations	53.28 (36.68)	0-100
Personal relationships	61.03 (32.79)	0-100
Emotions	53.36 (32.10)	0-100
Sleep/energy	68.82 (30.29)	0-100
Severity measures	27.83 (23.96)	0-100
Symptom severity scale	11.61 (6.46)	0-30

Table 4-14: A descriptive evaluation of the health-Related Quality of life of the cohorts using the Kings Health Questionnaire (n=147). Females (137): Males (10)

Parts	Domain	Ratings	N (%)
I	General Health Perception	Very good	17 (11.6)
		Good	62 (42.2)
		Fair	38 (25.9)
		Poor	19 (12.9)
		Very poor	11 (7.5)
	Incontinence impact	Not all	15 (10.2)
		A little	29 (19.7)
		Moderately	38 (25.9)
		A lot	65 (44.2)
II	Role limitations (household tasks)	Not at all	40 (27.2)
		A little	43 (29.3)
		Moderately	34 (23.1)
		A lot	30 (20.4)
	Role limitations (jobs)	Not at all	18 (12.2)
		A little	34 (23.1)
		Moderately	37 (25.2)
		A lot	58 (39.5)
	Physical limitations (walk, sport, run or gym)	Not all	18 (12.2)
		A little	39 (26.5)
		Moderately	35 (23.8)
		A lot	55 (37.4)
	Physical limitations (travels)	Not at all	8 (5.4)
		A little	29 (19.7)
		Moderately	45 (30.6)

Parts	Domain	Ratings	N (%)
		A lot	65 (44.2)
	Social limitations (social life)	Not all	26 (17.7)
		A little	41 (27.9)
		Moderately	32 (21.8)
		A lot	48 (32.7)
	Social limitations (visit friends)	Not at all	37 (25.2)
		A little	40 (27.2)
		Moderately	29 (19.7)
		A lot	41 (27.9)
	Personal relationship (partner)	Not applicable	4 (2.7)
		Not at all	30 (20.4)
		A little	37 (25.2)
		Moderately	38 (25.9)
		A lot	38 (25.9)
	Personal relationship (sex life)	Not applicable	12 (8.2)
		Not at all	13 (8.8)
		A little	19 (12.9)
		Moderately	24 (16.3)
		A lot	79 (53.7)
	Personal relationship (family life)	Not applicable	4 (2.7)
		Not at all	30 (20.4)
		A little	44 (29.9)
		Moderately	33 (22.4)
		A lot	36 (24.5)
		Not at all	21 (14.3)

Parts	Domain	Ratings	N (%)
	Emotions (feel depressed)	A little	50 (34.0)
		Moderately	38 (25.9)
		Very much	38 (25.9)
	Emotions (feel anxious or nervous)	Not at all	21 (14.3)
		A little	39 (26.5)
		Moderately	42 (28.6)
		Very much	45 (30.6)
	Emotions (feel about yourself)	Not at all	43 (29.3)
		A little	38 (25.9)
		Moderately	28 (19.0)
		Very much	38 (25.9)
	Sleep/energy (affect sleep)	Not at all	17 (11.6)
		A little	33 (22.4)
		Moderately	33 (22.4)
		Very much	64 (43.5)
	Sleep/energy (make you feel worn out or tired)	Not at all	7 (4.8)
		A little	32 (21.8)
		Moderately	40 (27.2)
		Very much	68 (46.3)
	Severity measures (wear pads to keep dry)	Never	86 (58.5)
		Sometimes	31 (21.1)
		Often	10 (6.8)
		All the time	20 (13.6)
		Never	31 (21.1)
		Sometimes	49 (33.3)

Parts	Domain	Ratings	N (%)
	Severity measures (careful how much fluid to drink)	Often	31 (21.1)
		All the time	36 (24.5)
	Severity measures (change your clothes because they get wet)	Never	94 (63.9)
		Sometimes	34 (23.1)
		Often	13 (8.8)
		All the time	6 (4.1)
	Severity measures (worry because you smell)	Never	92 (62.6)
		Sometimes	36 (24.5)
		Often	10 (6.8)
		All the time	9 (6.1)
III	Symptom severity scale (frequency of urination)	None	12 (8.2)
		Mild	30 (20.4)
		Moderate	57 (38.8)
		Severe	48 (32.7)
	Symptom severity scale (Nocturia)	None	30 (20.4)
		Mild	37 (25.2)
		Moderate	44 (29.9)
		Severe	36 (24.5)
	Symptom severity scale (Urgency)	None	23 (15.6)
		Mild	43 (29.3)
		Moderate	45 (30.6)
		Severe	36 (24.5)
	Symptom severity scale (Urge incontinence)	None	85 (57.8)
		Mild	29 (19.7)
III		Moderate	17 (11.6)

Parts	Domain	Ratings	N (%)
		Severe	16 (10.9)
	Symptom severity scale (Stress incontinence)	None	87 (59.2)
		Mild	38 (25.9)
		Moderate	13 (8.8)
		Severe	9 (6.1)
	Symptom severity scale (Nocturnal enuresis)	None	104 (70.7)
		Mild	17 (11.6)
		Moderate	13 (8.8)
		Severe	13 (8.8)
	Symptom severity scale (intercourse incontinence)	None	110 (74.8)
		Mild	17 (11.6)
		Moderate	7 (4.8)
		Severe	13 (8.8)
	Symptom severity scale (water works infection)	None	61 (41.5)
		Mild	36 (24.5)
		Moderate	28 (19.0)
		Severe	22 (15.0)
	Symptom severity scale (bladder pain)	None	9 (6.1)
		Mild	34 (23.1)
		Moderate	44 (29.9)
		Severe	60 (40.8)
	Symptom severity scale (post void dribble)	None	72 (49.0)
		Mild	37 (25.2)
		Moderate	20 (13.6)
		Severe	18 (12.2)

4.8 Discussion

4.8.1 Sociodemographic and diagnostic variables

The categorisation of IC/PBS into ulcer and non-ulcer variants has long been established and has both diagnostic and therapeutic implications. The histopathological and extra-urologic presentations of the two forms in addition to their response to therapy has necessitated reclassification of the former as a distinct urologic disease on its own (Akiyama and Hanno, 2019). The ulcer sub type has been reported to be more prevalent in the elderly with more urinary frequency compared to the non-ulcer subtype (Peters *et al.*, 2011; Van Moh, Vetter and Lai, 2018; Han, Shin and Choo, 2019).

The outcome of this survey has shown that more than one third of the sample do not know their type of disease. Patients' knowledge about their disease will aid the provision of bespoke care in a disease like IC/PBS that requires multi-modal management as this has been reported to significantly improve treatment outcomes in this patient population (Robert *et al.*, 2009). This is because behavioural therapy, which integrates a patient's knowledge and expectations of their bladder condition, and its treatment is the cornerstone of most treatment guidelines.

In this respect, care providers and IC/PBS related charities could step up educational programmes for their members to update their knowledge base. The study outcome shows that IC/PBS is more common in women than men, which is consistent with reported epidemiologic studies of the disease in other countries like the US and Canada (Held, Hanno and McCormick, 2018). This suggests that disease prevalence and distribution in these countries is like that seen in the UK. Although other studies have reported a much higher prevalence in men (Clemens *et al.*, 2005), the reason for this discrepancy may be related to the general perception that IC/PBS is a disease of women and as such clinicians are more likely to miss the diagnosis in male patients. Furthermore, the diagnosis of this bladder condition relies on an exclusion of related bladder pathologies and hence care givers may be more likely to ascribe the bladder symptoms in male patients to other related urologic pathologies. Our findings are limited by the fact that convenience sampling, was used which is consistent with cross-sectional design. Thus, as is typical of all non-probability sampling methods, the data should be interpreted with caution.

Regarding dietary regimes in the sample, certain foodstuffs and drinks have been found to exacerbates the symptoms of IC/PBS patients (Shorter *et al.*, 2007). In line with this, a progressive decrease in the quantities of both alcoholic and non-alcoholic beverages consumed has been observed in this study. Consequently, elimination of specific dietary items known to trigger flares has been an integral component of patient care in this disease type.

A limitation of our findings is that respondents were not further probed to see whether tea and coffee triggered their flares on case-by-case basis. This gives room for further studies to explore, in the form of focus groups or interviews, patient perspectives on specific dietary triggers of flares in this population.

Coffee is rich in caffeine which by its diuretic action has a more profound effect on LUTS experienced by patients compared to healthy controls. This in turn, may have the effect of both increasing the frequency and voiding volume (Bird *et al.*, 2005). There was no distinction made between caffeinated or non-caffeinated coffee in this survey, which limits the conclusion drawn. However, the fact that the anti-inflammatory properties of coffee are shared by both the caffeinated and decaffeinated form (Hang *et al.*, 2019), suggest that the bladder-triggering effect may be due to its diuretic action (but see below).

Having said this, the pattern of beverage consumption suggests this to be similar across the board. More importantly, alcohol consumption is noted in this sample. Dietary components such as alcohol, coffee, tea, and other offending foodstuffs are to be avoided in IC/PBS (Gordon *et al.*, 2015). However, complete abstinence from these poses' practical problems due to the nutritional and recreational value that these diets/drinks offer. Whether these items should be entirely avoided is still a subject of debate. However, we have explored the relationship between beverage intake and elevation of O'Leary Sant score (Table 4.5).

The non-significant difference in the O'Leary/Sant scores between those drinking and not drinking tea and coffee does not support any correlation between their use and IC/PBS symptoms. On the other hand, a significant decrease in O'Leary Sant scores in those drinking alcohol compared to non-alcoholic drinkers was observed. By itself this might be considered highly unexpected considering the diuretic effect of alcohol would be expected to translate into frequent urination with a consequent increase in the problem domain of the O'Leary/Sant scale. However, it might be that IC/PBS individuals in the cohort could be aware of the effects of alcohol and opt to avoid it, this being most marked in those with worse symptoms. A similar trend was noticeable amongst smokers having higher scores than non-smokers, this being most likely a direct effect of the chemical in tobacco on bodily systems notably, cardiovascular, circulatory, kidney and the bladder. However, this should not be regarded evidence of causality due to cross sectional nature of the study design. Moreover, dietary sensitivity amongst IC/PBS is individual specific and as such patients should keep flare trigger diaries to identify which foodstuffs exacerbate their bladder symptoms (Sutcliffe *et al.*, 2018).

A major strength of the current study is that the definitions of comorbid conditions was broadened to include both urologic (vulvodynia and endometriosis) and non-urologic diseases (asthma, chronic fatigue syndrome, fibromyalgia and Sjogren's syndrome). Furthermore, we tested to see if there were any difference(s) between the O'Leary/Sant scores of participants reporting comorbid diseases and those who had not. Our results showed that vulvodynia and chronic fatigue syndrome showed higher scores than those reported for participants without comorbidities. This underscores the finding that other chronic conditions often co-exist with IC/PBS.

In terms of history of pelvic surgery in the sample, our data corroborate earlier studies that reported a high prevalence of pelvic surgery amongst IC/PBS cohorts (Ingber *et al.*, 2008b). Although, the pathogenesis of the bladder condition is still unclear lower abdominal and pelvic surgeries are recognised as a posing a possible traumatic challenge to the bladder that could potentially start the initial phase of inflammation where the processes continue viciously subsequently culminating in the clinical manifestation of IC/PBS.

The type and nature of the surgery determines the risk associated with IC/PBS, for instance the risk of caesarean section was reported to be similar to normal vaginal delivery in a case control retrospective study of this condition (Chang *et al.*, 2018) but the risk of hysterectomy to the development IC/PBS is higher (Hall *et al.*, 2008; Lee *et al.*, 2016).

Our outcome is weakened by the fact that specific types of surgeries were not sought from study participants. Similarly, we were unable to establish whether the surgeries were prior to the development of IC/PBS or after it. In the same vein, we were unable to collect data with respect to number of surgical procedures performed on the participants. This may be of importance because Warren *et al.* (2014) in a case control study reported compelling evidence for the fact that a higher number of non-bladder surgeries increased the risk of IC/PBS. Similarly, the same study established that previous surgery secondary to chronic pelvic pain (CPP) increased the risk of IC/PBS. Although, the anatomical positions of these surgeries were identified by respondents, we couldn't confirm the exact nature of the surgery performed.

The reason for the lower proportion of people living with the disease for shorter period could be because diagnosis of the disease is difficult to establish and relies on an exclusion of confounding diseases. Moreover, recently diagnosed patients may not be aware of the charity (Bladder Health UK) and hence not available for contact via their members database.

These findings show that patients underwent several procedures before a diagnosis of IC/PBS was established. This corroborates earlier findings relating to the difficulties in diagnosis of this disease (Patnaik *et al.*, 2017).

Tirlapur and Khan (2016) reported that most diagnoses were made using a combination of the four methods in the sample. However, cystoscopic examination under anaesthesia appears to be the most predominant diagnostic procedure in this sample. This is not unusual considering cystoscopy under anaesthesia is used to exclude other confounding disease from IC/PBS for instance bladder cancer and carcinoma in situ. In addition, such practices are in line with major clinical guidelines such as those from the National Institute of Digestive Diabetes and Kidney Diseases (NIDDK) and the European Society for the Study of IC (ESSIC) with respect to the diagnosis and treatment of IC/PBS. These guidelines underscored the value of this test (cystoscopy) and classed it as the gold standard investigation (van de Merwe *et al.*, 2008; Homma *et al.*, 2016). Moreover, a previous survey that sampled the opinions of bladder specialists around the world indicated physician's preferences for this test over others (Mishra and Meijling, 2003). Particularly, it is the only procedure that distinguishes the two forms of the IC/PBS.

Bladder biopsy on the other hand is the least reported test after clinical history in the sample. This may be because of the poor sensitivity and specificity of the procedure in diagnosis and the fact it is only employed to exclude less common confounding diseases such as carcinoma in situ (Sant, 2002; Cheng, Rosamilia and Healey, 2012). The Royal College of Obstetricians and Gynaecologists (RCOG) guideline is the only one that addresses IC/PBS in the UK and it does not consider bladder biopsy as being useful in disease diagnosis (Malde *et al.*, 2018). This may in part explain, its lower incidence in the study sample.

The contribution of detrusor mastocytosis to the pathogenesis of the disease, even though reported in the literature is still poorly appreciated. Thus, bladder biopsy is used mainly to aid diagnosis. Theoharides, Kempuraj and Sant (2001) reported mastocytosis in various layers of the bladder particularly in the ulcer subgroup and this forms the basis for considering bladder biopsy after cystoscopy in the diagnosis. Subsequently, a case report by Roth (2007) showed that IC/PBS secondary to mastocytosis responded to PPS, which is thought to act via inhibition of mast cell degranulation. Importantly, recent studies have strengthened previous findings on the potential of this histopathology in the diagnosis of the disease (Malik *et al.*, 2018). In this study, mast cells proteases where statistically higher in all the layers of the bladder tissue particularly the detrusor muscles in IC/PBS patients compared to control. This finding emphasises the role of bladder biopsy in disease diagnosis. Even though the sample size was small, the result was strengthened by the random selection of the sample, the presence of a negative control group and the double-blind format of the investigation. Though highly sensitive for the diagnosis of IC/PBS, the specificity for the diagnosis is at best poor and bearing in mind that biopsies from bladder cancer may also have elevated mast cell counts (Kim *et al.*, 2011; Sari *et al.*, 2012).

A major limitation of this study is the self-reported manner of participant's responses to the items on the diagnostic domain without recourse to their clinical records. Hence, there is no way to substantiate the respondent's disease type. However, BHUK is a charity established for bladder related disorders and therefore most respondents have been consulting their GPs and/or specialists making it likely that the information provided is factual. In addition, IC/PBS diagnosis was strengthened by the O'Leary/Sant scores. Furthermore, the fact that respondents were given a "don't know" option in most items of the diagnostic domains and quite a few respondents opted for this suggests that bias arising from the patients' responses is minimal. Another limitation is that we don't have the data of survey non-responders. Consequently, we can't be sure how this will differ from sample population.

4.8.2 Respondents' perception of the disease and relationship with symptom severity

To the best of our knowledge, this is the first study to look at perception of IC/PBS using a standardized instrument (B-IPQ). The poor outcome ratings of most of the health indices highlights the burden of the disease and the effect it has on an individual patient's quality life, which was earlier reported to be poor (Hakimi *et al.*, 2017).

The negative impact of the disease in daily activities as reported by patients is consistent with an earlier cross sectional survey of IC/PBS in the UK (Tincello and Walker, 2005). Strikingly, most patients believe that the disease will continue indefinitely. Such a viewpoint is not unreasonable considering that treatment goals in this disease remain based on symptom relief rather than cure (Davis, Brady and Creagh, 2014). Such perceptions could contribute to the anxiety seen in IC/BPS patients which has also been reported by other researchers (Chung *et al.*, 2014).

Overall, this factor might explain why the emotional and concern components in these respondents is high. It is fair to hypothesise that anxiety levels in this cohort could be related to the ineffectiveness of treatments. Recently, Yu *et al.* (2019) compared the treatment outcomes of 85 IC/PBS patients with different levels of baseline anxiety and found that, irrespective of the background anxiety levels, effective treatment reduced anxiety scores. The fact that anxiety scores remain high underscores the ineffectiveness of current treatments.

Regarding disease knowledge, more than three-quarter of the participants had rated their understanding above average but it was observed that more than a quarter of the sample do not know their form of the disease. The reason for this discrepancy is still unclear and may reflect the uncertainty of their treating physician.

The relationship between illness perception and severity of symptoms in the cohort was explored. Not surprisingly, participants that were anxious about their illness, as represented by the concern item of the BIP-Q scale, had a 9.363 times greater likelihood of having severe IC/PBS. The identity item assesses the description or labelling of illness in IC/PBS; higher scores in this item were negatively associated (AOR=0.141) with the overall severity of the disease suggesting that the latter alone is not important in participants thoughts around being assigned a diagnosis of IC/PBS. Moreover, participants generally indicated a lack of belief in the role of current IC/PBS treatments (AOR=2.702). This contrasts the finding that half of the sample were happy, the reason for this discrepancy is still not clear. With respect to the lack of belief in the current treatments, this could be related to the need for hope that treatment will control symptoms, something that is more marked in those with more severe disease. In summary, the study found that 1. belief in the fact that available treatments will mitigate symptoms and 2. concern about the disease demonstrated a positive predictive value for severity of IC/PBS symptoms.

Regarding the factors thought to be responsible for causing IC/PBS in the sample, infection and inflammation featured prominently. Recurrent UTIs are a confounding diagnosis for IC/PBS and are known to coexist with it especially among post-menopausal women. The GAG layer serves as protection against the entry of microbes in the bladder through the epithelium. In the disrupted GAG layer this barrier function is lost. As a result, the entry and localisation of pathogenic microbes is facilitated and recurrent UTIs ensue. Previous studies have suggested that nanobacteria resident in the urine and bladder of IC/PBS patients are responsive to the antibiotic tetracycline, and the use of this agent significantly improve the symptoms of IC/PBS patients (Zhang *et al.*, 2010). This agrees with the findings that, there is a high prevalence of UTIs in this patient group (Bladder Health UK, 2018, September, 18). However, a study by Atuğ *et al.* (2004) was unable to establish the presence of a candidate bacterium Helicobacter pylori in the bladder samples of IC/PBS patients compared to controls using Polymerase Chain Reaction (PCR). Thus, the hypothesis that infection causes the disease is, to date, weak and poorly supported by empirical evidence. This finally led to the rebuttal of microbial infection theory as a possible cause of the disease by a consensus of expert opinion (Hanno and Dmochowski, 2009). However, some bladder specialists are still of the opinion that microbial infection is a cause of the disease (Personal communication). Having said this, most of the studies refuting an infective aetiology are underpowered, and therefore this topic deserves further research attention.

Whilst respondents perceived lifestyle such as holding urine for too long, dehydration and stress amongst others as possible causes of their bladder disease, these factors are, in practice, more likely to be flare triggers (Homma *et al.*, 2009).

In IC/PBS, increased urinary volume elevates the pressure in the bladder wall which may stimulate contraction of the detrusor muscles and activate the sensory nerve endings that mediate pain. In the same manner, dehydration increases the urinary solute concentrations thereby causing a higher concentration gradient and more leakage of solutes, especially potassium, across the compromised GAG layer with a resultant decrease in pain threshold.

Stress may also activate the HPA axis which causes the release of corticotrophin releasing hormone (CRH) from the adrenal medulla that mediates anti-inflammatory effects. However, outside the central nervous system (CNS) particularly the bladder, CRH exerts converse effects (Karalis and Sano, 1991). These include the activation of bladder mast cells and an inflammatory cascade resulting in symptoms characteristic of IC/PBS.

With respect to inflammation, it is a well-recognised that neurogenic inflammation likely plays a crucial role in the pathogenesis of this disease condition (Grover *et al.*, 2011). Steroids and other anti-inflammatory drugs have been used to treat IC/PBS with modest outcomes, thus lending credence to the role of inflammation in the disease. In addition, the mast cell activation implicated in the two forms of the disease affords the prospect of using inflammatory biomarkers in the diagnosis and treatment of the disease. Furthermore, it can be envisaged that targeted therapies for neurogenic inflammation could add substantially to existing treatment options (Cruz, 2012).

Respondents also identified previous pelvic surgery/procedures as potential causes of IC/PBS. This is quite revealing and aligns with established studies reporting that pelvic trauma is a contributory aetiologic factor for the disease (Hanno and Dmochowski, 2009). More importantly, a history of pelvic surgery has been reported in IC/PBS patients prior to the onset of disease (Ingber *et al.*, 2008a). Such findings are also in line with the survey outcomes showing that a substantial number of the respondents have had a history of pelvic surgery. However, this result should be interpreted with caution as the timing of surgery in relation to the diagnosis of IC/PBS could not be ascertained from this survey. This is because of large variations between pre-post diagnosis surgeries. For example, whilst caesarean section is entirely performed for non-LUTS reasons, cystoscopy and laparoscopy procedures in this patient group are mostly always disease related, being used for diagnosis and treatment of ulcer subtype and exclusion of endometriosis, respectively (Redmond and Flood, 2017; Cox and Wagner, 2018). Strikingly, a total of 9.4% participants reported having had surgery on the bladder. The use of cystectomy with urinary diversion in IC/PBS patients would suggest severe symptoms unresponsive to all other treatment options. Although, the benefit of cystectomy is still controversial (Nordling and Blaivas, 2014), the mere fact that some of these participants have undergone this procedure is another indicator of the absence of effective treatments.

4.8.3 Treatment variables

About one-fifth of respondents were not on any medication at the time of the survey. A similar trend was noted in the medication history of the participants. This outcome is similar to the Interstitial Cystitis Data Base (ICDB) study where 18% of the cohort were on no treatment at all (Rovner *et al.*, 2000). Although our result has limited the description of treatments to oral medications this is in sharp contrast to the ICDB study that used non-oral treatments as well. Despite this, the trend appears the same in both sets of results. The slight variation in the proportions of respondents without medication from the past to the current continues to indicate a lack of effectiveness of treatments underlined by the fact that the average O'Leary/Sant scores of the respondents indicates that cohorts are still symptomatic (moderate symptoms). This might be expected considering the poor Health-Related Quality of Life (HRQOL) metrics reported by respondents and consistent with generally held fact that treatment outcomes are generally poor in this disease. Although, the use of Complementary and Alternative Medicines which include dietary supplements and Chinese herbs in addition to behavioural treatments have been reported in 51% of IC/PBS patients surveyed in parts of the US (Anderson and Zinkgraf, 2013), the same cannot be said in the current sample where responses indicate little use of such treatments despite the scope for potential benefit.

Amitriptyline was the most prescribed medication in the sample. Previous surveys have also reported a preponderance of antidepressant usage for IC/PBS in the UK (Tincello and Walker, 2005). However, antidepressant use was not reported although other class members like imipramine have been shown to have good outcomes in disease treatment. Thus, it is not clear whether amitriptyline was the only medication used in that study. However, the result of an ICDB study supports the fact that amitriptyline is one of the more frequently prescribed treatments for this disease (Rovner *et al.*, 2000). It is worth mentioning that the clinical benefits of amitriptyline in this condition are not limited to its antidepressant actions because the prescribed doses (25-100 mg) are lower than those indicated for classical depression (van Ophoven *et al.*, 2004; Generali and Cada, 2014). It is thought that the pleiotropic effects of amitriptyline, which include muscle relaxant action (anti-cholinergic), mast cell degranulation inhibition, sedation and analgesia via neuronal inhibition play significant roles and make it an attractive treatment option for IC/PBS treatment (Baldessarini and Tarazi, 2001; Nishijima *et al.*, 2006). Another possible explanation for the widespread use of amitriptyline might be because of its tolerability at the lower doses used for IC/PBS treatment compared to the higher less well tolerated doses used to treat depression. In support of this a multi-centre randomised control trial in IC/PBS patients by Foster Jr *et al.* (2010) showed a non-significant difference (p=0.12) between amitriptyline and placebo in terms of adverse effects.

Lansoprazole and ranitidine were reported by the study cohort to be used for IC/BPS management. Indeed, cimetidine is recommended by most guidelines as a third line treatment for IC/BPS. However, such an indication is less a class effect than a rather generic effect of cimetidine as a drug molecule. Consequently, the use of either lansoprazole or ranitidine can't be substantiated despite their working via the same mechanism of gastric acid regulation as Proton Pump Inhibitors (PPI). The evidence in support of their usage for IC/PBS is just anecdotal. This is because cimetidine possesses mast cell stabilising effects which are lacking in older PPI congeners such as lansoprazole and ranitidine. Having said that, the studies reporting the benefits of cimetidine are weakened by poor design typified by low sample sizes and the absence of control arms (Meares, 1987; Lewi, 1995). One factor that may explain the high use of Cimetidine in the current patient sample is that it is now available as an over-the -counter-drug (OTC), and so can be used without prescription.

Despite weak evidence support the use of hydroxyzine in IC/PBS by the Royal College of Obstetricians and Gynaecologists (RCOG, 2017), it was used sparingly by the cohort. Hydroxyzine is conventionally used as an anti-allergic drug being a sedating anti-histamine but has been found useful in IC/PBS due to its mast cell stabilizing actions (Minogiannis *et al.*, 1998). In an open label uncontrolled case reports of 90 IC/PBS patients, the drug reduced symptom scores by 40% mainly due to its mast cell inhibitory actions on degranulation (Theoharides and Sant, 1997). Moreover, a pilot study to compare the effectiveness of PPS and hydroxyzine preparatory to a large multi-centre RCT showed non-significant differences (P=0.26) in the Global Response Assessment (GRA) score in the two arms (Sant *et al.*, 2003b). It is rather surprising that, despite weak evidence for the use of this agent as second line treatment that it has featured second to PPS in this survey. This suggests a possible untapped potential of this agent.

Somewhat alarmingly high rates of antibiotic use are reported by the respondents for IC/PBS despite weak evidence for this practice. Although the use of antibiotics in IC/PBS has significantly reduced compared to what was earlier reported by Tirlapur and Khan (2016), quinolones, penicillin and tetracycline were the major antibiotics reported to have been used in the current survey. So far, the justification for antimicrobials in IC/PBS is poorly supported by available empirical evidence. A small pilot study to evaluate the effectiveness of some antimicrobials over placebo for IC/PBS was performed by Warren *et al.* (2000). In this double-blind randomised placebo-controlled study, participants either received antibiotics (n=25) (rifampin, ciprofloxacin, doxycycline, erythromycin, metronidazole clindamycin, amoxicillin, or fluconazole) each in three-weeks consecutively and/or placebo (n=25) during the 18-week study period. The primary endpoint was symptom resolution. A non-significant improvement in symptoms resolution was recorded in the antibiotic arm compared to placebo (P=0.14) with side effects being higher in the antibiotic group (P≤0.05).

Despite this Hanno *et al.* (2010) in a review for the International Consultation for Incontinence (ICI) guideline enlisted doxycycline as one of the few oral agents that may be considered for IC/PBS. Potentially, the risk of antimicrobial resistance outweighs any anticipated benefits of symptom improvement. Consequently, antibiotic therapy should be discouraged due to likelihood of emergence of resistant strains and is an example of poor antibiotic stewardship at a time when inappropriate use of antimicrobials has resulted in a sharp rise of pathogenic urinary strains in some parts of England (Ironmonger *et al.* (2015).

Gabapentenoids and their congeners have been observed to have been consumed sparingly in the study sample. Gabapentenoids are indicated for neuropathic pain and related conditions due to plausible CNS involvement (Ke and Kuo, 2015; Patnaik *et al.*, 2017). The mechanism of action of Gabapentin is through increasing the inhibitory neurotransmitter GABA at post synaptic fibres and inhibiting calcium currents (Sills, 2006). It was reported to have shown significant reductions in visual analogue scale pain scores at both 12 and 24 weeks from baseline when compared to placebo in a randomised double blind control trial of CPP patients (AbdelHafeez *et al.*, 2019). The basis for its indication in IC/PBS is somewhat flawed since it is a different disease condition.

In respect to opioids, a slight shift in increased usage of opioids was documented in the sample (Table 2 and table 3). Recently, Zillioux *et al.* (2020) reported an exponential consumption of opioids amongst IC/PBS patients. This, together with our findings, suggests a gap in pain management in this patient group. Whilst the use of opioids outside cancer especially in those with IC/PBS is still under consideration (Nickel, 2006) there is a clear desire to optimise non-opioid pain management in IC/PBS using existing or novel (e.g., cannabinoid) drugs. One of the major limitations of our study is that participants were not probed further on how long they had been on opioids. Given the chronic nature of IC/PBS progression, the probability of chronic opioid consumption cannot be excluded. This raise concerns relating to tolerance, addiction, and misuse.

Whilst smooth muscle relaxants such as solifenacin have been tried in overactive bladder with modest outcomes (Aydoɬmuş *et al.*, 2014) there is no good evidence to justify their use in IC/PBS.

Overall, there was a trend towards the use of out of guideline medications to treat this frustrating urologic condition. This suggests a frustration on the part of caregivers who need to address the pain experienced by the patients under their care. The best we can offer is to try to provide an evidence base for the use of such medications using randomised controlled trials where this is possible or well-designed observational studies where this is practical.

Surprisingly, a subset of the participants indicated they are using herbal formulae and cannabis oil to control treat their disease.

This is an intriguing finding and suggests desperation on the part of the patients to get effective treatments for their urologic condition. Recently, Hassani *et al.* (2021) reported the preference of IC/PBS patients to try alternative medicine in a focus group study. Indeed, natural products are considered important sources of new treatments in various disease areas (Newman and Cragg, 2016). Although herbal formulae have been reported in a case report to improve symptoms of the disease (Taylor, Casteleijn and Gerontakos, 2018), scarce resources exist to demonstrate their effects in a well-designed experimental study. Importantly, cannabinoids, active compounds found in *Cannabis sativa,* have been shown to provide significant improvement in pain reduction compared to a placebo in a meta-analysis when used for chronic pain. (Yanes *et al.*, 2019). Given this, it is not surprising that cannabis oil is tried by some patients to control their IC/PBS symptoms. As IC/PBS is a disease with an inflammatory basis, it is reasonable to search for natural products with ethnomedical claims for chronic inflammation with a view to test them on models of IC/PBS with the aim of translational use in IC/PBS if outcomes seem favourable.

4.8.4 The O'Leary/Sant and PUF Scores

The PUF score assesses dyspareunia, amongst other symptoms, in patients with LUTS in addition to being used as a diagnostic and treatment monitoring tool in IC/PBS (Brewer *et al.*, 2007). The relationship between age of respondents, dyspareunia, symptoms, and disability in the sample were weak and inversely related. The reason for the inverse relationship between age and the PUF score could be explained by the fact that post-menopausal women are less likely to report dyspareunia with increasing age due to lower secretion of sex hormones and less sexual intercourse resulting in less dyspareunia. Although age distribution within the sample is symmetrical in respect to the PUF scores that may explain the reason why this relationship is observed in only 3.7% of the cohort since other participants are premenopausal. However, the low sample size of 36 limits generalisation of this findings. It is still unclear why more participants completed the O'Leary/Sant questionnaire in comparison to the PUF questionnaire. Probable explanation may be related to the face validity of the instrument as judged by these participants.

The relationship between pain sensitivity and ageing remains elusive with animal studies suggesting a linear and positive relationship whilst results in human studies suggest otherwise (Woodrow *et al.*, 1972; Jourdan *et al.*, 2000). When pain sensitivities are compared at extremes of ages, they are likely to have a positive relationship with pain, but the converse is true when all age groups are considered together. Not unexpectedly, O'Leary/Sant scores which assess pain in IC/PBS also produced a weak negative relationship. Possibly there may be down regulation of sensory nerve fibres with increasing age.

4.8.5 King's Health Questionnaire Outcomes

The King's Health Questionnaire (KHQ) has been used to assess HRQoL in LUTs, and urinary incontinence (Okamura, Nojiri and Osuga, 2009). The KHQ evaluates quality of life on a scale of 0-100, in a 21-item questionnaire spanning nine domains whilst the tenth domain, which assesses severity is scaled (0-30). In each case, lower values (0) correlate with a better QoL whilst higher values (100, 30) are associated with poorer QoL.

Table 4-13 shows that respondents rated their health to be poor. Apart from the General Health Perception and severity measures which were averagely scored at 40.6 and 27.83; all other domains were rated above 50 reflecting a marked deterioration of HRQoL in this patient group. The somewhat good scores of the severity measures should be interpreted with caution considering most items (pad usage, urinary odour, and change of underclothes) in this domain do not adequately capture IC/PBS symptoms but reflect more the problems relating to urinary incontinence as the KHQ was initially designed to assess this in part. The greatest contributor to poor quality of life in this cohort is the "sleep and energy" domain with a mean score of 68.2. This is because frequent urination especially at night causes sleep deprivation, which impacts negatively on energy and vitality. This result agrees with the PUF scores of participants which depicts bothersome frequency and urination as shown in Figure 4-3. Similarly, the symptom severity scale which has a ceiling value of 30, was scored at 11.61 translating to 38.7%. Lower ratings of this item were expected given that it consists of IC/PBS unrelated items such as bed-wetting, dribbling, urinary infections, and sexual incontinence, which are not classical symptoms of IC/BPS. The null response in these items could lower the cumulative weighting of this item. Despite, the utility of the KHQ tool in quality-of-life assessment, it is limited by the fact that some items such as bed-wetting and dribbling don't relate with IC/PBS.

The KHQ scores of the IC/PBS cohort in this study compared poorly with patients with multiple sclerosis confirming the poor HRQoL in these patients (Akkoc *et al.*, 2011). This is expected considering MS is not just a bladder-centric pathology, but rather CNS disorder with secondary urinary dysfunction. IC/PBS individuals have poor quality of lives in comparison to incontinent patients as assessed by this tool (Okamura, Nojiri and Osuga, 2009). This result agrees with the BIP-Q data (Figure 4-1 and Table 4-9) which show that HRQoL is significantly poor in this patient population and that this, in part, is due to absence of effective treatments for this disease.

The limitation of this study is that it provides only a time-prevalent data which may be subject to changes if taken at another period. Given that this data is consistent with the BIP-Q which is an entirely different tool in HRQoL assessment confirms the validity of this study outcome.

A major strength of this study is that the KHQ comprises a 21-item questions in several spheres of IC/PBS problematic themes that succinctly captured the QoL indices of IC/PBS patients, which makes the tool a holistic HRQoL evaluation assessor. Similarly, the tool is disease specific and has been used in different disease areas with both genders. In conclusion, the KHQ benchmarked HRQoL in IC/PBS individuals and provides solid data for future QoL analysis of this patient group.

4.9 Summary

The use of surveys to evaluate IC/PBS cohorts' is a valid tool especially in a disease with poorly developed diagnostic and prognostic tools. Participant evaluations can provide valid descriptions of treatment outcomes especially where they are used in lieu of invasive clinical techniques. We have, in this cross-sectional study, described the treatments of members registered with BHUK and living with IC/PBS in relation to diagnostic and therapeutic benchmarks.

Firstly, knowledge of disease classification into ulcer and non-ulcer is poor in about one-third of the participants. Key findings of this work also include poor ratings of HRQoL by participants. Notably, over 75% respondents can see no end to their symptoms.

The lack of belief that treatments will control disease symptoms and concern about the disease demonstrated are positive determinants of illness severity.

The consumption of beverages such as coffee, alcohol and tea were noted in the sample although, there were no significant differences between those consuming and not consuming these beverages with respect to O'Leary Sant scores. The ICSI and PUF scores are inversely related to the age of the respondents in more than 10% of the cases.

A history of pelvic trauma was identified in many of the respondents. Furthermore, cystoscopic examination under anaesthesia was the most common investigation participants underwent as an aid to diagnosis.

The ineffectiveness of current pharmacological interventions has been suggested by the fact that more than half of the sample were not on any treatment at all despite being symptomatic. The use of adjuvant medications was again observed in this sample. Treatments used in this sample shows disparity with the two only available national guidelines in the UK – from the RCOG and British Association of Urological Surgeons (BAUS) and in some instances agreement with other international guidelines such as those from the EAU, ESSIC, NIDDK and ICS.

The strength of this study is that it is adequately powered (adequate sample size) especially with respect to Illness perception and treatments used by respondents. Thus, firm hypotheses can be

generated which are generalisable and valid. A convenience sampling design was used, which is typical of cross-sectional studies, but participants diverse geographical distribution helps to counteract any bias arising from this.

In respect to the treatment's variables, the novelty in this survey lies in the multiple response pattern of the study questions that adequately capture all treatments in contrast to a single response questionnaire. Similarly, the "don't know, and others, and free text options" provided in the questionnaire makes it feasible to capture all the treatments used by the cohort and serves to adequately answer the research questions. In addition, participants were asked to provide the medications that had been used previously for their disease treatment, this helps to gauge ineffective medications as assessed by participants and possible attrition rates in treatments.

4.10 Conclusion

IC/PBS is a debilitating disease of bladder origin with a puzzling aetiology and poor prognosis. Despite wide arrays of treatments in the sample, it is noted that participants despair and experience hopelessness due to the ineffectiveness of available treatments. This is shown by the fact that mean O'Leary/Sant scores of the cohort (symptomatic) taking recommended treatment was not different from those who were not taking treatment.

Natural products have been identified as a new treatment strategy for IC/PBS. Thus, the development of targeted therapies using natural products with an ethnomedical claim for the treatment of inflammation are urgently needed.

Chapter 5 Medical Grade Manuka Honey and Degranulation of Mast Cells

5.1 Overview

It has been established in 4.10 that current available treatments for IC/PBS are largely ineffective in controlling symptoms. Similarly, natural products have been identified as hypothetical treatment options for this urologic condition.

Recently, manuka honey (MH) – a natural product - has been reported to have inhibitory effects on mast cells (Birch *et al.*, 2011a), which is an important therapeutic target for this disease. Medical grade manuka honey may also have pro-angiogenic and antibacterial effects, majorly due to its methyl glyoxal (MGO) composition, derived from metabolism of dihydroacetone in the nectar of Manuka trees (El-Senduny *et al.*, 2021). MGO is an important metabolite in determining the biological activity of MH especially antibacterial effects and inactivation of pro-inflammatory proteins (Majtan *et al.*, 2012).The dual antimicrobial and anti-inflammatory properties of this agent could be exploited in the management of IC/PBS since UTIs may coexist with the disease (Rossiter *et al.*, 2006; Nooh and Nour-Eldien, 2016). Mast cell modulators offer huge potential in the treatment of IC/PBS considering the role of neurogenic inflammation in the disease pathogenesis. More importantly, the widespread use of mast cell modulators has been observed in the previously reported survey with the more common preparations used being amitriptyline, cimetidine, and hydroxyzine (see 4.4).

How manuka honey mediates this effect is still unclear. The release of mediators from mast cells is achieved by either IgE or non-IgE mediated signalling pathways. G-Protein Coupled Receptor (GPCR) pathways mediate mast cell degranulation secondary to substance P (SP) challenge. Importantly, SP a neuropeptide released from sensory nerve endings and acts on both NK1 and NK2 receptors and the MRGPX2- GPCR receptor super family (McNeil *et al.*, 2015).

In IC/PBS in particular, activation of sensory nerve endings causes the release of SP which in turn activates bladder mast cells culminating in the inflammatory phase of the disease process. In this project, the signalling of GPCR via substance P (SP), and the non-immunologic activation of LAD2 cells will be investigated. The aim of the work is to investigate the effect of medical grade manuka honey (MH) in a cellular model of IC/PBS and determine how it affects critical downstream signalling proteins that are responsible for neural plasticity and inflammation. Thus, the effect of medical

grade manuka honey (MH) on degranulation and the MAPK and Akt signalling pathways will be investigated in IgE and non-IgE mediated stimulation.

5.2 Materials and Methods

5.2.1 Chemicals/Reagents/Drugs

All chemicals and reagents were of analytical and molecular grades. Stock solutions were made from appropriate diluents as directed by manufacturer(s). Solutions for cell culture were prepared from de-ionised water and ultra-pure water prepared using the Reverse Osmosis System Milli Q ultrapure water system apparatus for all other cell culture studies and ELISA respectively (Millipore, Billerica, USA).

Agents used include amitriptyline (Sigma), cimetidine (Sigma), hydroxyzine (Sigma), Radio immunoprecipitation assay (RIPA) buffer (cell signalling), leupeptin, sodium orthovanadate (Sigma), sodium pyrophosphate (Sigma), methanol, 10-DEBC, FR180204, GSK, glycine, Substance P (Sigma), Triton X (Sigma), Ethylene diamine tetra acetic acid (EDTA) (Sigma), Ethylene diamine glycolic acid (EGTA) (Sigma), Phenyl methyl sulphonyl fluoride (Sigma) sample buffer (Sigma), Tris HCl (Sigma), NaCl (Sigma), NaoH (Sigma), Citric acid (Sigma), Sodium bisphosphate (Sigma), Phosphate buffer saline (Sigma), Tween 20 (Sigma), leupeptin, Bovine Serum Albumin (Sigma), non-fat dry milk, running and transfer buffer (Sigma), electrophorator (Biorad), Nitrocellulose, pierce western blotting substrate (ThermoFisher), blotting paper, cassette, 4-12% precast gels (Sigma), Eazblue gel staining reagent (Sigma), LDS sample buffer (Sigma), pre-stained page ruler (Sigma), cytotoxicity detection kit-LDH (Roche), cysteinyl leukotrienes kit (Cambridge bioscience), histamine assay kit (Veratox), medical grade Manuka honey (Comvita, New Zealand Ltd)

5.2.2 Cell culture and antibodies

Agents used included penicillin 100U/ml, Streptomycin 100ug/ml, Stem Cell Factor (Peprotech), Stem Pro medium (ThermoFisher), beta actin Rabbit mAB (Cell signalling), Human p-Akt rabbit mAB (cell signalling), Human p-ERK1/ERK2 rabbit mAB (R and D system), p38 rabbit mAB (R&D System), Human MRGX2 (R and D system), anti-rabbit IgG HRP-linked (Cell signalling technology), Human myeloma IgE (Sigma), Anti-IgE, L-glutamine-Penicillin-Streptomycin (Sigma).

Laboratory of Allergic Disease 2 (LAD2) cells were gifted from Dr Arnold Krishenbaum of the National Institute of Allergic Disease, USA. The cells were maintained in Stem pro-34, equivalent of

foetal bovine serum (10 ml/l), supplemented with Stem Cell Factor (100 ng/ml), penicillin (100U/ml) and streptomycin (100U/ml). Cells were maintained at 70-80% confluency at a density of $0.5X10^6$ cells/ml by passaging every 10-14 days using hemi-depletion. Penicillin-streptomycin, SCF and growth medium were aliquoted and stored at -20°C. Aliquots of each were allowed to thaw at room temperature each time before use. LAD2 cells being a suspension were dislodged every two days to prevent the contact inhibition which slows cell division.

5.2.3 Cell counting

A 10 µl aliquot of the cell suspension was pipetted on an improved Neubauer haemocytometer after being thoroughly cleaned with water and dried. Viable cells were counted in four quadrants of the counting chamber and the average count taken. Cell number per ml was obtained by multiplying the average cell number by a dilution factor (10) and a correction factor (10^4).

5.2.4 Cytotoxicity assays

A range of cellular tests was used to investigate the toxicity of medical grade manuka honey and all other potential modulators (amitriptyline, hydroxyzine, and cimetidine) used in the cell culture at various doses (0.1 µM, 1 µM and 10 µM) of modulators and concentrations (2% w/v, 4% w/v, 6% w/v and 8% w/v) of manuka honey. Some of these included: β-hexosaminidase release, trypan blue exclusion and a lactate dehydrogenase assay. These are outlined below.

5.2.4.1 Beta hexosaminidase release

This is based on the principle that levels of this cytoplasmic enzyme are elevated in comparison to control when cells are exposed to a cytotoxic dose of investigational drug. MH was prepared at the concentrations of 2%, 4% and 8% whilst competitive modulators of mast cell functions were prepared at 0.1 µM, 1 µM and 10 µM. LAD2 cells were pre-incubated with these drugs for 30 minutes at 37°C, 5% CO_2 in a V-bottom 96-wells cell culture plate. The plate was centrifuged at 225 g at 4°C for 10 minutes. A 30 µl of the supernatant was transferred into a 96-well flat-bottom reading plate. Then a 50 µl of a β-hexosaminidase substrate (p-nitrophenyl-N-acetyl-β-D-glucosaminide, in 0.1M citrate buffer pH 4.5) was added to the supernatant for 60 minutes. The reaction was stopped by addition of 100 µl 0.1 M glycine. Absorbance was read at 410 nm on a plate reader.

Equation 1

$$\textbf{Net release} = \frac{\textbf{sample} - \textbf{spontaneous}}{\textbf{total}} \textbf{x } \textbf{100}$$

5.2.4.2 Trypan blue exclusion assay

This is based on the principle that viable cells have intact cell membrane, hence they do not take up the trypan blue dye, while a defective membrane allows the passage of trypan blue highlighting the cytolitic effect of investigational new drugs. LAD2 cells were pre-treated with Tyrodes buffer (vehicle) and 2%, 4%, 6% and 8% w/v concentrations of manuka honey as described above for 30 minutes in a humidified cell culture incubator at 37^{o}C and 5% CO_2. The 0% served as negative control.

After incubation, cells were washed with equal volume of PBSX1, and the resultant cell suspension centrifuged at 5000 g at 4 ^{0}C for 5 minutes. The cell pellets were re-suspended in Tyrodes buffer. A 1:1 ratio of cell suspension and trypan blue was gently mixed and a 10 µl of the homogenous suspension was put on a haemocytometer and the proportion of viable and dead cells counted for each of the treatments. Experiments were performed in duplicate and repeated three times

5.2.4.3 Lactate dehydrogenase assay

This assay assesses the effect of medical grade manuka honey (MH) on the membranes of LAD2 cells. Lactate dehydrogenase is a metabolic product that is released upon damage to the cell membrane. It is mostly employed in assessing the cytotoxic effect of new investigational agents. Consequently, it was used here to determine the effect of MH honey on the release of lactate dehydrogenase from LAD2 cells. Approximately 5X10^{4} LAD2 cells were pre-treated with vehicle (Tyrode's buffer), 2%, 4%, 6% and 8% concentrations of manuka honey as described above for 30 minutes in a humidified cell culture incubator at 37^{o}C and 5% CO_2. The vehicle served as negative control. After incubation, cells were washed with equal volume of PBSX1, and the resultant cell suspension centrifuged at 5000 g at 4 ^{0}C for 5 minutes. The cells pellets were re-suspended in Tyrodes buffer. A background control consisted of tyrodes buffer alone in the wells; the low control was a tyrodes buffer and cell suspension in 1:1 ratio while the high control (+ control) was a cell suspension and 1% triton (final concentration) in a 1:1 ratio. To the resuspended cells in Tyrodes buffer 100 µl of freshly prepared reaction mixture was added and the plate was incubated for 30 minutes protected away from light. The reaction is a colorimetric assay, a maroon colour developed which was proportional to the amount of lactate dehydrogenase released. Absorbance was read at 490 nm; the reference wavelength being set at 595 nm. Net release was calculated by the formula in Equation 2 after correcting for background control in each case.

Equation 2

- **LDH net release= (sample-low control)/(High control) x 100**

5.2.5 Degranulation assays

Various assays are used to investigate degranulation in cell culture studies. The most widely used are beta hexosaminidase and histamine release. These two were used in this study.

5.2.5.1 Beta hexosaminidase assay

Beta hexosaminidase is a catalytic enzyme used as a marker of mast cell degranulation (Fukuishi *et al.*, 2014). It is the most cost-effective method for measuring mediator release.

However, it requires large numbers of cells in comparison with other mediators such as histamine. Another, advantage of β-hexosaminidase is the cost effectiveness of the assay reagents.

About $1X10^5$ cells/ml were pre-treated with vehicle, 2%, 4%, 6% and 8% w/v concentrations of MH as described above for 30 minutes in a humidified cell culture incubator at 37^{o}C and 5% CO2. Meanwhile, total cell mediator released was obtained by treating the cells with Triton-X 1% Phosphate Buffer Saline PBS (Total). The cells in plates were challenged with Substance P, Anti-IgE (cells were pre-incubated with myeloma IgE for 24 hours) or A23187 for 40 minutes. The solution in buffer (Tyrodes buffer) served as negative control. After incubation, the plate containing cells was centrifuged at 225 g at 4 ^{0}C for 10 minutes. The percentage of released of β-hexosaminidase was calculated in Equation 3.

Equation 3

- **Net release= (sample-spontaneous)/total x 100**

5.2.5.1.1 IgE assays

For all IgE assays, an appropriate number of LAD2 cells were centrifuged at 225 g at room temperature for 10 minutes. Cells were re-suspended in half-strength medium and incubated with human myeloma IgE in a humidified cell culture incubator at 37^{o}C and 5% CO_2 for 24 hours. Cells were collected and washed with PBSX1 for 10 minutes then pre-treated with inhibitors or MH for 30 minutes in a humidified cell culture incubator at 37^{o}C and 5% CO_2. Treatments were challenged with sheep anti-human IgE for 40 minutes as described in 5.2.5.1

5.2.5.2 Histamine release assay

This is based on the principle that histamine is an important autocoid released after mast cell degranulation. So far, all the treatments that attenuate mast cell function act partly through

competitively inhibiting histaminergic action. Histamine release assays are fast, reliable and require low cell number but are expensive. Histamine ELISA (Neogene) was used for this assay.

About 7.5X0^4 LAD2 cells/ml were pre-treated with 0%, 2% and 4% Manuka honey and incubated in a humidified cell culture incubator for 30 minutes at 37^{o}C and 5% CO_2. Total cell mediator released was obtained by treating the cells with Triton-X 1% PBS. After incubation, the plate containing cells was centrifuged at 225 g at 4 ^{0}C for 10 minutes.

A 100 µl of the supernatant was used for histamine release assay according to manufacturer's instruction. The amount of histamine release (ppm) in each case was calculated.

5.2.5.3 Cysteinyl leukotrienes release assay

Cysteinyl leukotrienes are group of mediators released by the metabolism of arachidonic acid and contribute to the inflammation cascade in many immunologic reactions particularly allergic asthma.

Approximately 7.5X10^4 LAD2 cells/ml were pre-treated with vehicle (Tyrode's buffer), 2% and 4% MH and incubated in a humidified cell culture incubator for 30 minutes at 37oC and 5% CO_2. Total cell mediator released was obtained by treating the cells with Triton-X 1% PBS. After incubation, the plate containing cells was centrifuged at 225 g at 4 ^{0}C for 10 minutes.

A 50 µl of the supernatant was used for histamine release assay according to manufacturer's instruction. The quantity of Cysteinyl leukotriene release (pg/ml) in each case was calculated

5.2.6 Cytokine release assay

About 1X10^6 LAD2 cells/ml were pre-treated with vehicle, 2% and 4% MH and incubated in a humidified cell culture incubator for 30 minutes at 37oC and 5% CO_2. Total cell mediator released was obtained by treating the cells with Triton-X 1% PBS. After incubation, the plate containing cells was centrifuged at 225 g at 4 ^{0}C for 10 minutes. The cells in Eppendorf tubes were challenged with Substance P or A23187 for 24 hours. Tyrodes buffer served as negative control. After incubation, tubes containing cells were centrifuged at 225 g at 4 ^{0}C for 10 minutes.

100 µl of the supernatant was used for cytokine release assay according to manufacturer's (R & D system) instruction.

5.2.7 SDS PAGE Western blotting

This assay was designed to detect the signalling pathways through which mast degranulation and cytokine suppression were affected by manuka honey (MH). Protein kinase B (Akt) and Mitogen Activated Protein Kinase (MAPK) were exclusively studied in both SP and A23187 models.

5.2.7.1 Sample preparations

Cell suspensions (the number of cells being optimised at 1 x 10^6 after trying different cell numbers) were treated with Tyrodes buffer (negative control) or MH (2%, 4% and 6%) and incubated for 30 minutes after which they were challenged with SP, the concentration and time after challenge were optimised at 3 µM and 0.3 µM and 15 minutes for SP and A23187 respectively. Reaction was stopped by addition of equal volume of cold PBSX1, and the resultant mixture centrifuged at 5000 g for 5 minutes at 4^{o}C, the procedure was repeated twice, and lysis buffer added. The type of lysis buffer used was optimised to RIPA in 1 µM PMSF.

Samples were then vortexed for 3 minutes and sonicated on a water bath for 30 secs, the procedure was repeated twice. Samples were cooled on ice for two minutes and sonicated for 15 secs 3 times each with a 30 sec pulse interval.

5.2.7.2 Protein assay

The Pierce BCA reagent was prepared according to manufacturer's instruction in which 1 part of B was added to every 50 parts of A and the greenish solution was allowed to roll over for few minutes at room temperature to obtain a homogeneous solution - BCA reagent. Meanwhile, 190 µl BCA reagent was added to 10 µl of samples and serial dilutions of protein standard Bovine Serum Albumin (BSA) in a 96-well plate, and the plate incubated at 37^{o}C for 30 minutes. Absorbance was read at 595 nm and protein concentration of the samples were extrapolated from the standard.

5.2.7.3 PAGE

Samples were cooled on ice for 30 minutes and then added to 1x sample buffer, they were then heated on a hot plate for 5 minutes at 95^{o}C and cooled on ice for two minutes. Samples were centrifuged at 1000 g for 2 min to remove cellular debris.

An 8-12% Bis-Tris 17-well precast gels was arranged on a biorad electrophorator. Running buffer prepared as per manufacturers (direction) was added to the inner chamber of the apparatus set-up and allowed to stand for few minutes to check for any leakages if any. Thereafter, the running buffer was added to the set up to more than half of the apparatus height and to cover the precast gel at the inner chamber. Approximately 25 µg each of the sample, 5 µl each of MagicMark XP

Western Protein Standard (ThermoFisher) and Page Ruler Pre-stained NIR protein ladder (ThermoFisher) were added to wells as per each experimental template. The set-up was connected to a power source and run at 180V keeping the current constant for 90 minutes on average or when the process completes.

5.2.7.3.1 Coomassie staining

The same sample was subjected to electrophoresis, gel obtained was immersed in a tray containing Coomassie stain (Sigma). This was heated for 15 secs and allowed to rock gently for 10 min. This was then de-stained with de-staining reagent (50 ml methanol, 50 ml acetic acid and 400 ml H_2O). This was left overnight, and the de-staining solution drained, and gel observed.

It was evident that there was abundant protein in the sample. The protein assay was determined by both Pierce BCA and Bradford assay and Pierce BCA was chosen as the assay of choice due to its wider detection limit (20-2000 µg/ml) and consistency in contrast to the Bradford assay.

5.2.7.4 Blotting

Gel was removed with the aid of a knife from the precast gels. A sandwich was made on a transfer hinged cassette thus: A sponge, blotting paper, gel, nitrocellulose membrane, blotting paper and a gel in that order all in scotch-brit pads on a hinged cassette. A roller was gently used to roll over the sandwich to remove air bubbles that might interfere with the protein transfer. A transfer buffer solution was prepared according to manufacturers (Sigma) instructions. The sandwich in the cassette was put inside a transfer tank with the proximal gel end put to the anode while that of the membrane facing the cathode and transfer buffer added to cover the cassette completely. The transfer tank was connected to a power source (Biorad). The set-up was run at 90V for 1 hour and 100V for another hour after which the transfer was complete. Blotting was confirmed by staining the membrane in Ponceau S and the gel with Eazblue staining reagent (Sigma) each for 5 minutes and washed thoroughly with de-ionised water to see the presence of bands in the former and absence in the latter.

5.2.7.5 Blocking

The membrane was placed in either 5% BSA or non-fat dry milk to block non-specific proteins. This experiment was optimised for 5% non-fat dry milk. The membrane was put in a solution of 5% non-fat dry milk in washing buffer (TBSTX1). It was allowed to roll for one hour at room temperature. Thereafter, the membrane was washed three times each for five minutes.

5.2.7.6 Incubation

An appropriate dilution of the primary antibody was made in 5% BSA or 2% non-fat dry milk (according to manufacturer's instructions). The solution containing the primary antibody was added to the membrane in a 50 ml tube which was allowed to roll at 4^{o}C for 24 hours. A solution containing primary antibody was discarded and then washed three times each for five minutes. An appropriate dilution of the secondary antibody was made in either 5% BSA or non-fat dry milk depending on manufacturer's instructions. It was added to the tube containing the membrane and allowed to roll for one hour at room temperature. The solution was discarded and washed three times each for 5 minutes

5.2.7.7 Detection

A 500 µl sample of each of the two components of Pierce ECL Western blotting substrate (ThermoFisher) was thoroughly mixed and incubated with the membrane for 1 minutes, the solution was drained and put in between two clean photographic film papers. The membrane was visualised on Chemidoc (Biorad) and images taken.

5.2.7.8 Reprobing

On some occasions, the membrane was stripped with a stripping detergent for 15 minutes rolled at room temperature and the content drained and washed with PBSX1. The membrane was blocked, incubated, and detected as detailed above.

5.2.7.9 Statistical analyses

All experiments were repeated at least three times unless otherwise stated. Bar charts and line graphs were used to present data. Statistical analysis was performed using Graphpad prism 9.2.0 for Windows, San Diego, California, USA (www.graphpad.com). One way ANOVA was used to compare means of multiple groups and the p value was set at a significance level of $p \leq 0.05$.

Chapter 6 Results and Discussion for Degranulation Studies

6.1 Results of Cytotoxicity Assays

Basal beta hexosaminidase release, exclusion of Trypan blue dye and lactate dehydrogenase assay results are used as markers of cytotoxicity.

6.1.1 Lower concentrations of manuka honey do not elicit spontaneous degranulation

Medical grade manuka honey (MH) at 2% and 4% were tolerated by LAD2 cells (See Figure 6-1). A 6% MH concentration caused modest degranulation at baseline. Similarly, there were no changes between spontaneous release of β-hexosaminidase and that of hydroxyzine, cimetidine, and amitriptyline (0.1 µM, 1 µM and 10 µM); this showed that these doses were non-cytotoxic to the LAD2 cells (Figure 6-1). Thus, these doses of drugs and concentrations of MH can be used for degranulation and signalling assays.

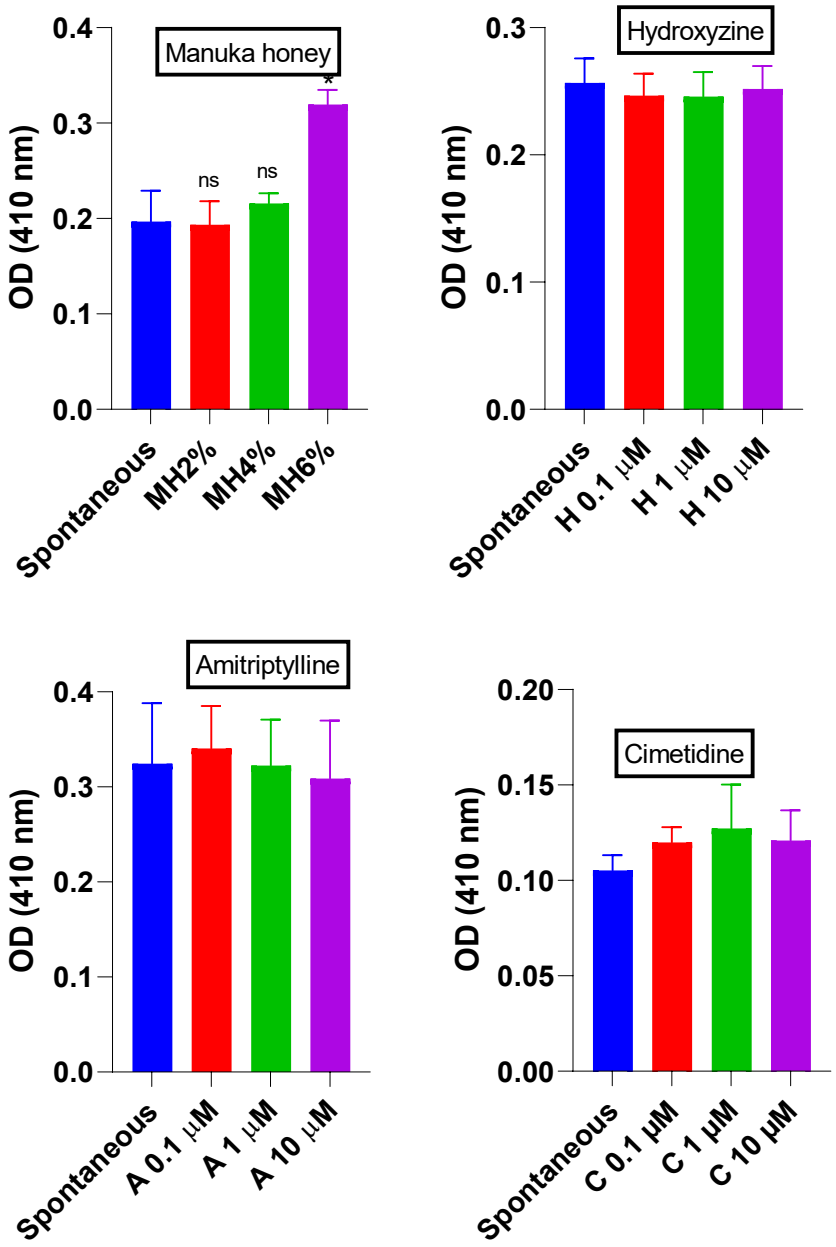


Figure 6-1: Effect of MH and drugs on basal release of β-hexosaminidase.

Basal release of beta hexosaminidase after pre-treatment with a manuka honey, b (hydroxyzine), c (amitriptyline), d (cimetidine) for 30 minutes. Absorbance was read on a plate reader at 410 and 595 nm. Data is presented as mean ± SEM. One way ANOVA was used to compare means from spontaneous group followed by Dunnett's post hoc test. Value of p was set at ≤0.05. In each case n=3. *p = 0.05.

6.1.2 Manuka honey at all concentrations tested are not taken up by the trypan blue dye

From Figure 6-2, there were no significant differences seen between medical grade Manuka honey (MH) at 2%, 4% and 6% when considering cytotoxicity using the spontaneous release of β-hexosaminidase or the Trypan blue method; suggesting these concentrations were not cytotoxic to the LAD2 cells. The higher concentration of MH 8% demonstrated significant ($p \leq 0.05$) cytotoxic action on LAD2 cells. Although, in the basal degranulation assay (Figure 6-1) MH 6% demonstrated significant release of β-hexosaminidase at baseline. However, in this method MH 6% was well tolerated by the cells. This reflects the differences in the biochemical endpoint assayed in each of the methods.

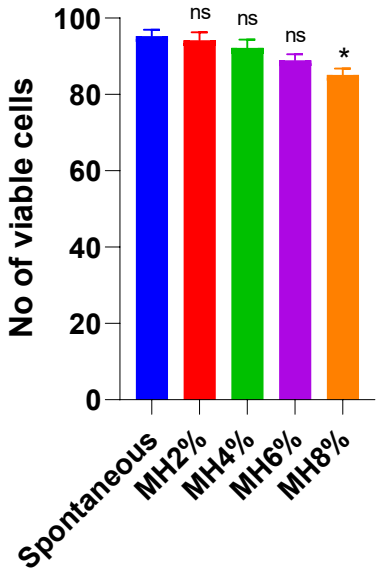


Figure 6-2: Effect of MH on viability of cells by trypan blue exclusion method.

Effect of various honey concentrations on trypan blue dye cell viability. Viable cells were counted under a light microscope under 10X. Experiment was carried out in triplicate. Data is presented as mean ± SEM. One way ANOVA was used to compare means from spontaneous group followed by Dunnett's post hoc test. Values of p was set at ≤0.05. In each case n=3. *≤0.05.

6.1.3 Manuka honey at lower concentration does not elicit the release of lactate dehydrogenase

Another commonly used marker of cell cytotoxicity to assay investigational drugs is the LDH assay – a cell membrane enzyme that is released upon cellular damage. Both 2% and 4% manuka honey concentrations were shown not to evoke the release of lactate dehydrogenase compared to the control group. However, 6% and 8% MH caused significant release of the enzyme suggesting that both concentrations are cytotoxic. This result agrees with the basal degranulation data (Figure 6-1). Thus, MH 2-4% are tolerated by the LAD2 cells.

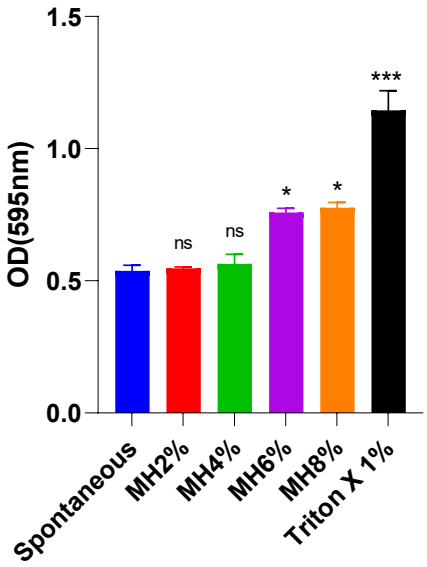


Figure 6-3: Effect of MH on the release of lactate dehydrogenase enzyme
Data were expressed as mean OD ± SEM. A one-way ANOVA was used to compare differences of treatments with spontaneous group followed by Dunnett's post hoc test. Value of P was set at ≤0.05. Experiment was done in triplicate and n=3. *≤0.05, ***=0.000, ns= non-significant.

6.2 Results of Degranulation Assays

The effect of manuka honey and inhibitors were studied following substance P (SP), IgE and A23187 challenge. The results of these assays are presented below.

6.2.1 Comparative effect of manuka honey and mast cell modulators on SP mediated stimulation

This assay was aimed at comparing the effect of MH and mast cell modulators commonly used to treat the disease as shown in Table 4-11. Manuka honey at 2% and 4% significantly inhibited β-hexosaminidase release from baseline. These degrees of inhibition were greater than that seen with amitriptyline and cimetidine but comparable to that seen with hydroxyzine (See Figure 6-4).

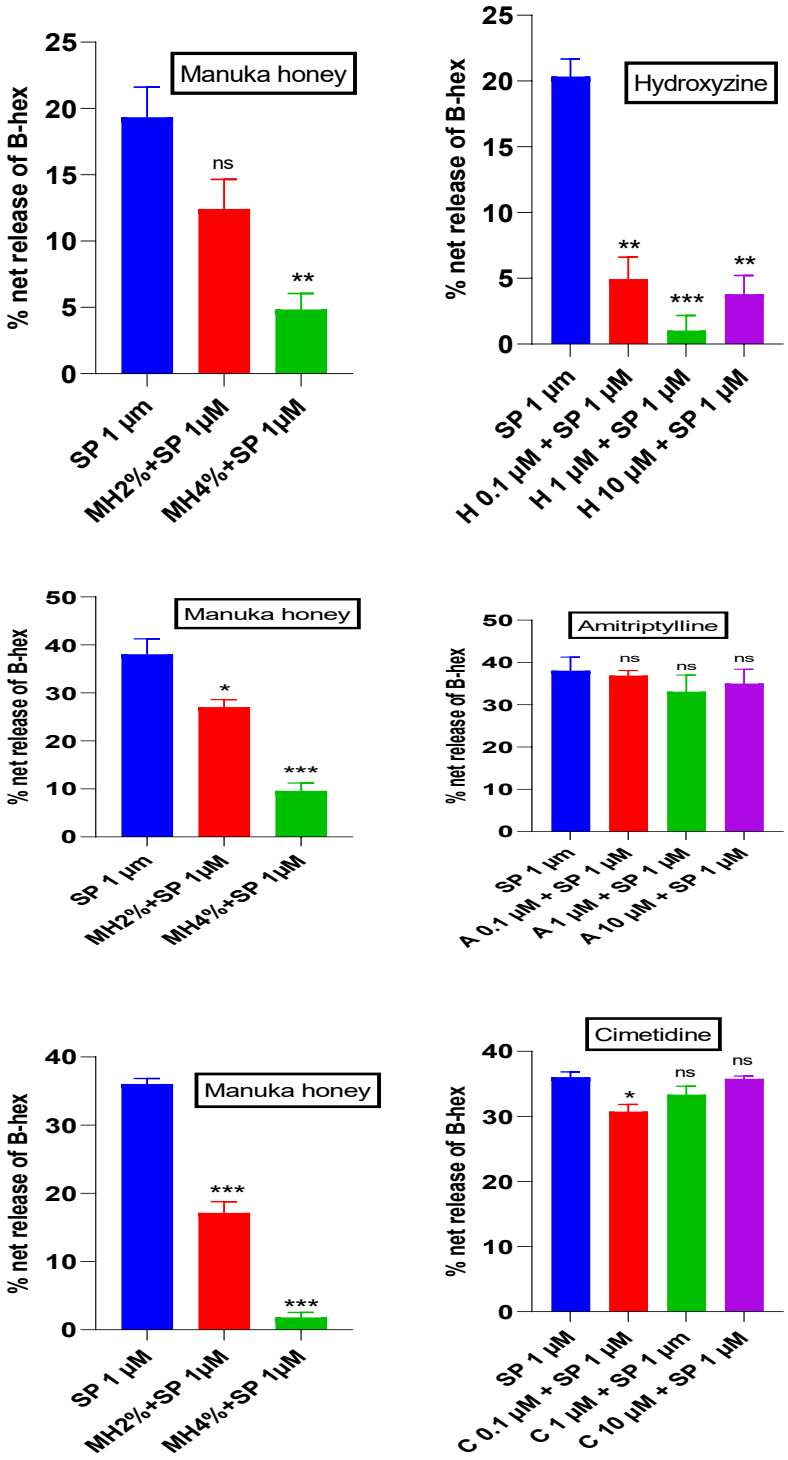


Figure 6-4:Comparative effect of MH and hydroxyzine; MH and amitriptyline; MH and cimetidine on the release of β-hexosaminidase.

LAD2 cells were pre-treated with graded concentrations of manuka honey (MH) or graded concentrations of MH for 30 minutes and stimulated by substance P 1µM. Absorbances were read on a plate reader at 410 and 595 nm and % release obtained. Net release of spontaneous group was null. Data is presented as mean ± SEM of the % inhibition n=3. Experiments were repeated three times. A one-way ANOVA was used to compare differences of treatments with SP group followed by Dunnett's post hoc

test. Value of P was set at ≤0.05. Experiments were done in triplicate and n=3. *≤0.05, ***=0.000, ns= non-significant.

6.2.1.1 Effect of manuka honey and signalling inhibitors on the release of beta hexosaminidase

Given that Akt and ERK are important signalling proteins that regulate degranulation. The effect of MH on the release of β-hexosaminidase release was compared with that of known inhibitors of Akt (10-DEBC) and ERK (FR-120804). At the concentrations tested (MH 2-4%) was comparable to Akt and ERK inhibitors 10-DEBC and FR-120804 respectively in inhibiting β-hexosaminidase release (See Figure 6-5).

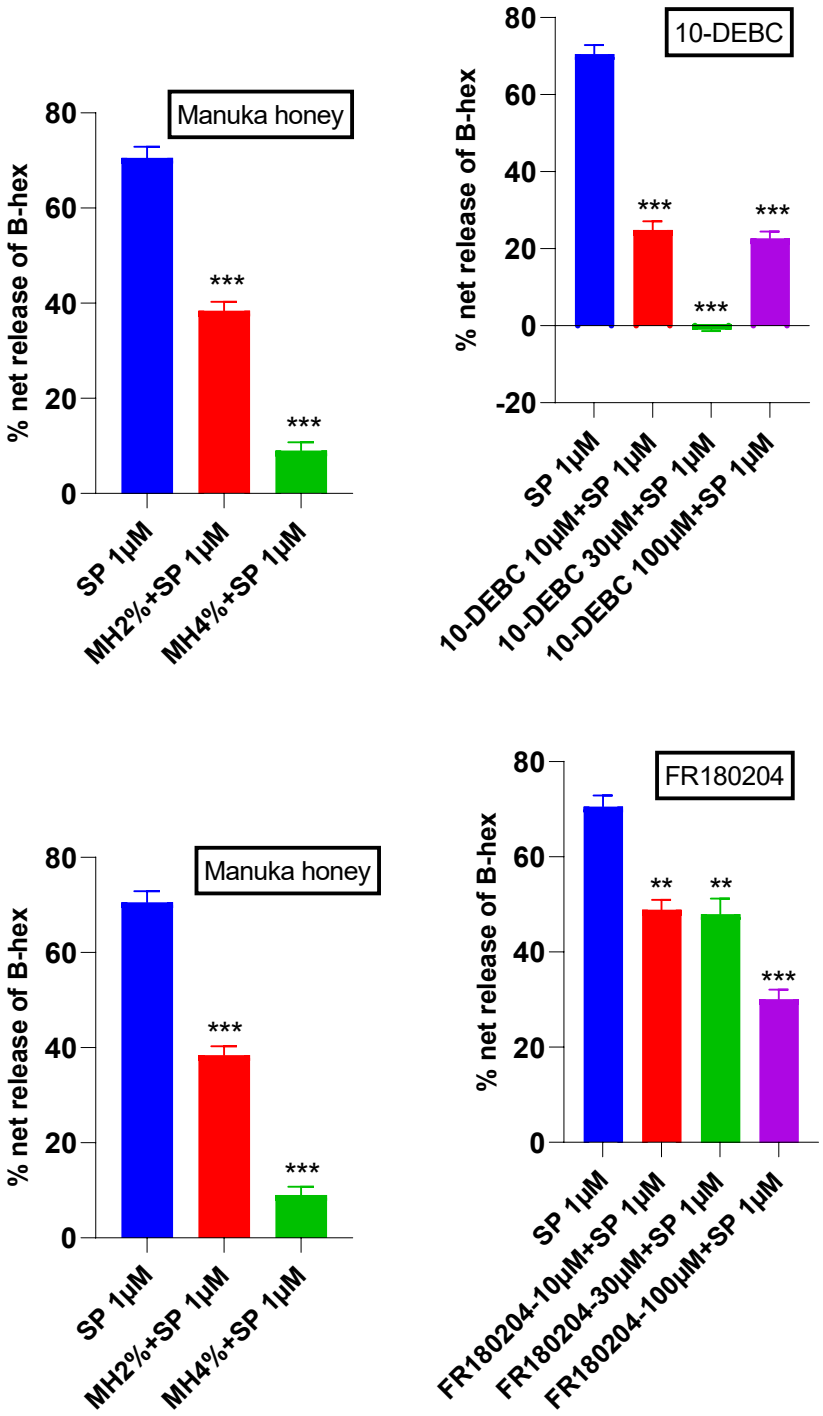


Figure 6-5: Effect of MH and signalling inhibitors on the release of β-hexosaminidase. Comparative effect of MH and 10-DEBC; MH and FR180204 on the release of β-hexosaminidase.

LAD2 cells were pre-treated with graded concentrations of manuka honey (MH) or graded concentrations of FR180204 (ERK inhibitor) for 30 minutes and stimulated by Substance P 1µM. Absorbance was read on a plate reader at 410 nm and 595 nm and % release obtained. Data was presented as mean ± SEM of the % net release n=3. Net release of spontaneous group was null. A one-way ANOVA was used to compare

differences of treatments with SP group followed by Dunnett's post hoc test Experiment was repeated three times. *≤0.05, ***=0.000, ns= non-significant.

6.2.1.2 Effect of medical grade manuka honey on histamine release

Histamine is an important mediator of inflammation and mediates the painful nociception in this disease. The effect of MH on the release of histamine was investigated using a histamine assay kit ELISA. MH demonstrated significant (p≤0.05) suppression of SP mediated histamine release at both 2% and 4% compared to control (See Figure 6-6).

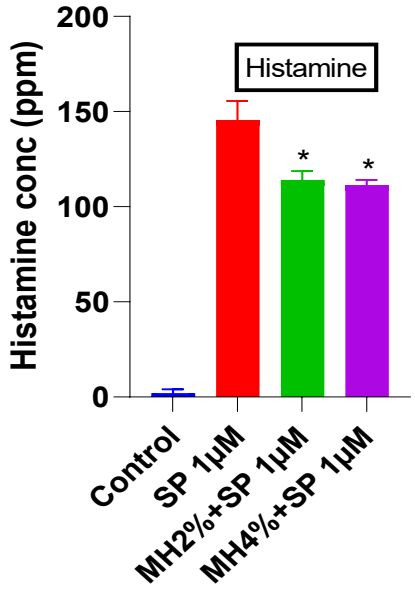


Figure 6-6: Effect of Manuka honey on the release of histamine.
LAD2 cells were pre-treated with graded concentrations of manuka honey 30 minutes and stimulated by substance P 1µM for 40 minutes. Supernatants analysed with histamine ELISA kit. Absorbance was read on a plate reader at 410 and % release obtained. Data is presented as mean ± SEM of the histamine concentration n=3. A one-way ANOVA was used to compare differences of treatments with SP group followed by Dunnett's post hoc test. Experiment was repeated three times. *≤0.05.

6.2.2 Effect of manuka honey on IgE mediated degranulation

The effects of MH and several signalling protein inhibitors on mast cell degranulation induced by IgE were studied.

6.2.2.1 Effect of MH concentrations on β-hexosaminidase using different α-IgE concentrations

The actions of MH on αIgE-induced release of β-hexosaminidase was investigated. At both 0.1% and 0.3% αIgE challenge, Manuka honey (MH 4%) significantly (p≤0.001) suppressed β-hexosaminidase release (See Figure 6-7). This demonstrate that MH could be useful in the disease condition considering the role of IgE in the disease aetiology and allergic diseases are comorbid with IC/PBS.

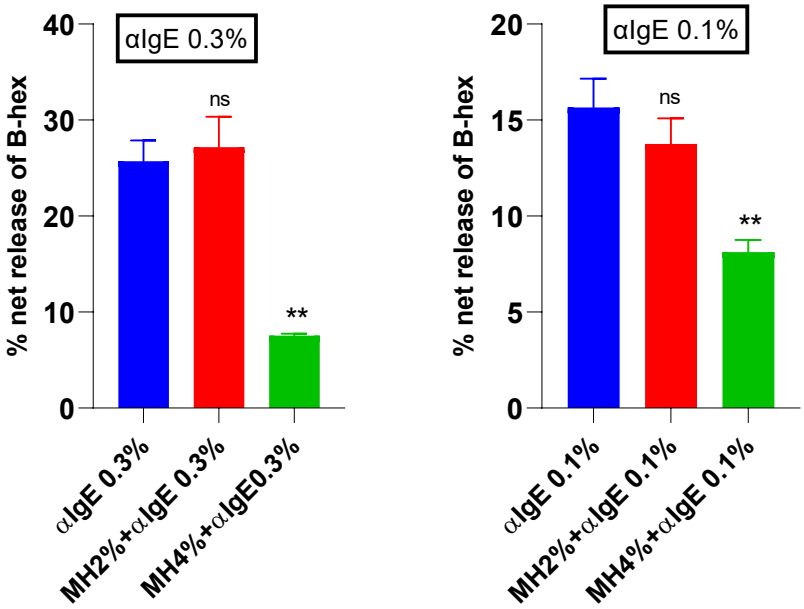


Figure 6-7: Effect of MH on αIgE induced release of β-hexosaminidase.
LAD2 cells were pre-treated with graded concentrations of manuka honey 30 minutes and stimulated by αIgE 0.3% and αIgE 0.1% for 40 minutes. Absorbances were read on a plate reader at 410 and 595 nm and % release obtained. Data is presented as mean ± SEM of triplicate readings of net release. Net release of spontaneous group was null. A one-way ANOVA was used to compare differences of treatments with αIgE group followed by Dunnett's post hoc test. In each case n=3. **≤0.01, ns= non-significant.

6.2.2.2 Comparative effect of manuka honey and signalling protein inhibitors on β-hexosaminidase release induced by α-IgE

Next, we compared the net effect of MH, the Akt inhibitor (10-DEBC) and the ERK inhibitor (FR-180204) on the release of β-hexosaminidase. Both MH 4% (p≤0.05) and 10-DEBC (10-30μM) (p≤0.001) significantly inhibited the release of β-hexosaminidase as shown in Figure 6-8.

However, 10-DEBC at 100 µM enhanced release of the enzyme (Figure 6-8) suggesting that at this concentration it was cytotoxic. Likewise, MH 4% significantly ($p \leq 0.05$) inhibited the release of β-hexosaminidase whilst FR-180204 (10-30 µM) again enhanced release suggesting that FR-180204 at the 2 lowest concentrations was cytotoxic in this model (Figure 6-8).

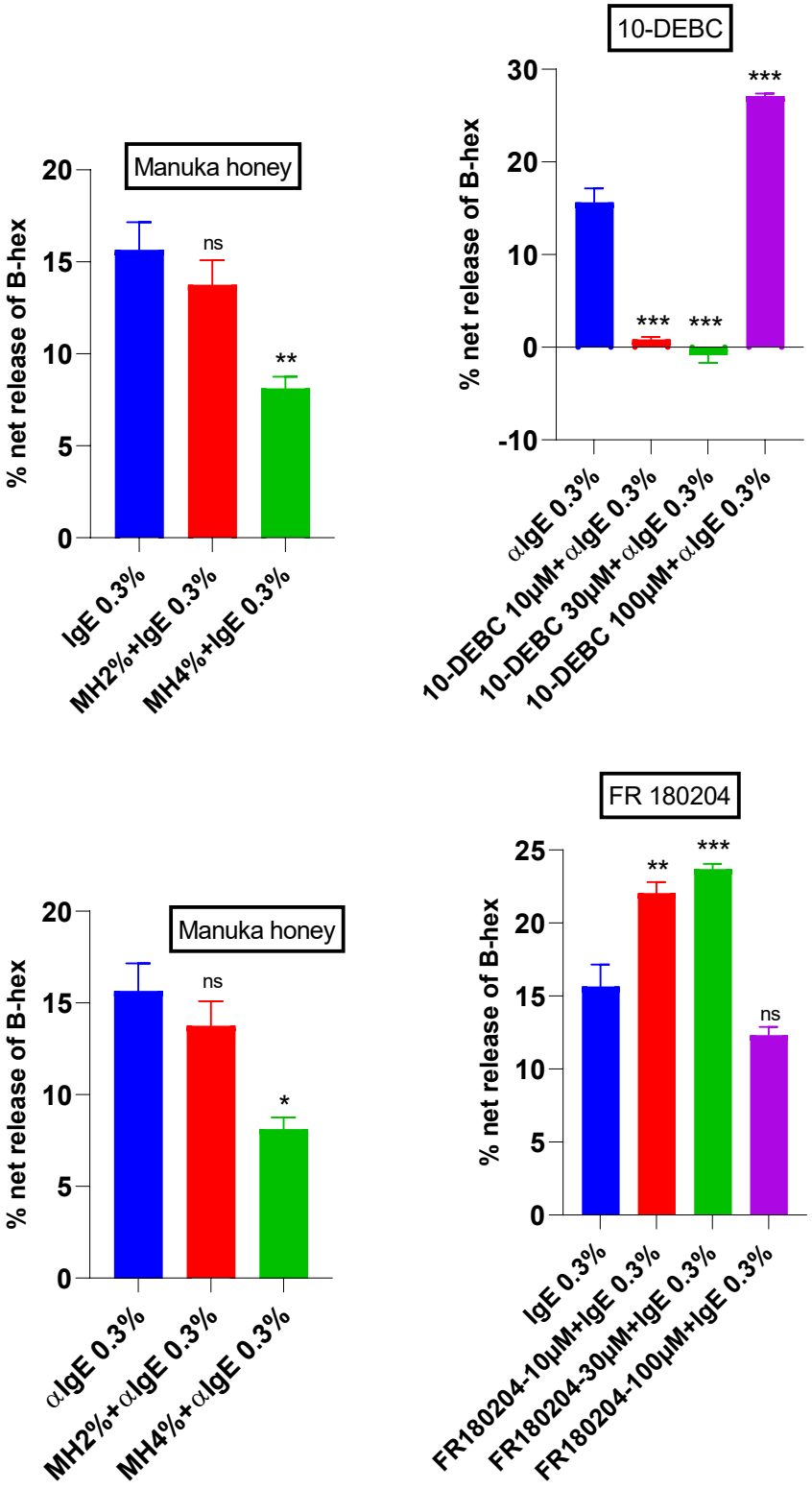


Figure 6-8: Comparative effect of MH and FR180204 in αIgE induce release of β-hexosaminidase.

LAD2 cells were pre-treated with graded concentrations of MH or 10-DEBC, and FR 180204 for 30 minutes and stimulated by αIgE 0.3% for 40 minutes. Absorbance was read on a plate reader at 410 and 595 nm and % release obtained. Data was presented as mean ± SEM of triplicate readings of net release. Net release of spontaneous group was null. A one-way ANOVA was used to compare differences of treatments with αIgE group followed by Dunnett's post hoc test. In each case n=3. **≤0.01, ***=0.000, ns= non-significant, *≤0.05.

6.2.2.3 Comparative effect of manuka honey and Syk inhibitor on degranulation on β-hexosaminidase release induced by α-IgE

The effect of MH and GSK 143 – a Syk inhibitor was investigated. Syk is an important adaptor protein that is tethered on the mast cell membrane and initiates the downstream events leading to mast cell degranulation via the IgE FcεRI receptor. As expected, GSK 143 significantly (p=0.000) inhibited β-hexosaminidase release at all doses tested (b) (Figure 6-9). Likewise, MH at 4% significantly (p≤0.05) inhibited the release of the mediator (Figure 6-9). Conversely, no effect was observed in the SP stimulated model because SP acts on the MRGPRX2, receptor which blockade by GSK produced null effect (Figure 6-9). Thus, Syk is a mediator of IgE not SP degranulation and MH 4% affect both pathways. This is supported by the current model.

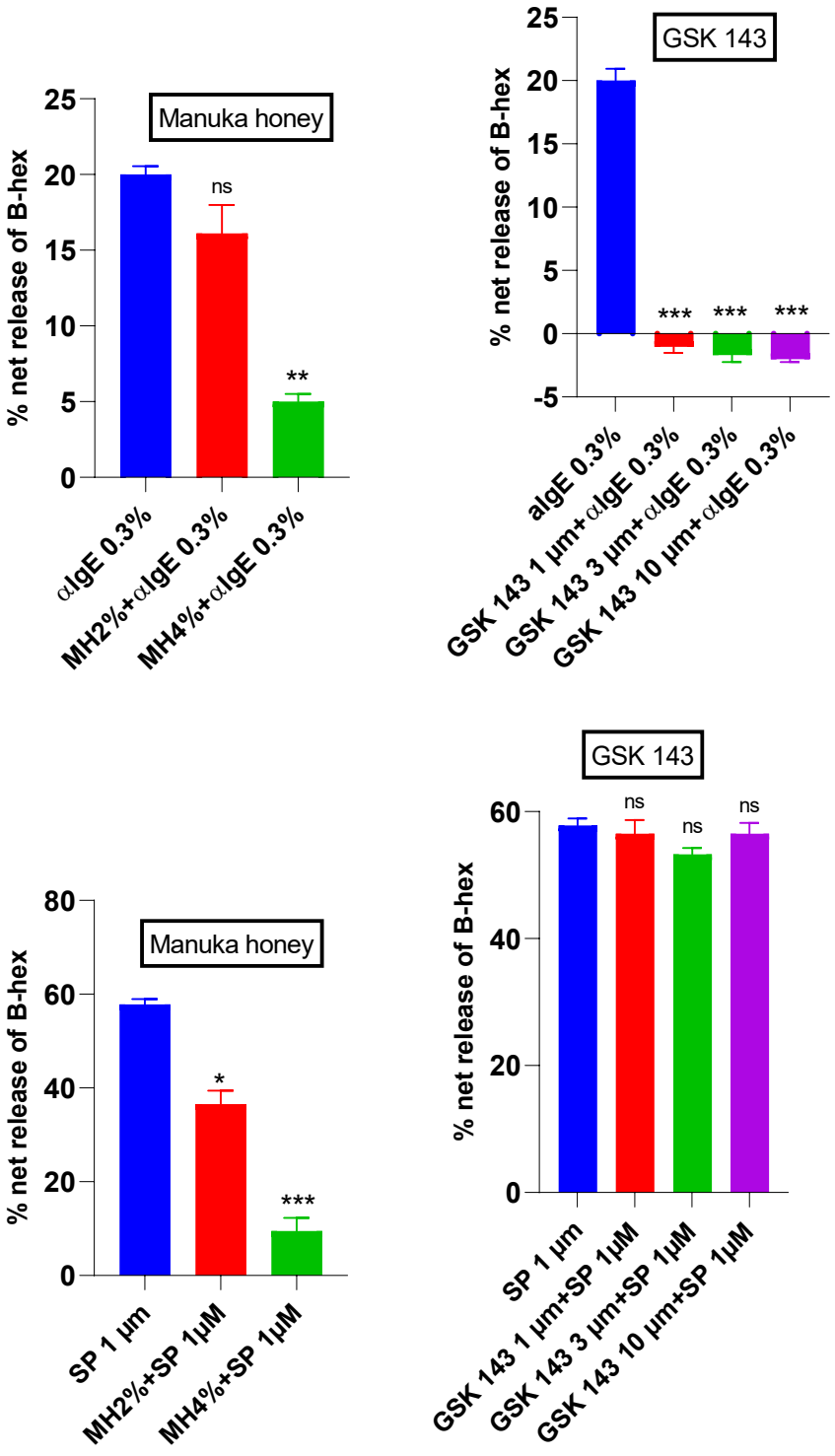


Figure 6-9: Comparative effect of MH and GSK (Syk inhibitor) on IgE and SP induced release of β-hexosaminidase.

LAD2 cells were pre-treated with graded concentrations of MH or GSK 143 for 30 minutes and stimulated by αIgE 0.3% or SP for 40 minutes. Absorbances were read on a plate reader at 410 and 595 nm and % release obtained. Data is presented as mean ± SEM of triplicate readings of net release. Net release of spontaneous group was null.

A one-way ANOVA was used to compare differences of treatments with SP group followed by Dunnett's post hoc test. In each case n=3, ***=0.000, ns= non-significant, *≤0.05.

6.2.2.4 Mast cell responsiveness to IgE

The responsiveness of the LAD2 cell to the effect of αIgE is continuously tested, as FcεRI functions decreases over time and with increasing passage number. In both myeloma IgE sensitised and non-sensitised cells. The cells were no longer responsive the effect of αIgE due to FcεRI receptor downregulation as shown in Figure 6-10. Generally, % net release of β-hexosaminidase below 5% indicates hibernation of cells. In other words, these cells have lost their physiological function and can no longer be used to model the disease process under review.

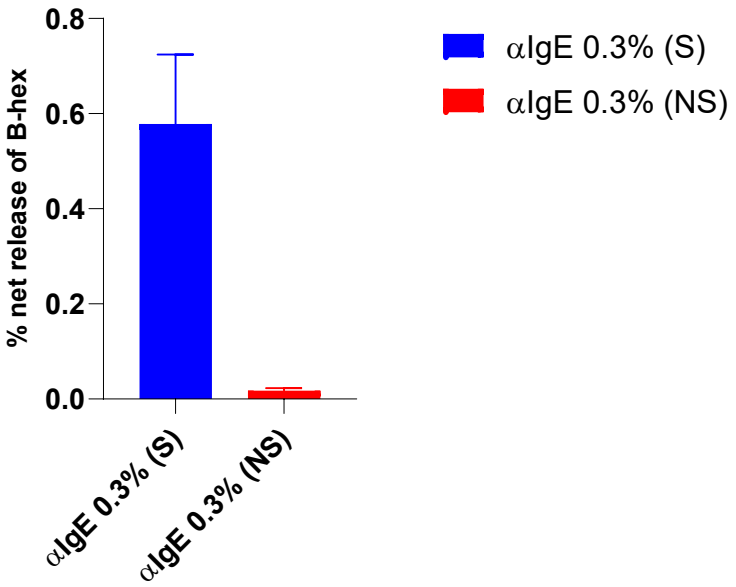


Figure 6-10: Effect of αIgE on the release of β-hexosaminidase in sensitised and non-sensitised cells

Equal numbers of LAD2 cells were divided into two wells of a 6-well plate. Myeloma IgE was added to the first plate and labelled Sensitised (S) while the second was devoid of myeloma IgE. Half strength medium was added to the plates and incubated overnight in an incubator. Cells were centrifuged and challenged with αIgE. The release of β-hexosaminidase was quantified and compared between the two groups. Net release of spontaneous group was null.

6.2.2.5 IL-4 treatment of LAD2 cells to restore receptor functions

From Figure 6-10, LAD2 cell IgE functions become been depleted over time due to downregulation of FcεRI. To restore the receptor function, the cells were pre-treated with IL-4 in the presence of full-strength medium for 28 days. After the 28 days treatment period, β-hexosaminidase release function was tested. The release of β-hexosaminidase was over 25%-40% from the baseline across various αIgE concentrations (See Figure 6-11) suggesting the restoration of receptor function.

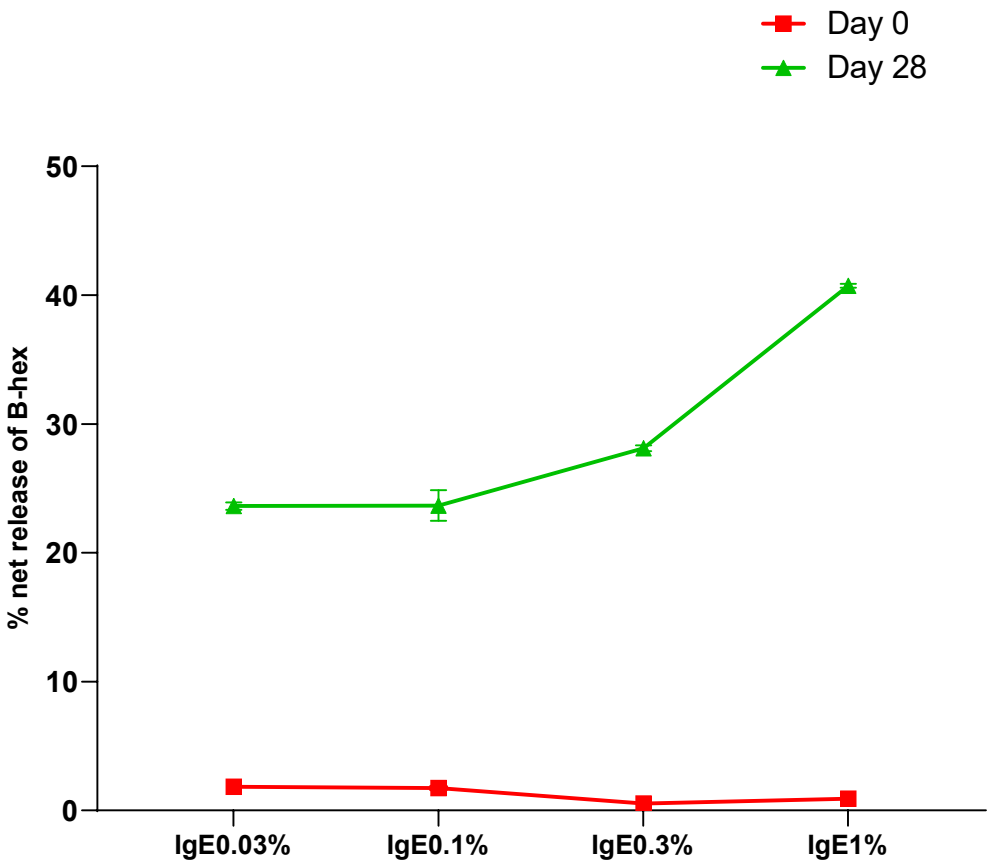


Figure 6-11: Effect of IL-4 treatment after 28 days on β-hexosaminidase release.

6.2.3 Effect of manuka honey on calcium ionophore mediated degranulation

Calcium ionophore is a non-immunologic inducer of mast cell degranulation. To investigate whether MH had any effect on non-immunologic degranulation; the effect of manuka honey after calcium ionophore (A23187) challenge was investigated. The effect on β-hexosaminidase, histamine and cysteinyl leukotrienes were studied.

6.2.3.1 Effect of Manuka honey on calcium mediated release of β-hexosaminidase

Manuka honey demonstrated significant inhibition of β-hexosaminidase at a concentration of 2% ($p=0.000$) and 4% ($p\leq0.05$) (See Figure 6-12). A stronger action was observed at MH 2%, suggesting the inhibition was not linearly related.

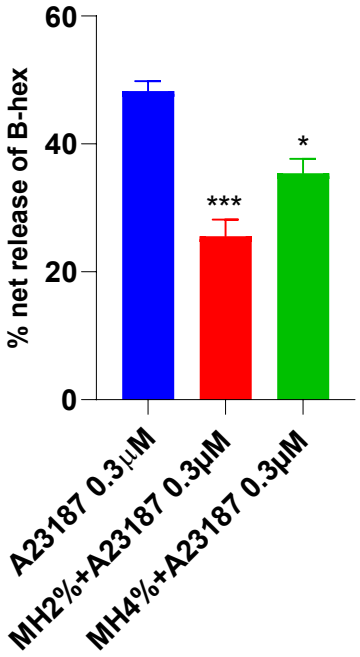


Figure 6-12: Effect of MH on A23187 stimulated release of β-hexosaminidase.
LAD2 cells were pre-treated with graded concentrations of MH for 30 minutes and stimulated by A23187 3 µM for 40 minutes. Absorbances were read on a plate reader at 410 and 595 nm and % release obtained. Data is presented as mean ± SEM of triplicate readings of net release. Net release of spontaneous group was null. A one-way ANOVA was used to compare differences of treatments with A23187 group followed by Dunnett's post hoc test. In each case n=3, ***=0.000, *≤0.05

6.2.3.2 Effect of manuka honey on calcium ionophore mediated release of histamine

Histamine is a known mediator of pain in IC/PBS. Thus, the effect of MH on A23187 release of this cytoplasmic autacoid was assayed. There was no statistically significant difference between the A23187-challenged group and the MH pre-incubated graded concentrations (See Figure 6-13). This suggest that MH does not inhibit the release of histamine when challenged by A23187. This result contrasts with the SP model where MH concentration-dependently inhibited the release of histamine (Figure 6-6). This suggest different pathways are involved in both SP and A2387 induced degranulation.

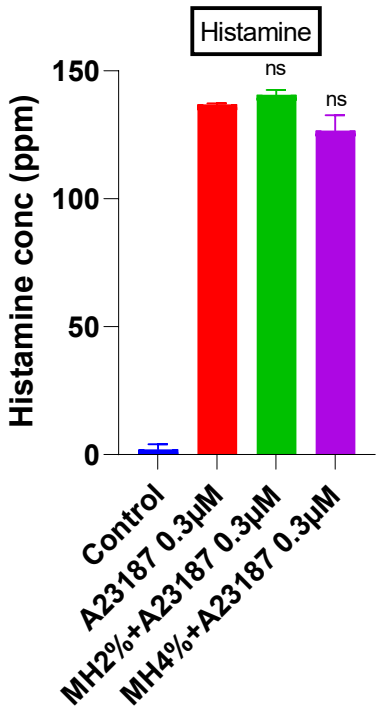


Figure 6-13: Effect of MH on A23187 stimulated release of histamine.
LAD2 cells were pre-treated with graded concentrations of MH for 30 minutes and stimulated by A23187 3 µM for 40 minutes. Absorbances were read on a plate reader at 410 and 595 nm and histamine concentrations obtained. Data is presented as mean ± SEM of triplicate readings of histamine concentrations. A one-way ANOVA was used to compare differences of treatments with A23187 group followed by Dunnett's post hoc test. In each case n=3, ns= non-significant.

6.2.3.3 Effect of manuka honey on calcium ionophore release of cysteinyl leukotrienes

Cysteinyl leukotriene is a metabolic by-product of arachidonic acid that mediates inflammation. It is an important target in most allergic diseases. This was targeted by MH. At 4%, MH significantly ($p \leq 0.05$) increased the release of Cysteinyl leukotrienes (See Figure 6-14).

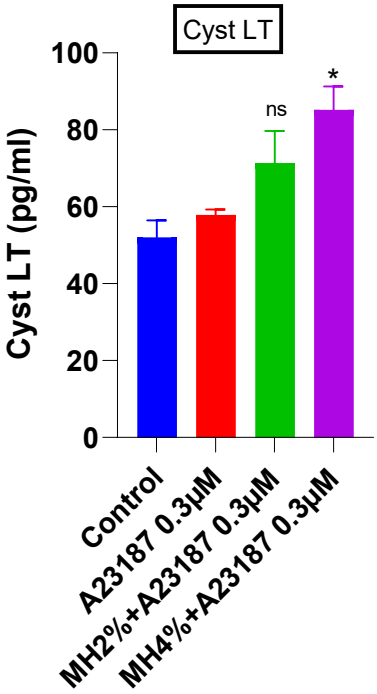


Figure 6-14: Effect of MH on A23187 stimulated release of cysteinyl leukotrienes. LAD2 cells were pre-treated with graded concentrations of MH for 30 minutes and stimulated by A23187 3 µM for 40 minutes. Absorbance was read on a plate reader at 410 and 595 nm and cysteinyl leukotrienes concentrations obtained. Data is presented as mean ± SEM of triplicate readings of Cyst LT. A one-way ANOVA was used to compare differences of treatments with A23187 group followed by Dunnett's post hoc test. In each case n=3, ns= non-significant.

6.3 Cytokine release assay

Inflammatory cytokines are important mediators that may potentiate degranulation through autocrine effects. The effect of MH on these cytokines was investigated.

6.3.1 Effect of manuka honey on the release of cytokine

To test the effect of MH on the release of pro-inflammatory cytokines, ten cytokines were studied: Tumor necrosis factor α (TNF-α), monocyte chemoattractant protein 1 (MCP-1), interleukin 1 β (IL-1β), interleukin 8 (IL-8), macrophage inflammatory protein-1 (MIP1), inducible protein 10 (IP10), inducible T-cell alpha chemoattractant (ITAC), interleukin 17A (IL-17A), growth regulated oncogene (GRO) and granulocyte monocyte colony stimulating factor (GM-CSF). However, only three cytokines: IL-1β, GM-CSF and IL-8 were detected in the two pathways tested (SP and A23187-induced).

Effect of manuka honey on SP mediated release of pro-inflammatory cytokines

MH 4% significantly inhibited the secretion of IL-8 ($p \leq 0.001$) and GM-CSF ($p \leq 0.000$) as shown in Figure 6-15. Similarly, spontaneous inhibition of IL-8 ($p \leq 0.001$) and GM-CSF ($p \leq 0.000$) was observed at baseline with same concentration (Figure 6-15.). Conversely, MH 4% significantly ($p \leq 0.05$) increased the secretion of IL-1β. More importantly, MH 4% spontaneously evoked the release IL-1β ($p \leq 0.05$) as shown in Figure 6-15.

Effect of manuka honey on A23187 mediated release of pro-inflammatory cytokines

Similarly, MH 4% decreased the concentration of IL-8 ($p \leq 0.001$) and GM-CSF ($p \leq 0.05$) following A23187 stimulation but had no effect on the release of IL-1β (See Figure 6-16). In the same vein, 4% significantly decreased the concentration of IL-8 ($p \leq 0.001$) and GM-CSF ($p \leq 0.05$) from baseline but no effect on the concentration of IL-1β (Figure 6-16).

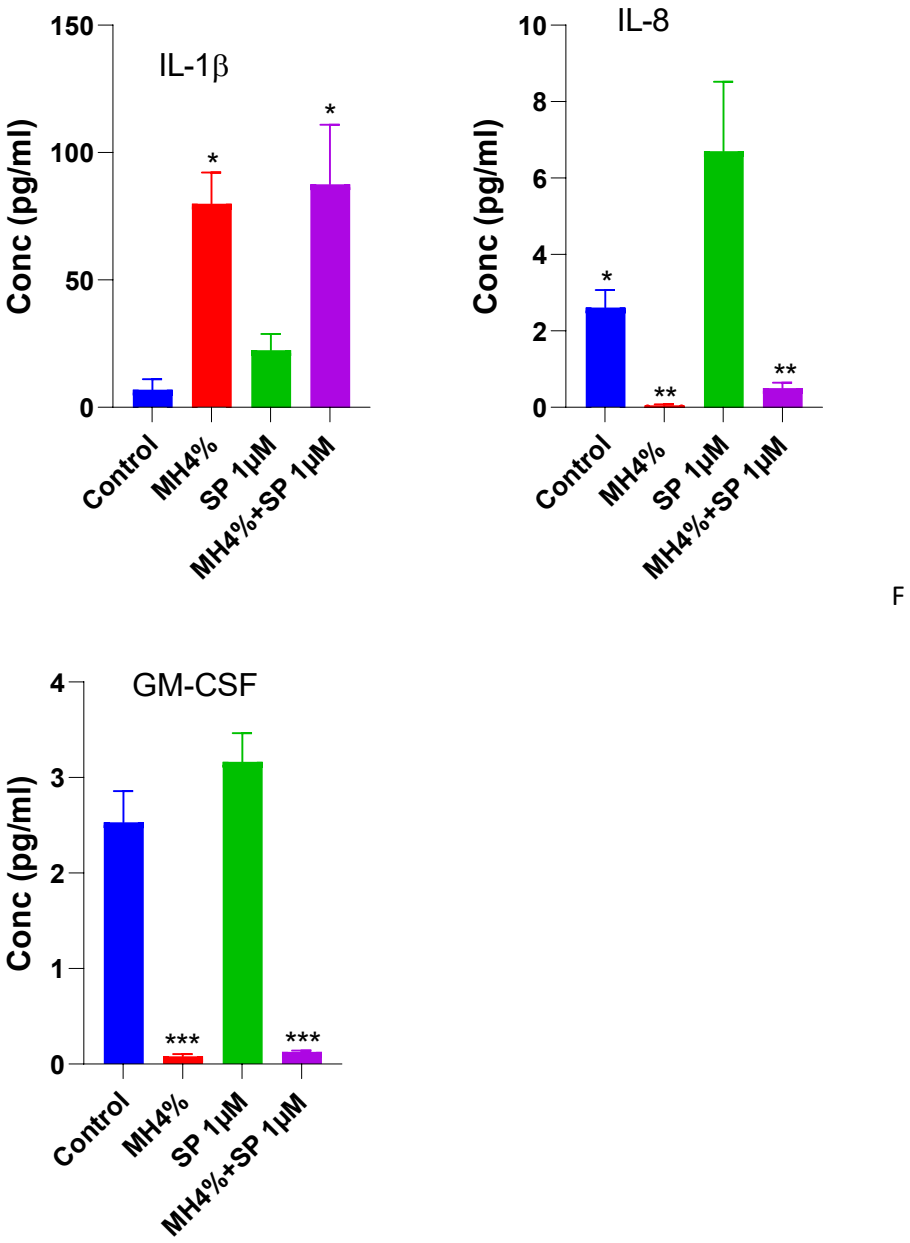


Figure 6-15: Effect of manuka honey on SP mediated release of cytokines. Result is presented as mean ± SEM of the concentration (pg/ml) n=3. A one-way ANOVA was used to compare differences of conditions with SP group followed by Dunnette post hoc test. Experiment was repeated three times. *≤0.05, ***=0.000, ns= non-significant.

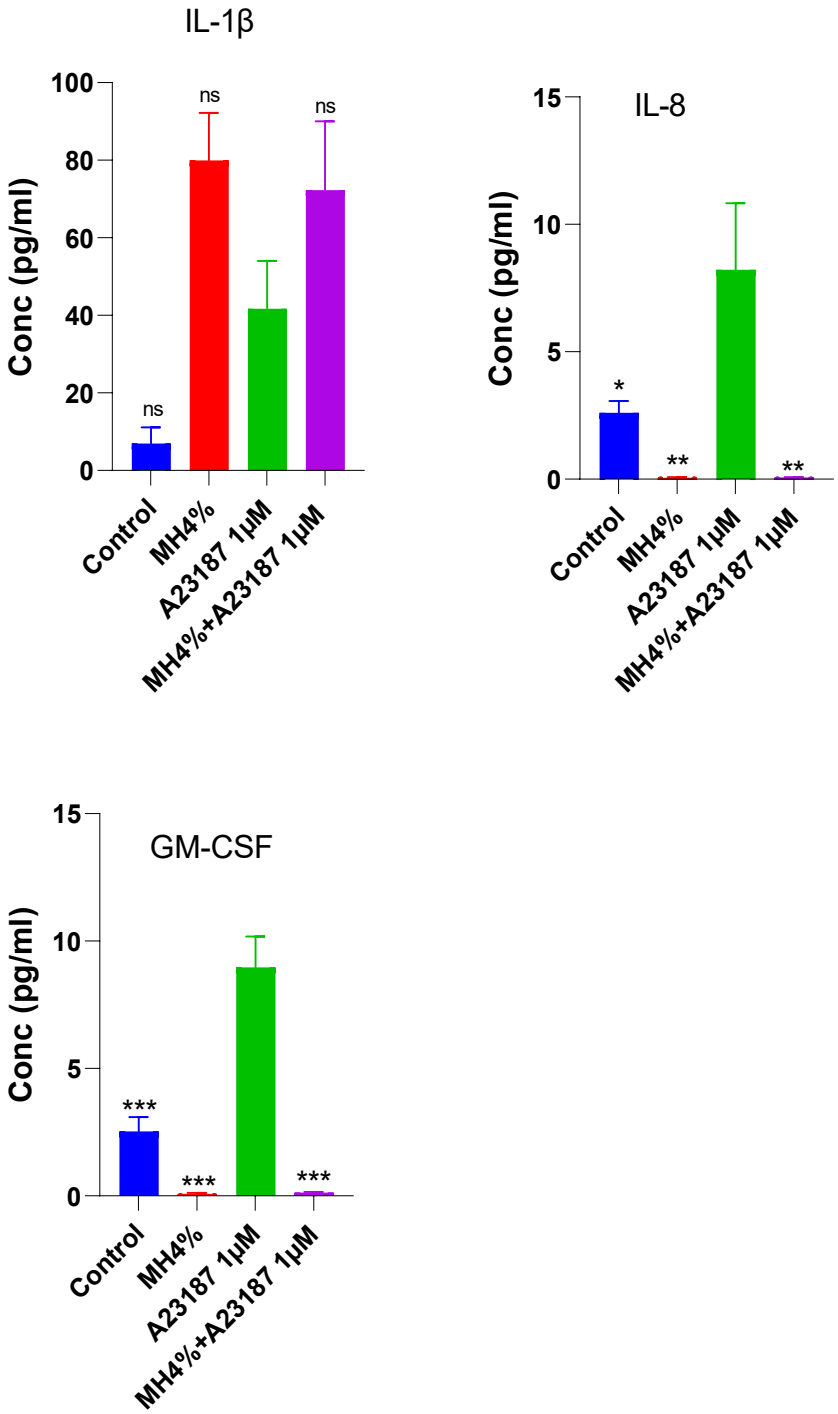


Figure 6-16: Effect of manuka honey on A23187 mediated release of cytokines.

Result is presented as mean ± SEM of the concentration (pg/ml) n=3. A one-way ANOVA was used to compare differences of conditions with SP group followed by Dunnett's post hoc test. Experiment was repeated three times. *≤0.05, ***=0.000, ns= non-significant.

6.4 Signalling studies

Degranulation and cytokine release in mast cells is a complex process that is relayed via a signal transduction mechanism, which involves activation of intracellular proteins. These proteins are targets of drug action that affects the final mediator release. How MH affects important signalling proteins in degranulation and cytokine production was studied in SP, A23187 and (partly) IgE pathways.

6.4.1 SDS PAGE optimisation

6.4.1.1 Optimisation of cell number for SDS PAGE

The study underwent a lot of optimisations to arrive at the appropriate number of cells and lysis buffer to be used in the signalling studies. At $0.5X10^6$ cells/ml and $1X10^6$ cells/ml all the lysis buffer used expressed abundant proteins in the gel (See Figure 6-17). However, intense bands were visible with the latter with LDS sample buffer and RIPA buffer. Consequently, LDS sample buffer and RIPA buffer were the variables of choice as lysis buffer at that cell number. RIPA buffer was more appropriate because it contains all the necessary protease and phosphatase inhibitors, which ensure the structural configuration of the phosphorylated form of the proteins being studied.

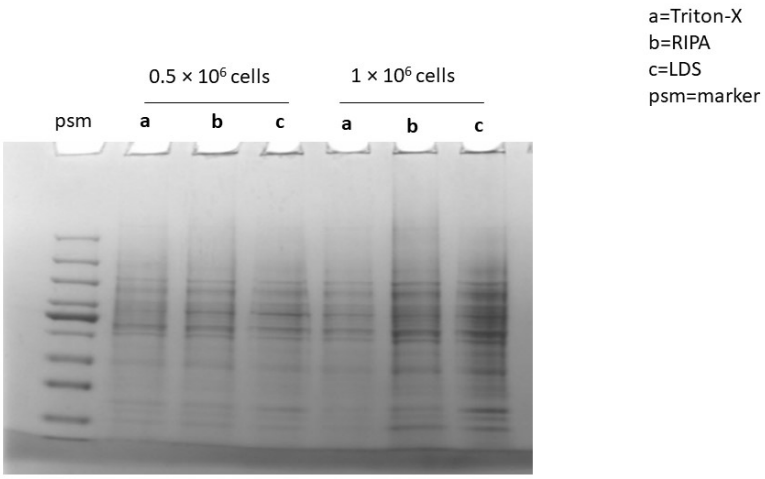


Figure 6-17: A blot showing protein density under different lysis condition.

6.4.1.2 Optimisation of stimulator dose and kinetics

Substance P was used to stimulate the cells at different doses and time points. Lysed cells were electrophosed and blots detected. SP 3 µM at 15 minutes produced a more visible band for ERK1/2 (See Figure 6-18). This dose and time were optimised for the SDS PAGE studies.

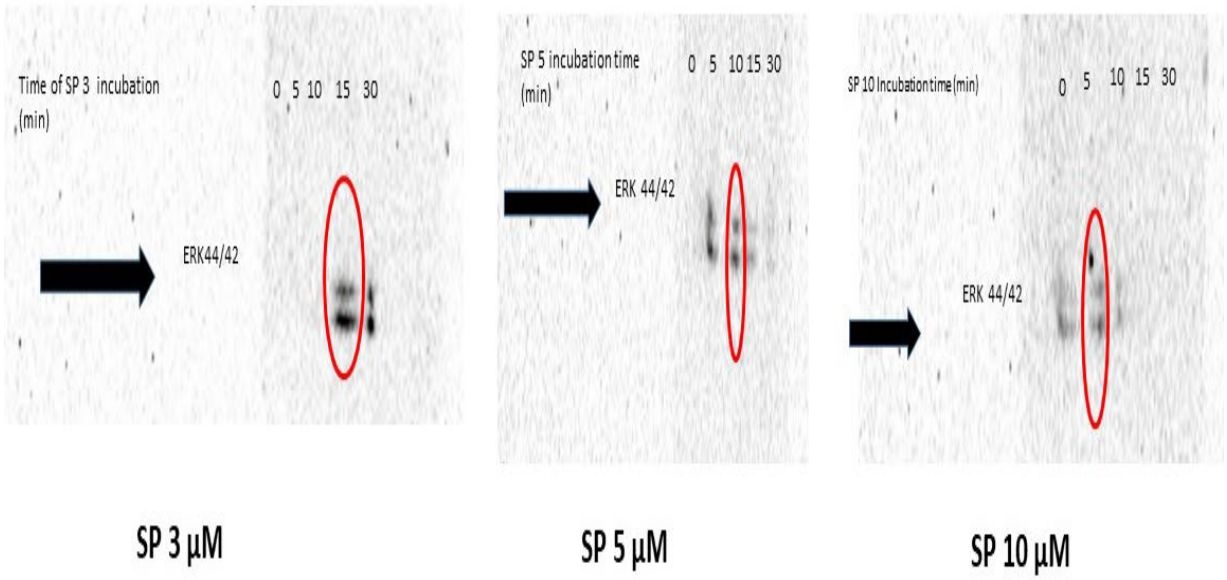


Figure 6-18: Effect of SP doses on ERK kinetics.

6.4.1.3 Optimisation for sample viscosity

Lysed samples were loaded and to the gel and electrophosed. Blot was developed as per protocol and detected as shown in Figure 6-19. A house keeping protein (β-actin) was used which should produce equal bands irrespective of treatment conditions. However, MH4%+SP 3µM and MH6%+SP 3µM samples in these conditions were viscous and difficult to load on the gel. In order, to overcome this. samples were all sonicated on ice as detailed in 5.2.7.1.

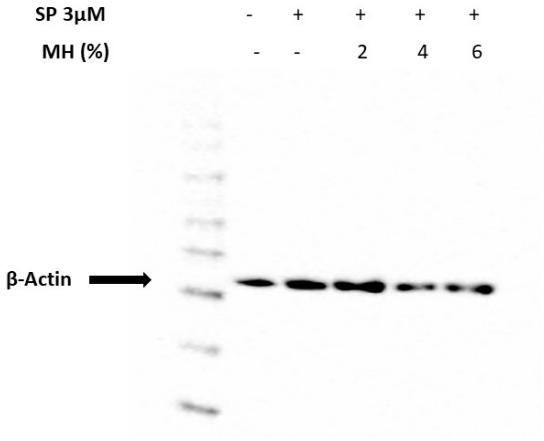


Figure 6-19: A blot showing unequal bands due to sample viscosity**.**

6.4.1.4 Optimisation for protein concentration

This assay was optimised for 25 µg of protein to be loaded in each well of the pre-cast gel. Protein determination was in each case using the Pierce BCA assay kit as illustrated in Figure 6-20. Protein concentration were read from the standard curve as shown below.

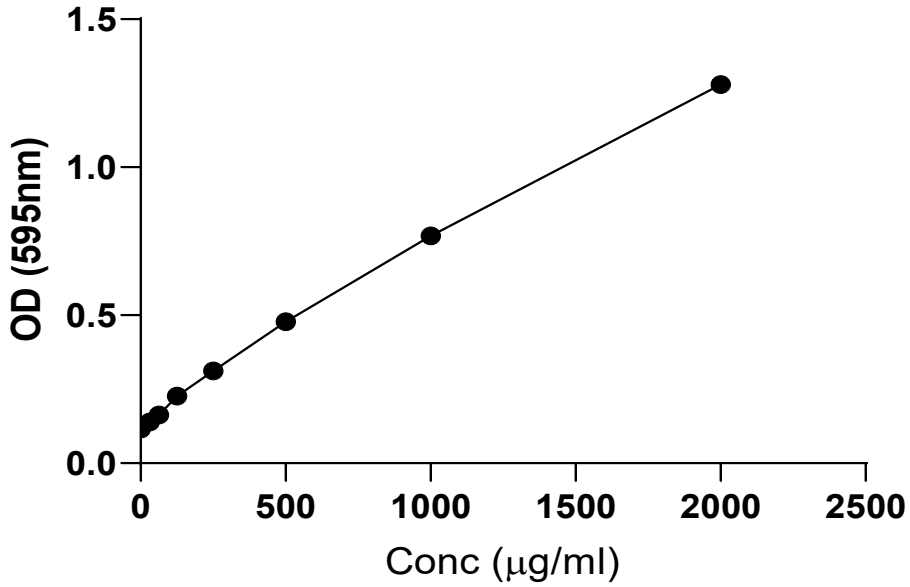


Figure 6-20: A representative BCA protein standard curve.

6.4.2 Manuka honey effect on SP mediated signalling

6.4.2.1 Effect of manuka honey on SP mediated expression of Akt

Akt is an important downstream protein target in degranulation. Thus, the effect of MH on this target was investigated. At the physiological concentrations of MH 2-4%, MH had no effect on this protein target (See Figure 6-21). However, at 6% there was significant ($p \leq 0.001$) inhibition of Akt expression (Figure 6-21). However, this concentration (6%) was shown to evoke significant release of lactate dehydrogenase but not Trypan blue. This suggest that MH 6% concentration is cytotoxic.

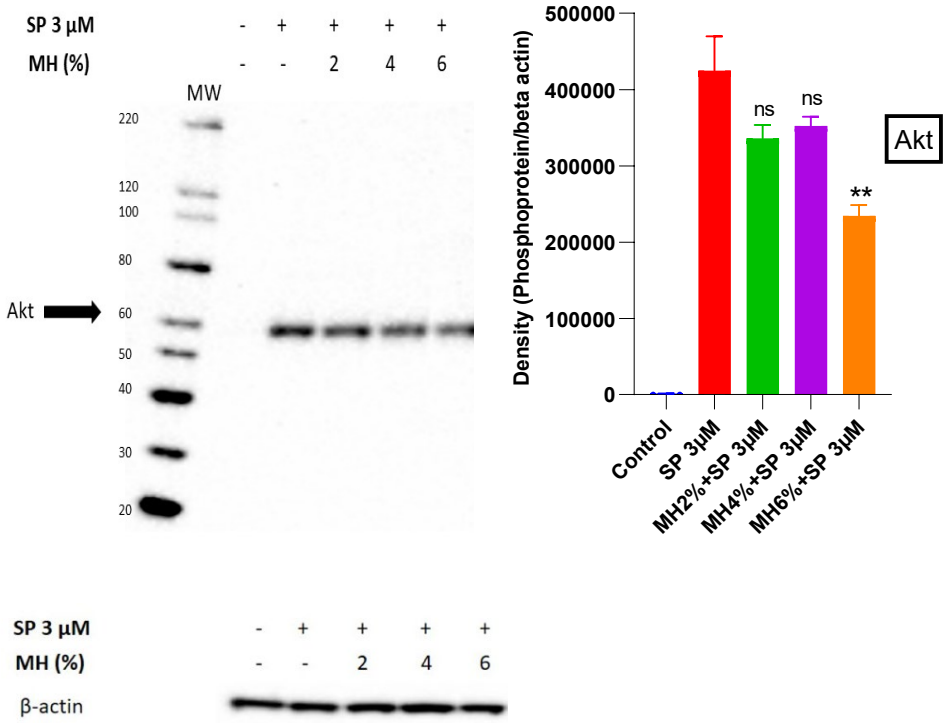


Figure 6-21: Effect of MH on SP-induced Akt expression.

Akt represents densitometric analysis of protein density of three different blots normalised to β-actin. Results were presented as mean ± SEM. ANOVA was used to compare differences of conditions with SP group followed by Dunnett's post hoc test. Experiment was repeated three times. **=0.001, ns= non-significant in comparison to SP group.

6.4.2.2 Effect of manuka honey on SP mediated expression of p38

Manuka honey at 2% and 4% significantly (p≤0.05) attenuated the expression of p38, but MH 6% increased it (p≤0.001) likely due to its cytotoxic effect (See Figure 6-22).

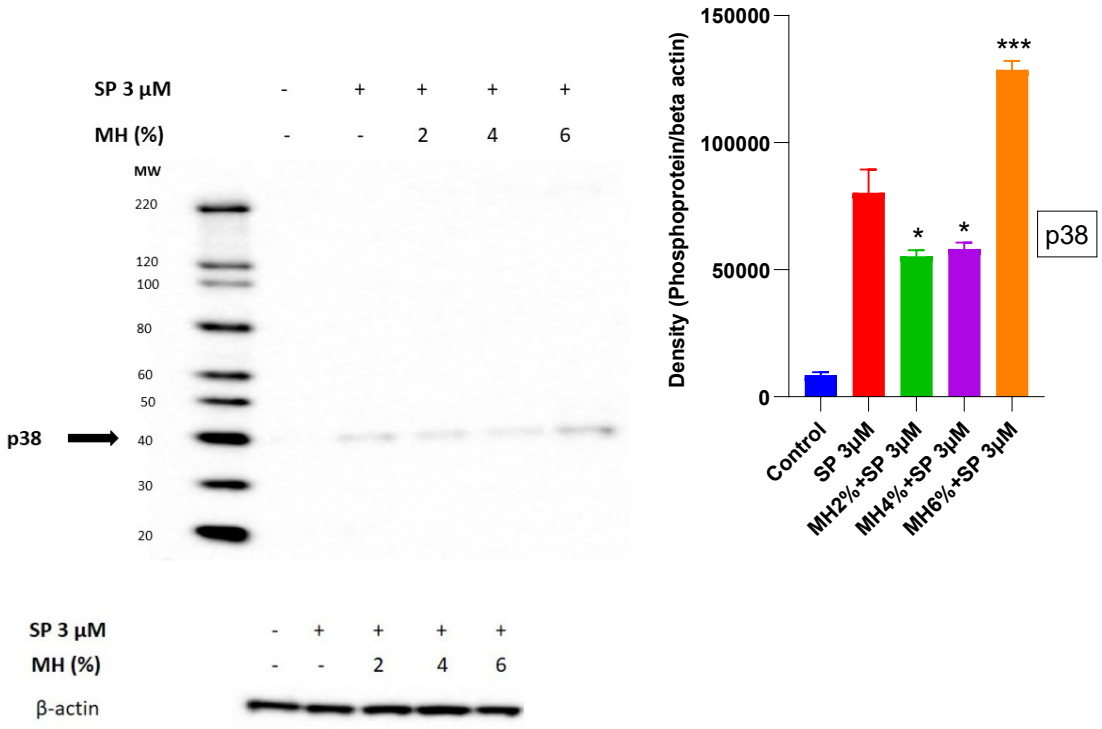


Figure 6-22: Effect of MH on SP-induced expression of p38**.**

p38 represents densitometric analysis of protein density of three different blots normalised to β-actin. Results were presented as mean ± SEM. ANOVA was used to compare differences of conditions with SP group followed by Dunnett's post hoc test. Experiment was repeated three times. *≤0.05, ***=0.000.

6.4.2.3 Effect of manuka honey on SP-mediated expression ERK

Manuka honey concentration dependently (2%, 4%) and 6% (cytotoxic concentration) significantly (p≤0.05, p≤0.001 and p=0.000), significantly suppressed the expression of ERK I (See Figure 6-23). The effect on ERK II was a non-significant attenuation.

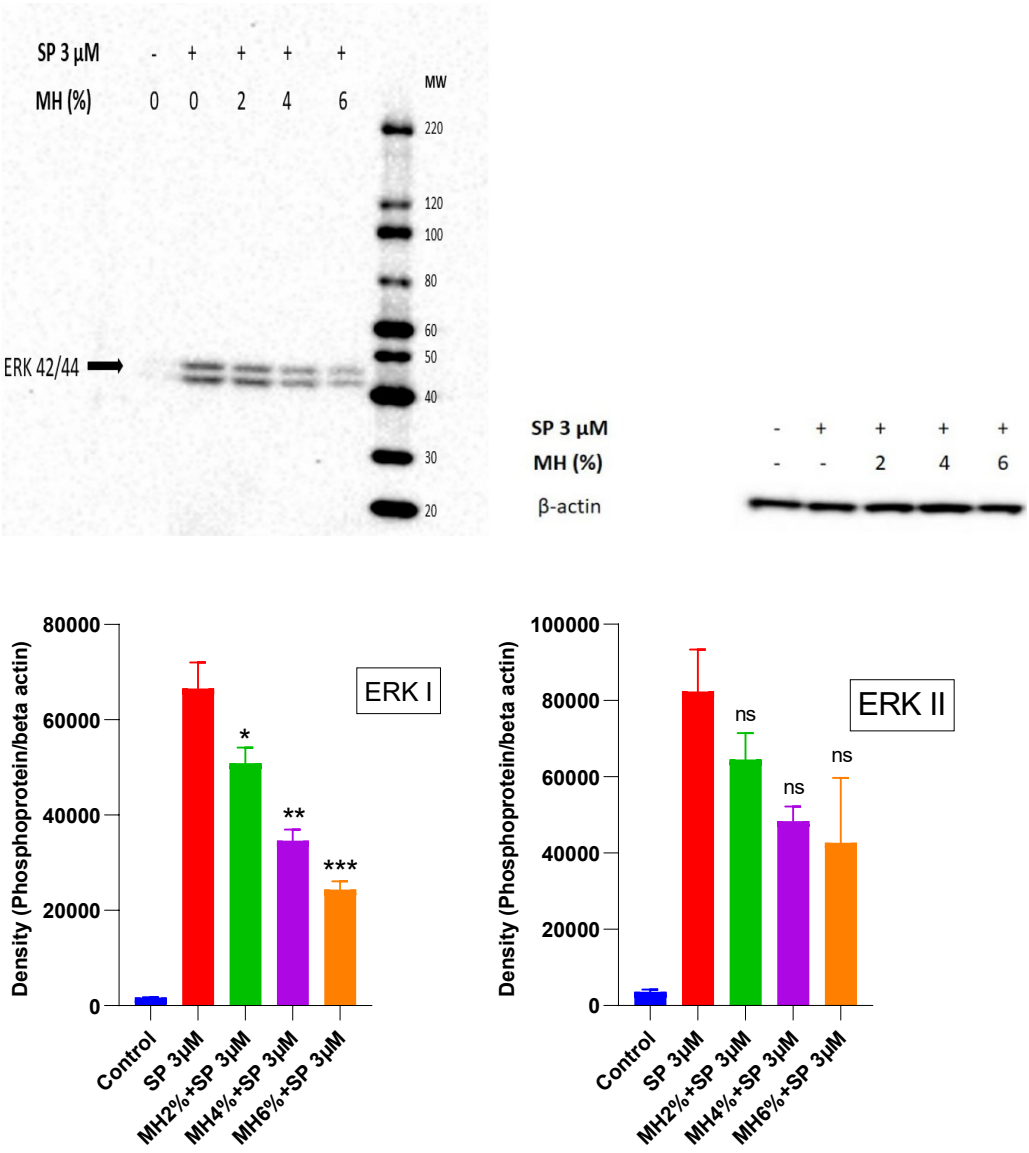


Figure 6-23: Effect of MH on SP-induced expression of ERK protein expression.
ERK I and ERK II represent densitometric analyses of protein densities of three different blots normalised to β-actin. Results are presented as Mean ± SEM. ANOVA was used to compare differences of conditions with SP group followed by Dunnett's post hoc test. Experiment was repeated three times. ***=0.000 **≤0.001, *≤0.05, ns= non-significant in comparison to SP group.

6.4.2.4 Effect of manuka honey SP mediated expression JNK

Manuka honey (MH) at 2% and 4% did not have inhibitory effect on JNK expression. When using MH at the cytotoxic concentration of 6% it was significantly ($p \leq 0.05$) upregulated (See Figure 6-24).

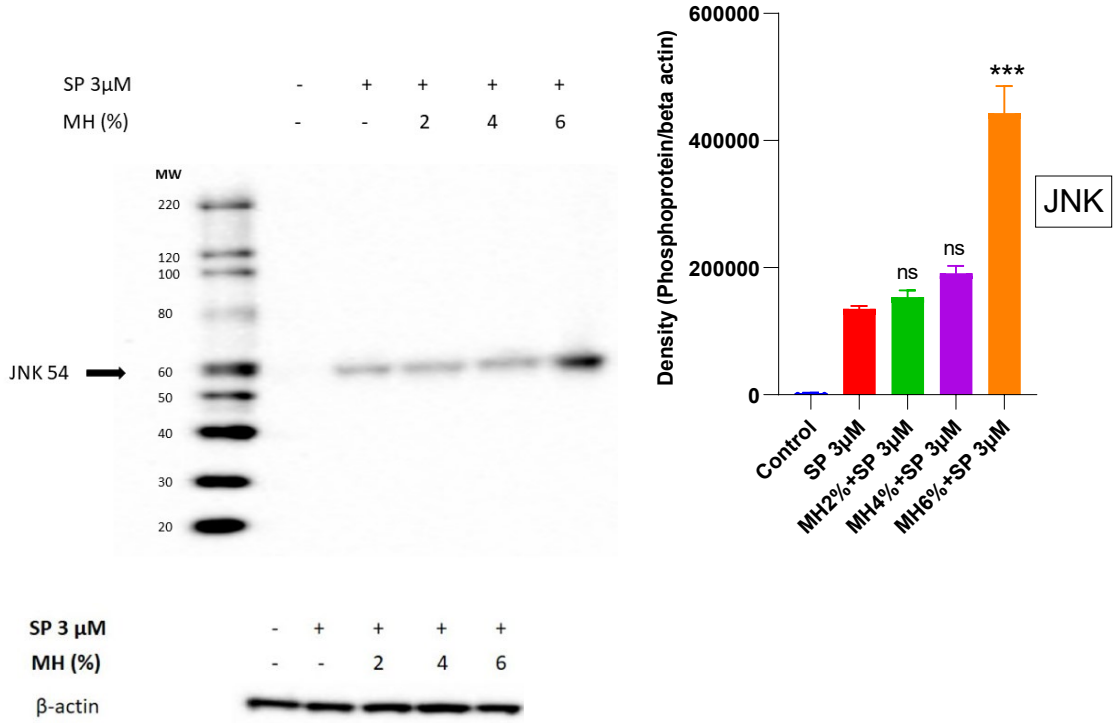


Figure 6-24: Effect of MH on SP-induced expression of JNK protein expression. JNK represents densitometric analysis of protein density of three different blots normalised to β-actin. Results were presented as mean ± SEM. ANOVA was used to compare differences of conditions with SP group followed by Dunnett's post hoc test. Experiment was repeated three times. ***=0.000, ns= non-significant in comparison to SP group.

6.4.3 Manuka honey effect on calcium ionophore mediated signalling

How manuka honey affects calcium mediated signalling in degranulation and cytokine release were investigated.

6.4.3.1 Effect of manuka honey on calcium ionophore mediated expression of Akt

Manuka honey (MH) had no effect at either physiological concentration (MH 2-4%) or cytotoxic concentration (MH 6%) (See Figure 6-25). This suggest the net effect of MH on A23187 is mediated via a different pathway

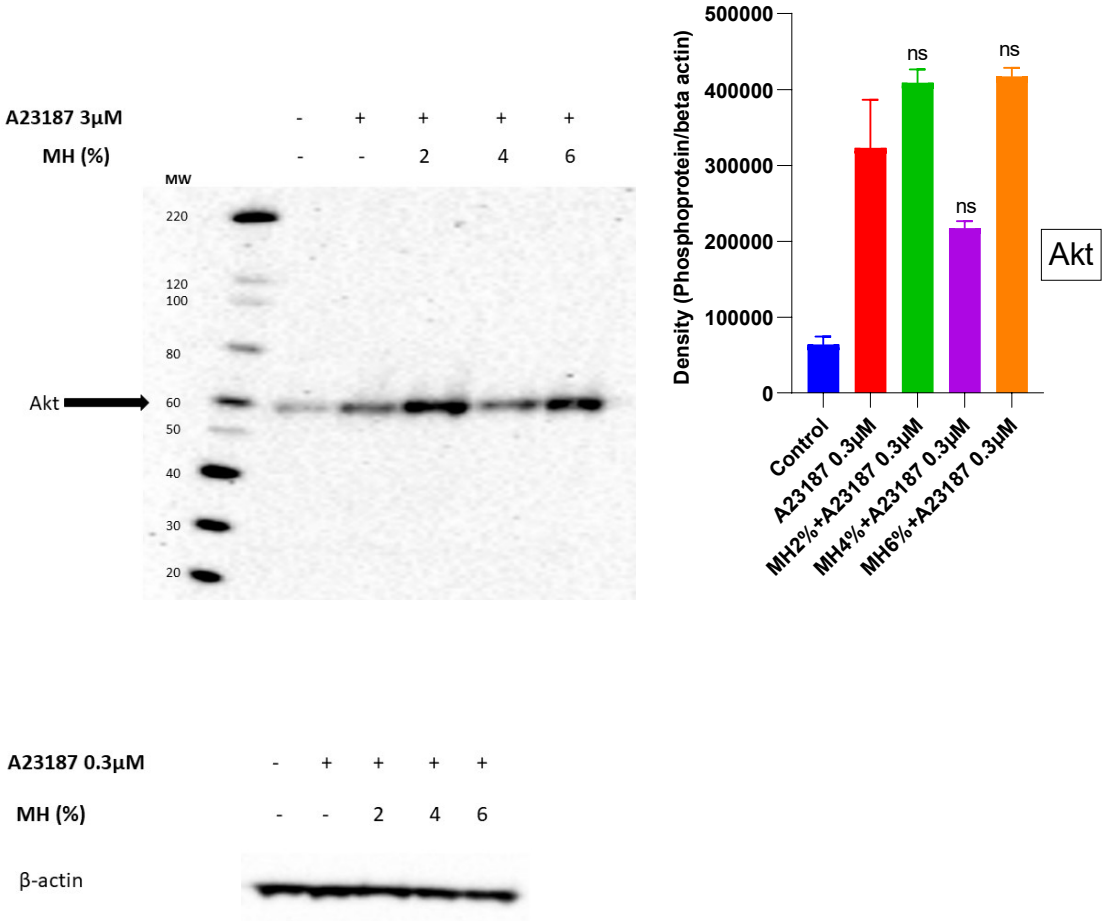


Figure 6-25: Effect of MH on A23187-induced expression of Akt**.**

Akt expression following A23187 stimulation of LAD2 cells after MH pre-treatment

Akt represents densitometric analysis of protein density of three different blots normalised to β-actin. Results are presented as mean ± SEM. ANOVA was used to compare differences of conditions with A23187 group followed by Dunnett's post hoc test. Experiment was repeated three times. ns= non-significant in comparison to A23187 group.

6.4.3.2 Effect of manuka honey on calcium ionophore mediated expression of ERK

Manuka honey at all the concentrations (2% and 4%) tested significantly (p=0.000) attenuated the expression of both ERK I and ERK II (See Figure 6-26).

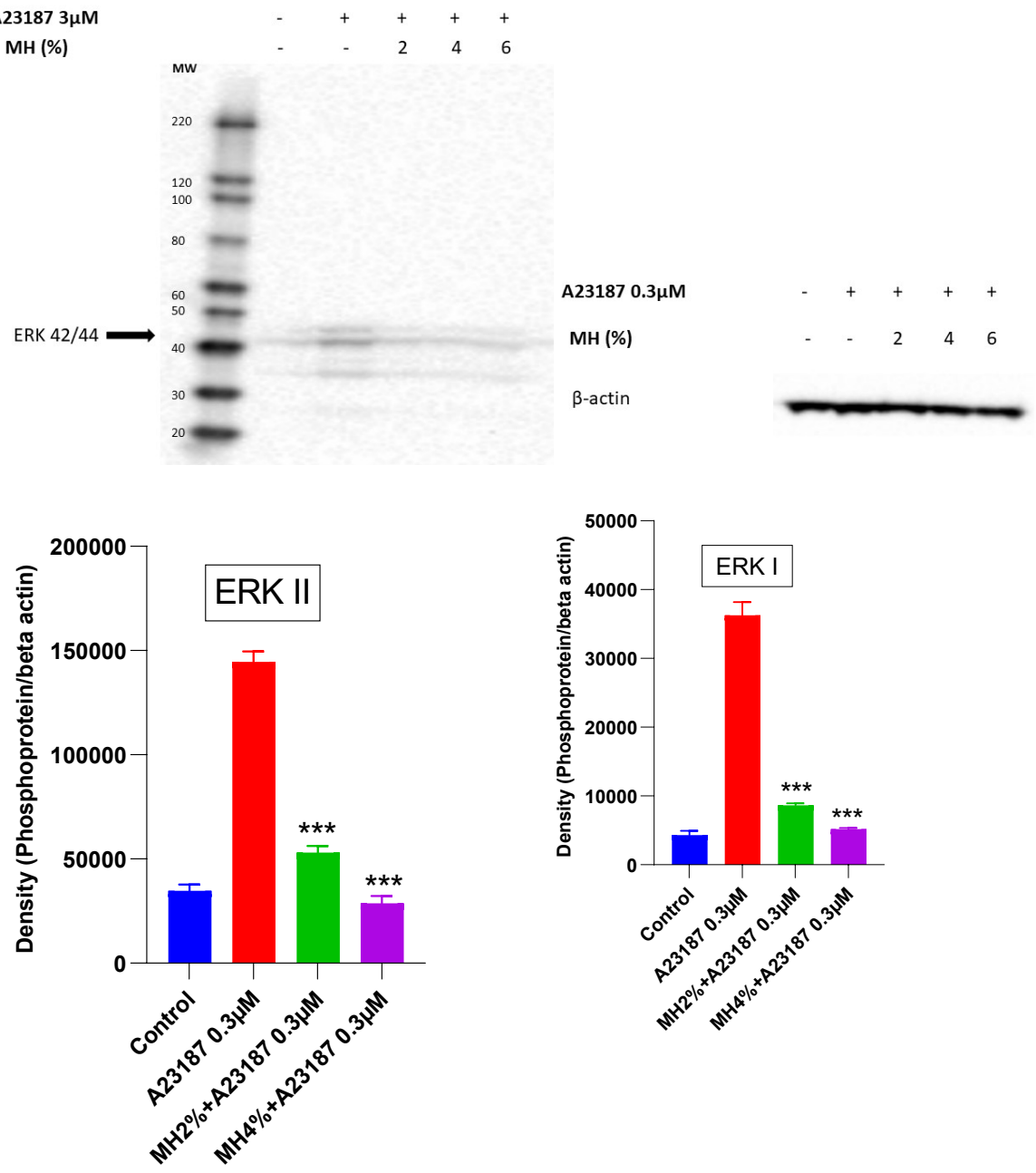


Figure 6-26: Effect of MH on A23187-induced expression of ERK**.**

ERK I and ERK II represent densitometric analyses of protein densities of three different blots normalised to β-actin. Results were presented as mean ± SEM. ANOVA was used to compare differences of conditions with A23187 group followed by Dunnett's post hoc test. Experiment was repeated three times. ***=0.000, non-significant in comparison to A23187 group.

6.4.3.3 Effect of manuka honey on calcium ionophore mediated expression of p38

Manuka honey at all the concentrations significantly (p=0.000) upregulated the expression of p38 proteins (See Figure 6-27). This shows that cytokines regulated by this protein could be secreted following MH pre-treatment.

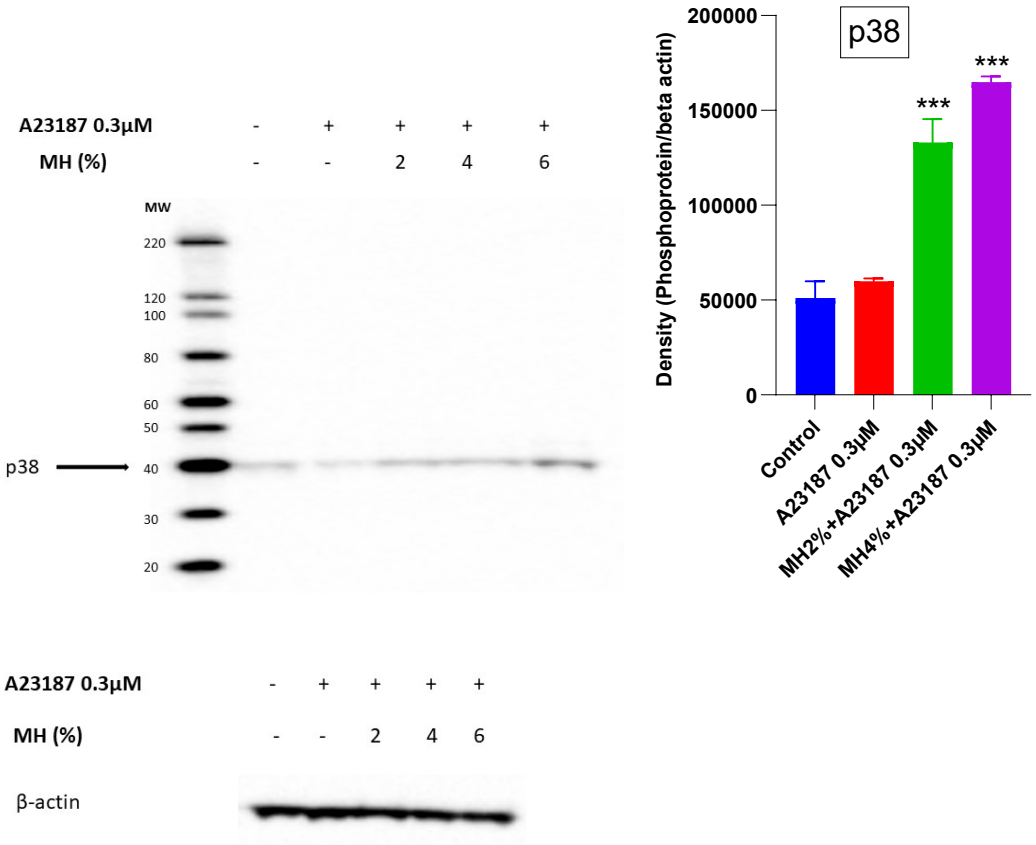


Figure 6-27: Effect of MH on A23187-induced expression of p38**.**

P38 represent densitometric analyses of protein densities of three different blots normalised to β-actin. Results are presented as mean ± SEM. ANOVA was used to compare differences of conditions with A23187 group followed by Dunnett's post hoc test. Experiment was repeated three times. ***=0.000, in comparison to A23187 group.

6.4.3.4 Effect of manuka honey on the expression of JNK

Manuka honey (MH) at all the physiological concentrations (MH 2-4%) had no effect on the expression of JNK. However, MH 6% significantly (p=0.000) upregulated the expression of JNK (See Figure 6-28) almost certainly due to the cytotoxic effects seen at this concentration.

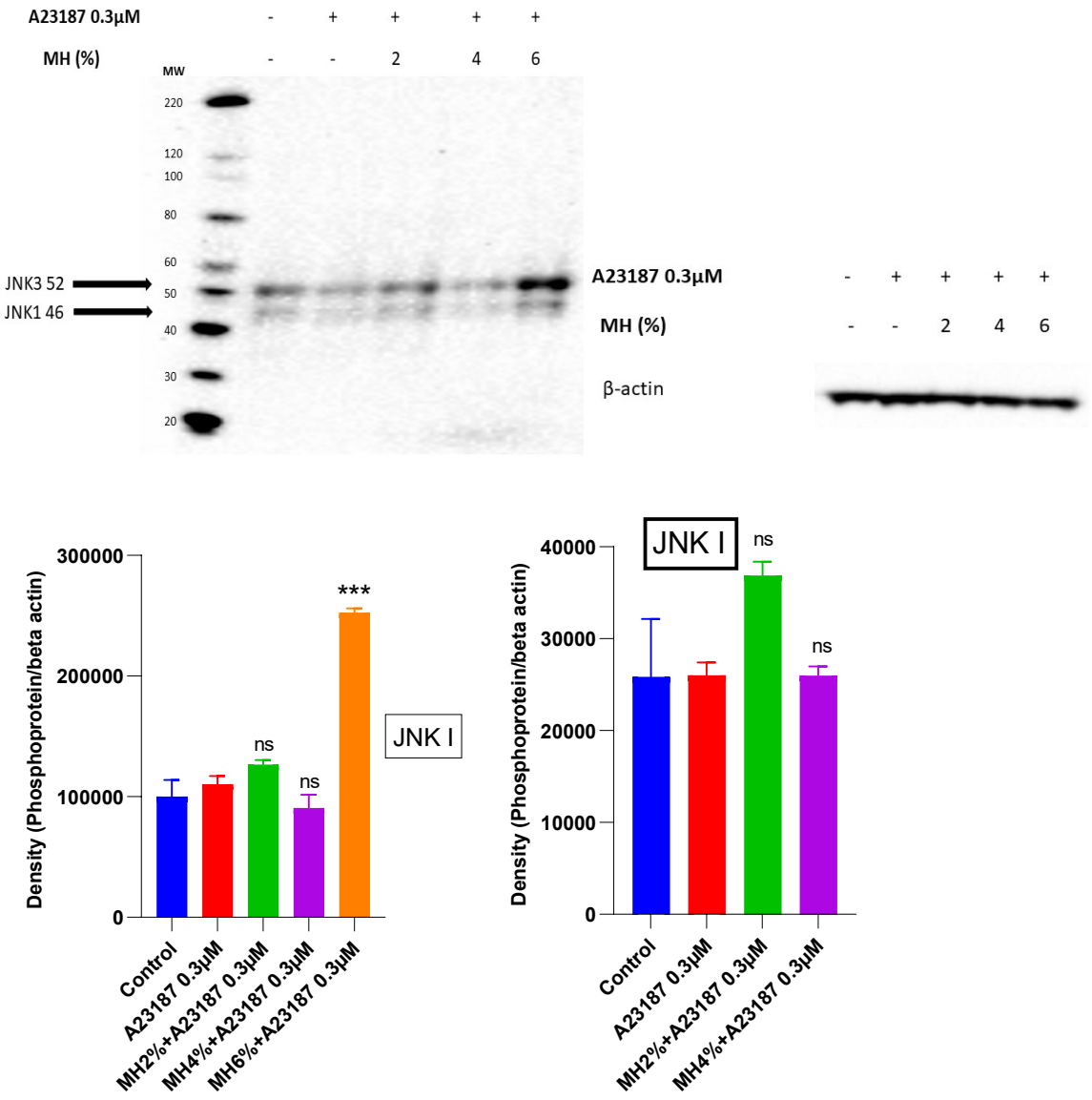


Figure 6-28: Effect of MH on A23187-induced expression of JNK**.**

JNK I and JNK III represent densitometric analyses of protein densities of three different blots normalised to β-actin. Results are presented as mean ± SEM. ANOVA was used to compare differences of conditions with A23187 group followed by

Dunnett's post hoc test. Experiment was repeated three times. ***=0.000, ns=non-significant in comparison to A23187 group.

6.4.4 Manuka honey effect on IgE mediated signalling

The effect of Manuka honey on αIgE-induced signalling protein was investigated.

6.4.4.1 Effect of manuka honey on IgE mediated expression of ERK

Manuka honey at all the concentration tested significantly downregulated the expression of ERK I and ERK II in a concentration dependent fashion as shown in Figure 6-29 below. Even the cytotoxic concentration (MH 6%) demonstrated downregulation of the protein. Thus, ERK may suggest a point of convergence related to in the molecular mechanism of MH actions on degranulation and cytokine production.

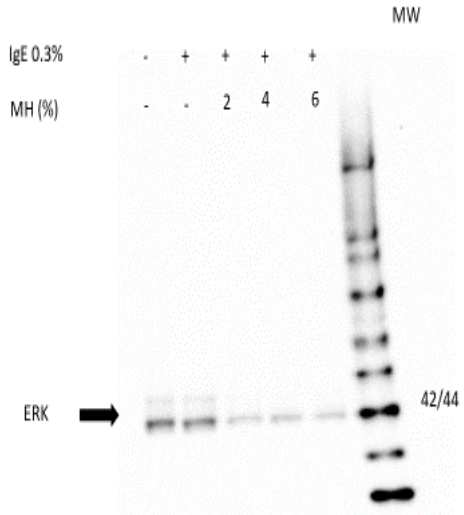
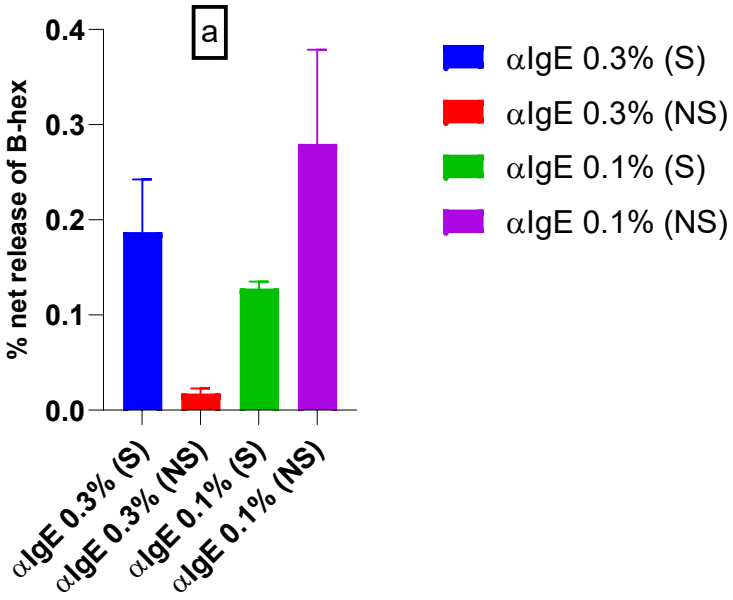


Figure 6-29: Effect of MH on αIgE-induced expression of ERK**.**
ERK I and ERK II blots normalised to β-actin. Experiment was performed once.

6.4.4.2 LAD2 cells lost IgE functions

LAD2 cells have lost IgE function after 12 passages as indicated by poor responsiveness of the cells to αIgE as shown in Figure 6-30. Stock was IL-4 treated to generate enough cells to look at the effect of MH on cysteinyl leukotrienes, histamine, and MAPK via the αIgE pathway. However, the receptor function was not restored as shown in Figure 6-30.



Beta hexosaminidase release after challenge with IgE.

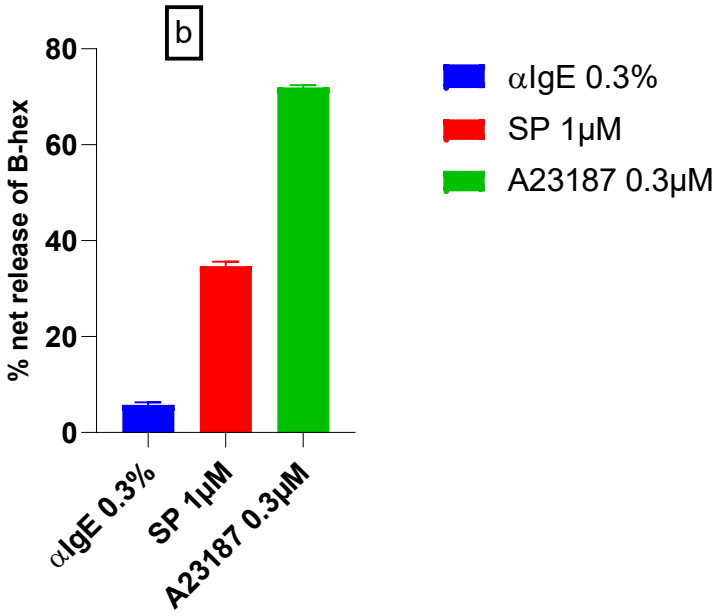


Figure 6-30: Release of β-hexosaminidase by different secreatagogues following IL-4 treatment**.**

Data was expressed as % net release of β-hexosaminidase. Experiment was done in triplicate and n=3.

6.5 Discussion

Substance P and αIgE stimulation of LAD2 cells displayed varied patterns of mediator release specifically with respect to cytokines and lipid mediators. In addition, SP causes rapid exocytosis as compared to αIgE as measured by time-lapse confocal microscopy (Gaudenzio *et al.*, 2016). Moreover, mast cell response to SP is short lived with poorly formed granules and fast exteriorisation while that of IgE is long lasting with fully formed granules with slow exteriorization. With regard to A23187 degranulation patterns are is similar to SP, and are rapid due to a direct action on the calcium channel and endoplasmic reticulum (Foreman, Mongar and Gomperts, 1973).

Akt is necessary in PI3K-mediated degranulation (Takayama *et al.*, 2013). Our data on Akt shows results, which are not in agreement with the assumption of Gaudenzio *et al.* (2016) that AKT-PKC pathway is exclusively IgE mediated. We have shown that substance P can also activate the AKT IgE-Independent pathway. The probable explanation for this might be related to the cell line used in the two different experiments where in the former LAD2 cells were used whilst BMMC cells were used in the one reported by Takayama *et al.* (2013). In addition, the sensitivity of the two techniques, Co-immunoprecipitation (less sensitive) against SDS-PAGE western blotting (more sensitive) account for the variation of the results. Whilst both SP and A23187 activated the Akt protein, the effect of MH on Akt at therapeutic concentrations (2% and 4%) did not reach statistical significance. Although, MH 6% was associated with increased LDH release, however it down regulated Akt expression in the SP induced model. The same pattern was observed with both isoforms of ERK in the three models (SP, A23187 and αIgE) tested. The reason for this unusual finding is still unclear.

The MAPK pathway does not evoke the release of histamine via degranulation, but rather the release of arachidonic acid and cytokines (Kimata *et al.*, 2000a; Kimata *et al.*, 2000b). However, Erk was reported to possess supplementary roles in IgE-mediated degranulation (Takayama *et al.*, 2013). This suggests that stabilisation of mast cell degranulation by MH in both the SP and A23187 induced models is through the downregulation of ERK. Notably TNF-α and IL-4 were elevated following the activation of the MAPK pathway (Fu *et al.*, 2019). In this respect it is interesting to note that the pre-treatment of mast cells with TNF-α inhibits degranulation (Gao *et al.*, 2017) and might be another route by which MH affects this process.

Generally, two receptors exist for SP: MRGPRX2 and NK1R. However, the SP mediated degranulation of LAD2 cells is exclusively mediated through the MRGPRX2 receptor due to the failure of NKIR specific inhibitors to block degranulation. MRGPRX2 receptors are abundantly expressed in the bladder, skin, colon, adipose tissues, as well as mast cell lines such as Human Mast Cell - 1 (HMC-1) and LAD2 cells (Tatemoto *et al.*, 2006).

In the same manner, they are activated by biologically diverse substances such as neuropeptides and plasma products - specifically albumins and HRP (Karhu *et al.*, 2017). Bladder mast cells are associated with nerve fibres which are rich in SP. Stimulation of these fibres causes degranulation of mast cells with consequent release of preformed mediators such as histamine and cysteinyl leukotrienes. These preformed mediators act in an autocrine fashion to activate their respective receptors on mast cells further inducing neuropeptide release that sustains the neurogenic inflammation cycle. MH has demonstrated significant reduction in the release of β-hexosaminidase and histamine in both SP and A23187 challenged cells. This suggests that MH might be useful in the treatment of IC/PBS where mast cell degranulation underlies the disease pathogenesis

Furthermore, neurogenic inflammation can also occur in an IgE mediated model, which has been demonstrated in this project where it is seen that both 2% and 4% MH reduce the degranulation of mast cells. Overall, the actions of MH on neurogenic inflammation have been extensively studied and reported. Thus, it is fair to conclude that MH could also be useful as a lead compound for the treatment of other neurogenic inflammatory conditions such as allergic rhinitis, psoriasis, allergy, and autoimmune diseases in addition to IC/PBS.

It is noteworthy, that effect of MH on the MRGPRX2 receptor was not elucidated. Given the fact that it is the only receptor activated by SP there remains the possibility MH could function through competitive antagonism of this receptor (Snider *et al.*, 1991). This is because significant reduction of the upstream signal Akt was demonstrated in SP induced signalling. Furthermore, MH could be beneficial in pseudo allergic drug reactions given that this event is primarily a function of the MRGRPX2 stimulation (McNeil *et al.*, 2015).

Degranulation by any pathway leads to release of preformed mediators particularly histamine. Histamine is reported to increase afferent nerve signal induction and smooth muscle contraction. In the bladder especially of the IC/PBS patient, it may be responsible for the bouts of episodic pain seen clinically in this condition. MH 2% and 4% showed significant inhibition of SP induced stimulation of histamine, suggesting it could be useful as an intravesical instillation in this condition. However, the same concentrations of MH failed to inhibit A23187 induced release of histamine in this model. This is an intriguing finding, given that both histamine and β-hexosaminidase are contents of mast cell granules. This differential action of MH on degranulation could be due to the fact that histamine is a biogenic amine and stored with serglycin proteoglycan, which exhibit morphological and phenotypical heterogeneity with storage sites being different for the two mediators (Wernersson and Pejler, 2014). This contrasts with β-hexosaminidase, which is stored exclusively in lysosomes.

This may account for the differential action of MH on histamine. In addition, it could be related to the stimulus, as exocytosis of a mast cell cytoplasmic granules is dependent on stimuli (Gaudenzio *et al.*, 2016). Moreover, A23187 is a strong stimulator of mast cell degranulation, and the effect of MH might not be strong enough to reduce the subsequent activation of exocytosis (Foreman, Mongar and Gomperts, 1973; Wightman *et al.*, 2002).

A more striking effect of MH is the inhibition of immunologic degranulation (IgE-degranulation). Degranulation induced by this route causes tissue damage and common disorders such as allergic asthma and hypersensitivity reactions. IgE degranulation is consequent upon cross-linking of IgE with the FcεRI receptor, which is tyrosine kinase dependent. This leads to phosphorylation of LAT (Linker for Activation of T-Cells) that activates PLC, which in turn hydrolyses P_1P_2 to IP_3 and DAG leading to mast cell degranulation (Gilfillan and Tkaczyk, 2006).

On the other hand, RAS activation via PKC leads to activation of MAPK with the resultant synthesis of cytokines. MH demonstrated significant inhibition of αIgE mediated degranulation and down regulation of ERK I and ERK II. This might be considered highly beneficial considering the fact that IgE levels have been found to be increased in the serum of 11% of IC/PBS patients and that allergic conditions such as asthma are comorbid associations with IC/BPS (Jhang and Kuo, 2015a). However, an effect on other signalling proteins and cytokines was not investigated due to poor responsiveness and loss of high affinity IgE receptor FcεRI of the LAD2 cells. This is one of the limitations of this project.

The mitogen activated protein kinases (MAPK) are a diverse class of kinases that play a crucial role in signal transduction, neural plasticity, and the inflammatory response. The members of this group include: extracellular signal-regulated kinase (ERK), p38 and c-Jun N-terminal kinase (JNK) (Ji *et al.*, 2009). Activation of MAPK leads to gene transcription that regulates the synthesis of cytokines in the nucleus. For instance, ERK regulates the production of GM-CSF in human cultured mast cells while p38 regulate the synthesis of IL-1 and TNF-α and this could explain why down-regulation of ERK by MH leads to inhibition of GM-CSF following both SP and A23187 challenge (Kimata *et al.*, 2000a). Similarly, MH increased the production of IL-1β, a not unexpected finding given that IL-1 and TNF-α are direct downstream targets of p38, and given that MH increases the expression of p38 (Kyriakis and Avruch, 1996). Furthermore, TNF-α may, itself, reduce mast cell degranulation (ref needed here). Such findings help to validate the results of the current study.

With regard to the effect of MH on cytokines, IL-8, also referred to as CXCL8, is a chemoattractant that recruits monocytes, lymphocytes, basophils, neutrophils and eosinophils to sites of inflammation (Baggiolini, Imboden and Detmers, 1992).

Furthermore, IL-8, via GPCR receptors, can activate MAPK and as a consequence can regulate gene expression and secondary degranulation (Turner *et al.*, 2014). Thus, the observed suppression of IL-8 by MH 2% and 4% might provide benefit in the context of inflammatory diseases such as IC/PBS. Likewise, a strong and positive correlation was observed between symptoms of IC and GM-CSF levels in a cyclophosphamide induced model of IC. This is in addition to the role as GM-CSF as a promoter of the inflammatory response through its attraction of monocyte and granulocytes to sites of inflammation (Smaldone *et al.*, 2009; Bhattacharya *et al.*, 2015). Thus, the MH inhibition of GM-CSF in A23187 and SP stimulated LAD2 cells reported in this study suggests a potential benefit for MH as a drug candidate in this the treatment of IC/BPS.

Activation of the MAPK pathway has been well demonstrated in various pain models (Cui *et al.*, 2008; Ji *et al.*, 2009). Of note, ERK upregulation was demonstrated in cyclophosphamide induced IC in a rat model, which correlated strongly with visceral hyperalgesia (Qiao and Gulick, 2007). Given that MH attenuated the expressions of ERK in SP, A23187 and αIgE stimulated cells suggests further potential benefit for this agent in the treatment of IC/PBS.

The actions of MH on phosphorylated ERK are not entirely unexpected given that a refined compound derived from MH – quercetin - was shown to abrogate ERK expression following non-immunologic stimulation by SP (Ding *et al.*, 2019). This study has shown for the first time that MH downregulates ERK expression following immunologic and non-immunologic stimulation in LAD2 cells.

PI3K, in addition to controlling the pathway for degranulation through calcium immobilisation (Okkenhaug and Vanhaesebroeck, 2003), controls varied biological functions which include, growth, metabolism and autophagy amongst others. Conversely, AKT PI3K dependent protein after receiving a signal via mTORC2 causes degranulation while MAPK intercepts the signal from MTORC1 causing activation and release of cytokines (Ashwell, 2006; Rakhmanova *et al.*, 2020)

Medical grade manuka honey demonstrates a good cell tolerability profile at lower doses. This is expected, in part, of natural products that have been consumed over long periods of time for both nutraceutical and therapeutic purposes. Secondary metabolites obtained from natural products have been identified as inhibitors of mast cell degranulation in several disease models particularly allergic asthma. Thus, Thymoquinone, a monoterpene isolated from *Nigella sativa,* suppressed pro-inflammatory cytokine release in LPS-induced RBL-2H3 cells (El Gazzar, 2007). In the same manner, reservatrol, a polyphenol from grapefruit, inhibits IgE dependent degranulation and histamine release in a murine model of passive cutaneous anaphylaxis through downregulation of PLCγ and PKC (Han *et al.*, 2013).

In addition, sinomenine, an alkaloid from a Chinese plant *Sinomenium acutum,* stabilised IgE mediated degranulation, inhibition of TNF-α and IL-4 through attenuation of Akt and p38 in RBL-2H3 cells (Huang *et al.*, 2008). Furthermore, isolated flavonols such as quercetin, kaempferol, myrecetin and morin inhibit immunologic degranulation, and cytokines (IL-4, IL-6 and TNF-α) release in human umbilical cord derived cultured mast cells (Kempuraj *et al.*, 2005). The above examples illustrate how active constituents of natural products can be effective in stabilising mast cell degranulation. However, most of the effects related to IgE dependent degranulation; little work was done in IgE-independent degranulation, which seems to drive interstitial cystitis via a neurogenic mechanism. Our data has shown that MH can also stabilises mast cell degranulation in an IgE-independent mast cell model – this may be particularly relevant to patients with IC/PBS.

This cellular model of IC/PBS is not without limitations. Firstly, LAD2 cells are cell line not primary cells. Consequently, there are some distinct differences with the primary cells. This can be seen in the observation that that TNF-α and MCP-1, despite being pre-formed mediators, are not detected in LAD2 cells supernatants. However, LAD2 was the most suitable cell to be use in our study considering that the experiments require many cells (in the tens of millions), something that is not feasible when using primary cells that lose responsiveness with fewer passage numbers and have a finite lifespan. In addition, LAD2 cells afford more consistent degranulation studies than other cell lines such as HMC-1, which is an immature cell line with poor responsiveness (Kirshenbaum *et al.*, 2003) or RBL-2H3, which is a murine cell line with varied human homology making extrapolation to human disease such as IC/PBS problematic. Another limitation of the LAD2 cells is the intermittent loss of FcεRI function which necessitated halting this study at one point. However, at the initial stage, loss of receptor function was restored by IL-4 treatment. However, with further increase in passage number, receptor function could not be restored.

6.6 Summary

In this chapter, the potential of MH in inhibiting degranulation of mast cells was clearly demonstrated. First, the cytotoxicity profiles of MH at various graded concentrations were tested using biochemical assays and microscopy. Then the effect of MH on degranulation using different routes was investigated. The actions of MH on the release of pro-inflammatory cytokines were investigated using ELISA. Lastly, how MH affects downstream signalling events was elucidated using SDS-PAGE western blotting.

6.7 Conclusion

Medical grade MH was shown to be tolerable to LAD2 cells at 2%-4% concentrations and inhibit the degranulation of mast cells, inhibit the release of histamine in an SP induced model, inhibit the synthesis of IL-1β and GM-CSF through regulation of ERK and p38 (in a SP induced model) and ERK alone in an A23187 induced model. Thus, MH shows considerable promise for use as a potential agent for use in the treatment of IC/PBS.

Chapter 7 Conclusion and future work

7.1 Overview

Chapter 5 provided a summary and conclusion of the epidemiological arm of the work whilst chapter 6 outlined conclusions based on the potential of MH as a useful therapeutic strategy for the treatment of IC/PBS. This chapter will review the purpose of the study, literature review, research questions/hypotheses and present the findings of this work together with a discussion and recommendations for further research.

7.2 Conclusion

This work was conceptualised to address a research gap in the epidemiology and treatment of IC/PBS. Given that this is a urologic condition with no identifiable aetiology and cure, it necessitates a trial of candidate agents with the hope that patient's quality of life could be improved. Moreover, there is paucity of epidemiological data relating to treatment and quality of life indices of these patients particularly in the UK.

In this project, literature relating to the epidemiology, aetiology and treatments of IC/PBS were reviewed. There was a significant research gap in areas of patient reported outcomes in IC/PBS; particularly in perception of illness (relationship between illness perception and severity of symptoms) and description of treatments. Consequently, the research questions in the PhD project were aimed at describing illness and treatments among IC/PBS cohorts. To answer these research questions, a cross sectional study was deemed an appropriate research tool. Thus, this was used to collect data using various validated questionnaires such as the O'Leary/Sant questionnaire, PUF, BIP-Q and KHQ in addition to a researcher developed questionnaire (lifestyle and diagnostic measures). The project was successful, and members of the IC/PBS cohorts responded to the survey questions. This provided the data necessary to bridge the gap between purely objective outcomes and patient reported outcomes in various spectrum of the disease. The study has shown that patients living with IC/PBS have a poor quality of life occasioned by increased concern and hopelessness that available treatments are ineffective in managing the disease. One of the main findings of this study was that the two domains "expectation that treatments will control symptoms" and "concern levels" were both predictive of symptom severity. With regards to the oral treatments used by participants for the disease, Amitriptyline was the most common used either alone or in combination with other medications. Likewise, many participants were not any treatments recommended by various urological association guidelines.

There were no significant differences between the mean O'Leary/Sant scores of participants on recommended guideline treatments and those who were not. Regarding beverages and alcohol consumption. There were no significant differences between the O'Leary/Sant scores of those drinking tea and coffee and those not drinking the beverages. On the contrary, there was decrease (improvement) in the O'Leary/Sant scores of those drinking alcohol compared to those drinking it. The survey identified that treatments were in ineffective, and participants enlisted natural products as potential treatments for the disease. Consequently, this latter hypothesis was tested by examining the potential role of manuka honey (a natural product in a cellular model of IC/PBS.

A cellular model of IC/PBS was developed based on the principle that mast degranulation underlies the aetiology of the disease. The degranulation of mast cells in IC/PBS is secondary to neuropeptide stimulation. Thus, SP – a neuropeptide was used to stimulate a human mast cell line – LAD2. Other, mast cell stimulators such as αIgE and A23187 were also used to examine the effect of MH on the degranulation of LAD2 cells.

It was hypothesised that manuka honey could be used as an intravesical instillation to prevent mast cell degranulation in IC/PBS. Thus, mast cells were pre-incubated with MH and challenged with stimulators and effects on degranulation were quantified by enzyme immunoassay with effects on histamine, cytokines and leukotrienes release measured using ELISA. The effects on downstream signalling were investigated using SDS PAGE western blotting.

MH inhibited the degranulation of LAD2 cells significantly in SP, A23187 and αIgE stimulated cells. MH also inhibited the release of histamine in SP but not A23187 stimulation. MH decreased the release of GM-CSF and IL-8 but increased IL-1β in both SP and A23187 induced models of the disease. MH down regulated the release of ERK, p38 but increase JNK in the SP induced model. MH attenuated the expression of ERK but increased p38 and JNK in an A23187 induced model. Moreover, MH reduced the expression of ERK in αIgE induced cells.

Taken together, MH demonstrated significant reduction of β-hexosaminidase release in all models used but decreased the release of histamine in an SP model but not an A23187 model. This suggests a discriminatory effect of MH on mast cell mediators, which orchestrate the inflammatory response. Hence, MH could be a valuable natural remedy for the treatment of IC/PBS. The differential effects on downstream signalling proteins are reflected by differential actions on cytokines, which are the immediate nuclear target of the preceding transducing proteins. This validates the use and outcomes of the chosen models. The significant effect of MH on ERK expression demonstrate that it could be a valuable agent in the treatment of IC/PBS given that ERK is the protein of interest that mediates hyperalgesia in a cyclophosphamide induce model of the disease.

This project is classical example of bedside-to-bench approach to treatments, where hypotheses generated from epidemiological data (bed) in the field were tested in the lab (bench) to improve disease treatment. This contrasts with the conventional bench-to-bedside approach that is time-consuming, capital-intensive, and laborious. In addition, the bedside-to-bench approach provided real time data.

7.3 Future work

IC/PBS is an under-researched disease of the urinary bladder, and this is apparent in the vague aetiologies of the disease, which is manifest as multiple differing treatments all with poor clinical outcomes.

7.3.1 Epidemiological data

This project has identified this treatment gap in IC/BPS via patient reported outcomes and tested the hypotheses generated thereof. Typical of cross-sectional study data, a potential for bias due non-randomisation and lack of causality could preclude risk associations between lifestyle indices and symptoms of IC/PBS. Non-randomisation is counteracted by use of the Randomised Control Trial (RCT), which provides the highest grade of medical evidence. However, this is not feasible in many cases due to ethical considerations. Thus, observational studies are mostly used to address these needs in research. Consequently, future work should build on these hypotheses and look at whether these indices have causality links using a prospective longitudinal cohort study. This is achieved by following up IC/PBS and non-IC/PBS cohorts over a time frame that will circumvent the causality limitation inherent in the cross-sectional design.

Another area that is worth researching in epidemiological data is the perceived causes of IC/PBS by respondents. Importantly, pelvic surgery and other surgical interventions have been enlisted as possible causes of the disease. In this study the survey data was limited by the fact that questions regarding these surgeries were not structured to distinguish whether IC/BPS occurred secondary to the surgeries or not. As a result, cohort longitudinal studies are needed to relate the risk of these surgeries to the onset of disease.

7.3.2 Manuka Honey as therapeutic strategy for IC/PBS

Manuka has demonstrated many potentially beneficial actions in cellular models of IC/PBS. However, one of the major limitations of this findings was that the effect of Manuka honey on IgE mediated signalling was inconclusive due to down-regulation of the FcεRI receptors.

Future work should investigate the effect of manuka honey on p38, JNK and Akt. Similarly, the mechanism(s) by which manuka honey regulates upstream signalling at receptor levels, ion channels and other phosphorylation targets such as PLC was not investigated. Thus, future research should consider these areas. In the same vein, the mechanistic pathway of manuka honey action in IC/PBS could be better predicted by an examination of gene expression profiling of the mRNA extracted from MH treated samples in comparison with non-treated samples following IC/PBS induction; genes downregulated by MH could suggest possible pathways of MH action. Similarly, future studies should consider other mast cell lines particularly primary cells.

Furthermore, our data could not explain the differential actions of MH on mediator release particularly on histamine and leukotriene. Future work should focus on the effect of MH on serglycin-dependent storage of histamine to see how this proteoglycan regulates MH effect. Moreover, our work looked at manuka honey as an entire product. Future work should isolate and characterise using quantitative structure activity relationship (QSAR) the bioactive components in MH such as MGO and their effects on this model. Mechanistic studies to investigate gene expression (mRNA) of tested concentrations using Polymerase Chain Reaction (PCR) would add substantially to the future direction of the project.

Another limitation of this work is that IC/PBS was modelled at a cellular level which could have significant variations at tissue and organ levels due to networks of cellular and tissue communications, which have crosstalk and feedback regulations. As result, future work should consider modelling IC/PBS in mammalian model such as rat, mice, or feline models which have similar homology with humans; only then might firm inferences be made as to the potential of manuka honey in the treatment if IC/BPS.

Appendix A Databases

Database	Number of hits	Search terms	Filter	Exclusion
Medline (Ovid)	133	"Interstitial Cystitis" OR "Pain* bladder syndrome" OR "Bladder pain syndrome" AND Epidemiology AND Treatment OR Management OR Honey	English	No exclusion
AMED	2			
CINNAHL	41			
EMBASE (Ovid)	186			
Web of Science	67			
PsychINFO	6			
TRIP	120	Treatment of Interstitial Cystitis OR Painful bladder syndrome OR bladder pain Syndrome	Primary research	No exclusion
Cochrane	526	Treatment of Interstitial Cystitis OR Painful bladder syndrome OR	Primary research	Trials

		bladder pain Syndrome		

Appendix B Questionnaire

A WEB BASED SURVEY ON CURRENT AND PAST TREATMENTS AND OUTCOME FOR PEOPLE LIVING WITH INTERSTITIAL CYSTITIS/PAINFUL BLADDER SYNDROME

This is a study by group of researchers from the University of Southampton on the treatment and outcome for people living with Interstitial Cystitis/Painful Bladder Syndrome (IC/PBS). Confidentiality of your data would be maintained throughout the study period and beyond. Participation in this survey is voluntary and participants are free to withdraw at any point of the study. Data obtained from this survey would be treated with the highest level of confidentiality by inputting into an excel spread sheet which will be password-protected, accessible only to the researchers. The survey takes approximately 20 minutes to complete.

The survey is made up of 8 sections viz:

1. Health history (12-questions)
2. Treatment (5-short questions)
3. The Pelvic Pain and Urgency/Frequency Index (12-questions)
4. The O'Leary/Sant IC Symptom Index (4-questions)
5. The O'Leary/Sant Problem Index (4-questions)
6. The King's Health Quality of Life Index (10-questions)
7. The Brief Illness Perception Index (9-questions)
8. Demographic Information (5-questions)

Instructions for completing the survey

1. Your consent for taking part in this survey will be indicated by checking the box provided. Thereafter, if you wish to continue you press the next button.
2. Please answer each question by checking at the appropriate box. You should answer the correct option by checking at the appropriate box for most question. However, where multiple answers are required it will be clearly specified by the side of the question in italics.
3. In some instances, an open question will be asked where a participant is asked to answer in his/her own words. In such circumstances, a free text column would be highlighted and the participant asked to make an appropriate comment.
4. Proceed through the survey using the 'save and continue' button at the end of each page
5. The 'save and quit' button will ensure your responses are saved and you can continue completing the survey at another time by following the instructions given
6. When you have finished the survey, please click the 'save and finish' button at the end of the survey.

CONSENT

1. I have read and understood the information sheet and have had the opportunity to ask questions about the study.
2. I agree to take part in this research project and agree for my data to be used for the purpose of this study
3. I understand my participation is voluntary and I may withdraw (at any time) for any reason without my participation rights being affected.
4. I understand that I will not be directly identified in any reports of the research.
5. I understand that should I withdraw from the study then the information collected about me up to this point may still be used for the purposes of achieving the objectives of the study only.
6. I understand that special category information i.e. health data, will be collected about me to achieve the objectives of the study.

Many thanks for your co-operation, we would be delighted if you could complete this survey by (07/03/19).

For further information, kindly contact:

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Supervisor, University of Southampton

Section 1. HEALTH HISTORY

The questions in this section will be about your medical history, we would like to have some background information relating to your bladder condition and lifestyle.

Question 1.1

What is your primary diagnosis? *(Please choose which one is your main concern)*

- ○ IC/PBS
- ○ Recurrent UTIs

Question 1.1b

What type is your IC/PBS?

- ○ Ulcer
- ○ Non-ulcer
- ○ I don't know

Question 1.2

For how long have you been living with IC/PBS?

- ○ Less than six months
- ○ More than six months less than one year
- ○ More than one year less than two years
- ○ More than two years less than five years
- ○ More than five years

Question 1.3

Do you know how your diagnosis of IC/PBS was made? *(You can check more than one option)*

- ☐ Cystoscopy (telescopic bladder check) awake
- ☐ Cystoscopy (telescopic bladder check) under anaesthesia
- ☐ Hydrodistension (stretching bladder under anaesthesia)
- ☐ Bladder biopsy
- ☐ Clinical history
- ☐ I don't know

Question 1.4

Apart from your bladder problem, have you been told if you suffer from any of the following condition(s)? *(You can check more than one option)*

- ☐ Vulvodynia (a condition of unexplained pain in the vulva)
- ☐ Endometriosis (a condition where tissues that houses the womb are found outside the womb)
- ☐ Asthma
- ☐ Fibromyalgia (a condition with widespread pain in the body)
- ☐ Chronic fatigue syndrome (a condition characterised by persistent tiredness)
- ☐ Systemic lupus erythematosus (a condition whereby the immune system attacks the body)
- ☐ Sjogren's syndrome (a disease affecting body parts producing fluids e.g. saliva and tears)

Question 1.5

Has anyone in your immediate or wider family ever diagnosed with IC/PBS?

- ○ Yes
- ○ No

Question 1.5b

Who among these in your family was ever diagnosed with this condition? *(you can check more than one option)*

- ☐ Father/Mother
- ☐ Grandfather(s)/Grandmother(s)
- ☐ Brother(s)/Sister(s)
- ☐ Child(ren)/Grandchild(ren)
- ☐ Uncle(s)/Aunt(y)ies

Question 1.6

Have you undergone any surgical operation(s) in your abdomen (tummy) or pelvis in the last ten years?

- ○ Yes
- ○ No

Question 1.6b

Select all that apply

- ☐ Operation on bladder
- ☐ Operation on womb (uterus) or ovaries

☐ Operation on bowel (intestine)

☐ Other operation on abdomen or pelvis)

Question 1.7

Do you have any form of allergy?

○ Yes

○ No

Question 1.7b

Please specify the food, medicine or substance you are allergic to

Question 1.8

How many cup(s) of coffee do you drink in a day?

○ None

○ 1-2

○ 3-4

○ 5-6

Question 1.9

How many cup(s) of tea do you drink in a day?

○ None

○ 1-2

○ 3-4

○ 5-6

Question 1.10

On a normal day, how many cigarettes do you smoke?

○ None

○ 1-5

○ 5-10

○ More than 10

Question 1.11

Do you vape electronic cigarettes?

○ Yes

○ No

Question 1.12

How many units of alcohol do you consume in a week? *(a unit of alcohol = 1 small glass of wine; or = 1 shot of spirit; or = half a pint of beer or lager)*

○ None

○ 1-5

- ○ 5-10
- ○ More than 10

Section 2. TREATMENT

In this section we will be asking questions relating to your medication(s) and other treatment(s) for your bladder condition.

Question 2.1

Which of these treatment(s) are you currently on for your bladder condition?

- ☐ Behavioural therapy
- ☐ Stress reduction
- ☐ Dietary manipulation
- ☐ Physical therapy
- ☐ Oral drug treatment
- ☐ Intravesical (in the bladder) drug treatment
- ☐ Others

Question 2.1b

Please specify

Question 2.2

Are you on any dietary modification therapy?

- ○ Yes
- ○ No

Question 2.3

Which of these medications have you ever taken for your bladder condition? *(you can check more than one option)*

- ☐ Pentosan polysulphate
- ☐ Amitriptyline
- ☐ Cimetidine
- ☐ L-arginine
- ☐ Cyclosporine
- ☐ Hydroxyzine
- ☐ Prednisolone
- ☐ Suplatast tosilate
- ☐ None
- ☐ Others
- ☐ I don't know

Question 2.3b

Please specify *(please enter your answer in the box provided)*

Question 2.4

Which of these medications do you normally take for your bladder condition? *(you can check more than one option)*

- ☐ Pentosan polysulphate
- ☐ Amitriptyline
- ☐ Cimetidine
- ☐ L-arginine
- ☐ Hydroxyzine
- ☐ Cyclosporine
- ☐ Prednisolone
- ☐ Suplatast tosilate
- ☐ None
- ☐ Others
- ☐ I don't know

Question 2.4b

Please specify *(please enter your answer in the box provided)*

Question 2.5

Has the treatment of your IC/PBS been undertaken by a GP alone?

- ○ Yes
- ○ No

Question 2.5b

Which of these health care professional manages your IC/PBS?

- ○ Urologist
- ○ Gynaecologist
- ○ Nurse
- ○ Others
- ○ I don't know

Section 3. THE PELVIC PAIN AND URGENCY/FREQUENCY INDEX

The questions in this section are related to the pain you feel around the lower part of your abdomen, the urge to urinate and how often you experience these symptoms.

Question 3.1

How many times do you go to the bathroom during the day?

- ○ 3-6
- ○ 7-10
- ○ 11-14
- ○ 15-19
- ○ 20+

Question 3.2

How many times do you go to the bathroom at night?

- ○ 0
- ○ 1
- ○ 2
- ○ 3
- ○ 4+

Question 3.3

If you get up at night to go to the bathroom, does it bother you?

- ○ Never
- ○ Mildly
- ○ Moderate
- ○ Severe

Question 3.4

Are you sexually active?

- ○ Yes
- ○ No

Question 3.4b

Do you now or have you had pain or symptoms during or after sexual intercourse?

- ○ Never
- ○ Mildly
- ○ Moderate
- ○ Severe

Question 3.4c

If you had pain, does it make you avoid sexual intercourse?

- ○ Never
- ○ Mildly
- ○ Moderate
- ○ Severe

Question 3.5

Do you have pain associated with your bladder or in your pelvis (vagina, lower abdomen, urethra, perineum, testis or sacrum)?

- ○ Yes
- ○ No

Question 3.5b

Is it usually

- ○ Mild
- ○ Moderate
- ○ Severe

Question 3.5c

Does your pain, bother you?

- ○ Never
- ○ Occasionally
- ○ Usually

Question 3.6

Do you have urgency after going to the bathroom?

- ○ Yes
- ○ No

Question 3.6b

Is it usually

- ○ Mild
- ○ Moderate
- ○ Severe

Question 3.6c

Does your urgency, bother you?

- ○ Occasionally
- ○ Usually
- ○ Always

Section 4. THE O'LEARY/SANT IC SYMPTOM INDEX

We are about to ask you questions relating to the symptoms of your bladder condition.

Question 4.1

How many times have you felt the strong urge to urinate with little or no warning?

- ○ Not at all
- ○ Less than I time in 5
- ○ Less than half the time
- ○ About half the time
- ○ More than half the time
- ○ Almost always

Question 4.2

During the past month, have you had to urinate, less than two hours after you finished urinating?

- ○ Not at all
- ○ Less than I time in 5
- ○ Less than half the time
- ○ About half the time
- ○ More than half the time
- ○ Almost always

Question 4.3

During the past month, how often did you most typically get up at night to urinate?

- ○ Never
- ○ Once
- ○ 2 times
- ○ 3 times
- ○ 4 times
- ○ 5 times
- ○ More than 5 times

Question 4.4

During the past month, have you experienced pain or burning in your bladder?

- ○ Not at all
- ○ Once
- ○ A few times
- ○ Fairly often
- ○ Almost always
- ○ Usually

Section 5. THE O'LEARY/SANT IC PROBLEM INDEX

The questions in this section will be regarding your concerns arising from your bladder condition.

Question 5.1

During the past month, how much has each of the following been a problem to you.

	No problem	Very small problem	Small problem	Medium problem	Big problem
Frequent urination during the day?	○	○	○	○	○
Getting up at night to urinate?	○	○	○	○	○
Need to urinate with little warning?	○	○	○	○	○
Burning pain, discomfort or pressure in your bladder?	○	○	○	○	○

Section 6. THE KING'S HEALTH QUALITY OF LIFE INDEX

We are about to ask you questions relating to your general well-being.

Question 6.1

GENERAL HEALTH PERCEPTION: How would you describe your health at present?

- ○ Very good
- ○ Good
- ○ Fair
- ○ Poor
- ○ Very poor

Question 6.2

INCONTINENCE IMPACT: How much do you think your bladder problem affects your life?

- ○ Not at all
- ○ A little
- ○ Moderately
- ○ A lot

Question 6.3

ROLE LIMITATIONS: Does your bladder problem affect your

	Not at all	A little	Moderately	A lot
household tasks e.g. cleaning, shopping etc?	○	○	○	○
job or normal activities outside the home?	○	○	○	○

Question 6.4

PHYSICAL LIMITATIONS: Does your bladder problem affect your

	Not at all	A little	Moderately	A lot
physical activities e.g. (going for walk, run, sports, gym, etc.)?	○	○	○	○
travel?	○	○	○	○

Question 6.5

SOCIAL LIMITATIONS: Does your bladder problem limit your

	Not at all	A little	Moderately	A lot
social life?	○	○	○	○
ability to see/visit friends?	○	○	○	○

Question 6.6

PERSONAL RELATIONSHIPS: Does your bladder problem affect your

	Not at all	A little	Moderately	A lot	Not applicable
personal relationship with your partner?	○	○	○	○	○
sex life?	○	○	○	○	○
family life?	○	○	○	○	○

Question 6.7

EMOTIONS: Does your bladder problem make you

	Not at all	A little	Moderately	Very much
feel depressed?	○	○	○	○
feel anxious or nervous?	○	○	○	○
feel bad about yourself?	○	○	○	○

Question 6.8

SLEEP/ENERGY: Does your bladder problem

	Not at all	A little	Moderately	A lot
affect your sleep?	○	○	○	○
make you feel worn out or tired?	○	○	○	○

Question 6.9

SEVERITY MEASURES: Do you do any of the following?

	Never	Sometimes	Often	All the time
Wear pads to keep dry	○	○	○	○
Be careful how much fluids you take	○	○	○	○
Change your underclothes because they get wet	○	○	○	○
Worry because you smell	○	○	○	○

Question 6.10

SYMPTOM SEVERITY SCALE: How much do they affect you?

	None	Mild	Moderate	Severe
Frequency of urination	○	○	○	○
Nocturia	○	○	○	○
Urgency	○	○	○	○
Urge incontinence	○	○	○	○
Stress incontinence	○	○	○	○
Nocturnal enuresis	○	○	○	○

Intercourse incontinence	○	○	○	○
Water works infection	○	○	○	○
Bladder pain	○	○	○	○
Post void dribble	○	○	○	○

Section 7. THE BRIEF ILLNESS PERCEPTION INDEX

The questions to be asked in this section will be related to how you perceive your bladder problem. The options provided are on a scale of 0-10. Kindly choose the one that best corresponds to your views.

Question 7.1

How much does your illness affect your life?

no effect at all

- ○ 0
- ○ 1
- ○ 2
- ○ 3
- ○ 4
- ○ 5
- ○ 6
- ○ 7
- ○ 8
- ○ 9
- ○ 10

severely affects my life

Question 7.2

How long do you think your illness will continue?

a very short time

- ○ 0
- ○ 1
- ○ 2
- ○ 3
- ○ 4
- ○ 5
- ○ 6
- ○ 7
- ○ 8
- ○ 9

○ 10

forever

Question 7.3

How much control do you have over your illness?

absolutely no control

○ 0
○ 1
○ 2
○ 3
○ 4
○ 5
○ 6
○ 7
○ 8
○ 9
○ 10

extreme amount of control

Question 7.4

How much do you think your treatment can help your illness?

not at all

○ 0
○ 1
○ 2
○ 3
○ 4
○ 5
○ 6
○ 7
○ 8
○ 9
○ 10

extremely helpful

Question 7.5

How much do you experience symptoms from your illness?

no symptoms at all

○ 0

- ○ 1
- ○ 2
- ○ 3
- ○ 4
- ○ 5
- ○ 6
- ○ 7
- ○ 8
- ○ 9
- ○ 10

many severe symptoms

Question 7.6

How concerned are you about your illness?

not at all concerned

- ○ 0
- ○ 1
- ○ 2
- ○ 3
- ○ 4
- ○ 5
- ○ 6
- ○ 7
- ○ 8
- ○ 9
- ○ 10

extremely concerned

Question 7.7

How well do you understand your illness?

don't understand at all

- ○ 0
- ○ 1
- ○ 2
- ○ 3
- ○ 4
- ○ 5
- ○ 6
- ○ 7

○ 8

○ 9

○ 10

understand very clearly

Question 7.8

How much does your illness affect you emotionally? (e.g. does it make you angry, scared, upset or depressed?)

not at all affected emotionally

○ 0

○ 1

○ 2

○ 3

○ 4

○ 5

○ 6

○ 7

○ 8

○ 9

○ 10

extremely affected emotionally

Question 7.9

Please list in rank-order the three most important factors that you believe caused your illness.

Section 8. DEMOGRAPHIC INFORMATION

Question 8.1

Age in years *(please type your response in the box provided)*

Question 8.2

Gender

○ Male

○ Female

○ Other

○ Prefer not to say

Question 8.3

Have you ever been involved in research related to IC/PBS?

- ○ Yes
- ○ No

Question 8.3b

Kindly indicate the type of the research.

- ○ Clinical trial
- ○ Survey
- ○ Others

Question 8.4

Kindly indicate if you would be happy to take part in any future surveys or other research related to IC/PBS.

- ○ Yes
- ○ No
- ○ Maybe

Please supply your email

Question 8.4b

Please supply your email

Thank you for taking part in this survey.

Appendix C Article No 1

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CLINICAL ARTICLE

WILEY

The relationship between illness perception and worsening of interstitial cystitis/painful bladder syndrome symptoms: A cross-sectional study

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Abstract

Objective: To evaluate disease perception in a cohort of patients with interstitial cystitis/painful bladder syndrome (IC/PBS) using the Brief Illness Perception-Questionnaire (BIP-Q) and to evaluate how this might relate to disease severity.

Materials and Methods: The study is a cross-sectional survey amongst members of Bladder Health UK who had previously received a clinical diagnosis of IC/PBS. A hyperlink containing the questionnaire was sent to the patient group's website and interested members accessed and completed the survey. Participants' inclusion was based on a prior clinical diagnosis of IC/PBS, current O'Leary Sant scores supportive of the diagnosis, and age between 18 and 80. A sample size of 171 was used in the study. The Brief Illness Perception Questionnaire (BIP-Q) and the O'Leary/Sant symptoms and problem indices questionnaire were used to collect data. A multivariable logistic regression analysis was used to test the relationship between items of BIP-Q and severity of IC/PBS. Content analysis was used for the causal domain and subsequently analysed as percentages.

Results: Six hundred and one members accessed the questionnaire of whom 159 returned completed questionnaires. One hundred and twenty-two of 159 (≥75%) respondents believe that their illness will continue indefinitely. The majority of the respondents indicated that IC/PBS had a negative impact on their daily lives, caused them worry and made them emotionally unstable. Of the 8 BIP-Q items, those most predictive of disease severity were (adjusted odd ratio and confidence intervals): consequence 0.094 (0.023–0.386); treatment control 2.702 (1.256–5.812); identity 0.141 (0.033–0.600); concern 9.363 (1.521–57.632).

Conclusions: Our findings show that IC/PBS negatively impacts participant's quality of life and emotional wellbeing. Higher expectation for treatment benefit and increasing levels of patient concern are predictive for severity of IC/PBS.

KEYWORDS
illness perception, interstitial cystitis/painful bladder syndrome, O'Leary Sant score, severity

1 | INTRODUCTION

Interstitial cystitis/painful bladder syndrome (IC/PBS) is a poorly understood, chronic, debilitating urological disorder that can significantly impair quality of life.[1] The precise etiology of the disease remains unclear, thus hampering the development of an optimum management strategy. It is, therefore, reasonable to broaden the scope of disease management by talking to patients to better understand and so address their unmet needs which could help improve overall quality of life.

The concept of patient's perception of illness was popularized by Leventhal et al.[2] in the Common Sense Model for self-regulation wherein a patient's set of beliefs regarding their illness was shown to be crucial in providing self-care for chronic diseases.[3] Thus, integrating these perceptions could help to form the basis of self-care in IC/PBS (chronic illness) management, which might then translate to an overall improvement of Health Related Quality of Life (HRQoL). More importantly, clinical assessment of disease morbidity is often difficult to establish due to the subjective nature of pain and discordance between patient and clinician perception of treatment outcomes in particular and the disease as a whole.[4] In this respect incorporating patient voices helps gain more insight into some of the constructs of IC/PBS, which are not open to clinical measurement. The end result being a better understanding of the impact of IC/PBS on patient lives.

Likewise, an individual's perspectives of their illness is as important as the caregivers views especially in chronic illnesses like IC/PBS with somatic components that necessitate physical and psychosocial evaluation.[5,6] Whilst much is known and utilised regarding Illness perception in many disease areas,[7] to the best of our knowledge no study exists in IC/PBS. Studies utilising the Illness perception tool are mostly descriptive and seldom shed insight into how these views might be predictive of established clinical benchmarks of illness progression.[8] Given this, we sought to describe Illness perception in IC/PBS and to asses any relationship between Illness Perception (IP) items of the Brief Illness Perception Questionnaire (BIP-Q) and IC/PBS severity to provide baseline data for patient's views relating to disease severity.

2 | MATERIALS AND METHODS

2.1 | Study design, sample and measurement

Approval for the study was obtained from the University of Southampton, via the Ethics and Research Governance Online (ERGOII) application system and review by The Faculty of Medicine Ethics Committee. Participants fully consented following full disclosure of the study aim and objectives. In addition, personal identifying data were not sought from the survey.

The survey hyperlink was made available for access to the participants between February 2019 and March 2019, on the website of Bladder Health UK (BHUK)—a charity for bladder-related problems in the United Kingdom. Convenience sampling was used in this survey. A sample size of 171 was projected based on confidence interval (CI) width of 0.15, CI of 0.5%, and planning proportion of 0.5. A sample size calculator developed by Naing et al.[9] was used to arrive at the sample size. The survey questions comprised the Brief Illness Perception Questionnaire (B-IPQ), the O'Leary/Sant Symptoms and Problem Indices Questionnaire and a record of the age and gender of the participants.

The O'Leary/Sant Symptoms and Problem (ICSI/ICPI) Questionnaire was used to ensure that participants reporting a previous clinical diagnosis of IC/PBS fulfilled the criteria for this diagnosis and was also used for stratification of illness severity. The O'Leary/Sant symptom and problem scale was developed as a tool to complement other noninvasive techniques in IC/PBS diagnosis and treatment monitoring. A cumulative score of 12 and above has a predictive value that is both positive and specific. Respondents were dichotomised into 0–18 (mild to moderate symptoms IC/PBS) and 19–36 (moderate to severe symptoms IC/PBS). Similarly, the 10-point scale of the BIP-Q was stratified into a lower level (0–5) and a higher level >5. The covariates from the BIP-Q scale were: consequence, timeline, personal control, treatment control, identity, concern, understanding, and emotional response.

2.2 | Statistical analysis

The study was supported by a medical statistician (Associate Professor Scott Harris). Data were collected using isurvey

software that automatically entered participant responses into a spreadsheet. In addition, it also recorded start and completion times, completion status, number of participants that accessed and completed the survey—the latter being used to calculate the response rate. The data was transferred to an excel sheet for cleaning and assessed for missing values and thereafter imported into IBM SPSS statistics for windows version 26.0 (IBM Corp.) for analysis.

Categorical variables and open-ended items following content analysis were analysed as proportions, while continuous variables as means (SD) and ranges.

Simple logistic regression was used to screen for each of the independent variables (consequence, timeline, personal control, treatment control, identity, concern, understanding, and emotional response) for inclusion in the multivariable analysis. Covariates with $p < 0.1$ at the univariable stage were included in the multivariable analysis. Multivariable logistic regression was used to test for BIP-Q items as risk factors for IC/PBS severity. The results of the univariable logistic regressions are presented as crude odds ratio (OR), 95% confidence intervals (CI) and corresponding p values, while those of the multivariable logistic regression are presented as adjusted OR, 95% CI and their corresponding p values. $p < 0.05$ were considered statistically significant. Goodness-of-fit model assumptions were checked using Hosmer-Lemeshow and Omnibus tests. Multicollinearity (to ensure that independent variables are not correlated) was checked by standard error of mean of the B coefficients.

3 | RESULTS

A total of 159 participants completed the questionnaire out of the 601 members that accessed the survey hyperlink, producing a response rate of 26.4%.

The mean age of participants was 55.40 years (SD = 15.38). In total, 92.6% of the participants were females. The mean (SD) O'Leary/Sant score of the participants was 19.93 (9.13).

3.1 | BIP-Q scores of the sample

Participants were of the opinion that the disease would proceed indefinitely, impact negatively on their well-being and had low belief that available treatments could help alleviate their symptoms (Table 1). In addition, participants felt a loss of control with respect to managing their disease and that their symptoms caused a high degree of both physical and emotional bother despite participants showing some understanding of their disease.

3.2 | Causal factors of IC/PBS

Respondents identified many factors perceived to be the cause(s) of their disease. All of the primary stated causes were grouped into categories by author's preference (Table 2). Infection and inflammation (UTIs, tonsillitis, cystitis, viral infection, auto-immune disease, and pain) and Lifestyle (diet, stress, anxiety, emotions, bereavement, holding on urine for too long, obesity, dehydration, and heatwave) accounted for more than half of all causes. Others included pelvic surgery/procedures (hysterectomy, surgery around the pelvis, cesarean section, and childbirth); hormonal dysregulation (changes in hormones as a result of menopause); and an anatomical defect (poor bladder lining). Major causes of IC/PBS as understood by the respondents are represented in Table 2. About one-quarter of the sample did not know the exact cause of their illness. Other ancillary causes

TABLE 1 Means (SD) of IC/PBS of perception from BIP-Q scale ($n = 159$)

Item	Mean (SD)	Scale (min-max)	Higher score means
Consequence	7.03 (2.64)	0–10	Greater perceived impacts of IC/PBS
Timeline	9.31 (1.67)	0–10	Stronger perception of chronicity of IC/PBS
Personal control	4.25 (2.96)	0–10	Greater personal control of IC/PBS
Treatment control	4.71 (3.17)	0–10	Stronger belief in the role of treatments on IC/PBS
Identity	7.04 (2.38)	0–10	Increased association of symptoms with IC/PBS
Concern	7.69 (2.48)	0–10	More bothered emotionally by IC/PBS
Understanding	7.68 (2.58)	0–10	Better understanding of IC/PBS
Emotional response	6.99 (2.79)	0–10	Highly affected emotionally by IC/PBS

Abbreviations: BIP-Q, Brief Illness Perception-Questionnaire; IC/PBS, interstitial cystitis/painful bladder syndrome.

TABLE 2 Showing major perceived causes of IC/PBS ($n = 159$)

Cause	N (%)
Infection and inflammation	46 (28.9)
Lifestyle	38 (23.8)
Pelvic surgery/procedure	14 (8.8)
Medication side effects	4 (2.5)
Genetics	3 (1.8)
Sjogren's syndrome	3 (1.8)
Anatomical defect	2 (1.2)
Endometriosis	2 (1.2)
Fibromyalgia	2 (1.2)
Hormonal dysregulation	2 (1.2)
Sexual activity/abuse	2 (1.2)
No Idea	41 (25.7)

Abbreviation: IC/PBS, interstitial cystitis/painful bladder syndrome.

included: Fibroids, constipation, Irritable bowel syndrome, chronic fatigue syndrome and ketamine abuse.

3.3 | Logistic regression outcomes

The logistic regression model is statistically significant $\chi^2(4) = 51.317$, $p = 0.000$ and explained 37% (Nagelkerke R^2) of the variance of IC/PBS severity and correctly classified 74.2% of the cases. Of the 8 BIP-Q items, only one item (understanding) was excluded in the multivariate analysis (Table 3; $p > 0.1$). Of those included, consequence (expected effects and outcome of the illness), treatment control (extent to which the patient believes that treatment can help recovery from or control of the illness), Identity (the label the person uses to describe the illness and the symptoms they view as being part of the disease) and concern (anxiety about the disease) had odds of 0.094, 2.702, 0.141, and 9.363 for predicting the severity of IC/PBS, respectively (Table 4). This equates to a 0.094 and 0.141 fold reduction in the odds of predicting disease severity for the consequence (disease effects and outcome) and Identity (labeling) items of the BIP-Q. While there was an increase in the odds for predicting disease severity by 2.702 and 9.363 times respectively for the treatment control and concern items.

4 | DISCUSSION

To the best of our knowledge, this is the first study that describes and explores illness perception in people living with IC/PBS and examines its relationship with disease

TABLE 3 A univariate logistic regression analysis of illness perception items as predictors of IC/PBS severity ($n = 159$)

Variable	OR	95% CI for OR		p value
		Lower	Higher	
Consequence	0.099	0.042	0.234	0.000
Timeline	0.270	0.069	1.060	0.061
Personal control	2.040	1.059	3.931	0.033
Treatment control	3.413	1.761	6.615	0.000
Identity	0.126	0.055	0.290	0.000
Concern	0.364	0.164	0.805	0.013
Understanding	1.266	0.551	2.913	0.578
Emotional response	0.308	0.143	0.662	0.003

Abbreviations: IC/PBS, interstitial cystitis/painful bladder syndrome; OR, crude odds ratio.

TABLE 4 A multivariate logistic regression analysis of illness perception items as predictors of IC/PBS severity ($n = 159$)

Variable	OR	95% CI for OR		p value
		Lower	Higher	
Consequence	0.094	0.023	0.386	0.001
Treatment control	2.702	1.256	5.812	0.011
Identity	0.141	0.033	0.600	0.008
Concern	9.363	1.521	57.632	0.016

Abbreviations: IC/PBS, interstitial cystitis/painful bladder syndrome; OR, adjusted odds ratio.

severity. The poor ratings of most of the items in the BIP-Q correlate with the negative impact on HRQoL seen in this disorder and are consistent with previous studies.[8,10]

Descriptively, the poor ratings of the psychological items of the BIP-Q point to the underlying mental health challenge experienced by these individuals. Psychological stress has long been reported as higher in IC/PBS cohorts compared to healthy controls.[11] Previous studies have also reported a higher prevalence of depressive disorders in IC/PBS patients compared to other lower urinary tract symptom sufferers.[12] Furthermore, the incidence of posttraumatic stress disorder (PTSD) and its associated psychological distress is higher in IC/PBS than in other Chronic Pelvic Pain conditions.[13] Deterioration of mental health as evaluated by depressive symptoms, isolationism, and helplessness amongst IC/PBS patients was shown to be risk factors for suicide that further complicates the psychiatric burden of the disease.[14,15] Not surprisingly, participants that were anxious about their illness, as represented by the concern item of the BIP-Q scale, had a 9.363 times greater likelihood of having

severe IC/PBS. The identity item assesses the description or labelling of illness in IC/PBS; higher scores in this item were negatively associated with the overall severity of the disease suggesting that the latter alone is not important in participants thoughts around being assigned a diagnosis of IC/PBS.

The higher scores of "concern" and "consequence" shown in response to the BIP questionnaire indicate depressive tendencies that have been recognised in IC/PBS patients.[16] Furthermore, the cohort reported higher timeline scores and lower treatment control scores, which correlate strongly with poor psychosocial and physical outcomes.[7] In the same manner, the cumulative BIP-Q scores in this sample were higher than those reported in cancer and periodontal diseases using a similar tool that serves to emphasise the poor quality of life associated with IC/PBS.[17,18] Participants generally indicated a lack of belief in the role of current IC/PBS treatments. This could be related to the need for hope that treatment will control symptoms, something that is more marked in those with more severe disease. Participants also indicated an inability to take personal control of their disease.

With respect to the causal factors—what did respondents believe to be the cause(s) of IC/PBS? It should be noted that, these are anecdotal views largely influenced by an individual participant's level of knowledge and education. In addition, mass media and physician-patient education are influential.[15] However, they are no less valid for that.

Most respondents reported infection and inflammation as the primary cause of their IC/PBS. Recurrent UTI's and other bladder infections are diseases that can be confused with IC/PBS and their presence usually excludes a diagnosis of IC/PBS.[19] Interestingly, participants identified inflammation as one of the causes of their illness, an outcome consistent with the mainstream understanding of IC/PBS pathogenesis, which is often characterised by activation of the bladder mast cells, cytokine, and nitric oxide release.[20,21] However, the unsatisfactory outcomes of available antihistamines and other immune-modulatory agents in the treatment of IC/PBS confound this theory. The presence of both histaminergic receptors on IC/PBS biopsies and pro-inflammatory mediators in biological samples point to the fact that these cell membrane proteins are critical therapeutic targets.

Lifestyle measures, identified by respondents, may be flare triggers, which could be misconstrued as causes. In the same manner, pelvic surgery was perceived as a major cause of the disease. This is consistent with other work reporting pelvic trauma as a contributory factor for the pathogenesis of the disease.[22] Equally important, a history of pelvic surgery was documented in IC/PBS cohorts before the onset of the disease.[23] Our data is limited by the fact that the timing of pelvic surgery related to the diagnosis of IC/PBS was not sought in the sample. Other causes of IC/PBS identified by respondents included endometriosis, fibromyalgia and Sjogren's syndrome

The strengths of this study include the fact that it is the first to look at illness perception and its relationship with severity of IC/PBS. Furthermore, data collection was from real-world IC/PBS sufferers and so helps to bridge the knowledge gap between patients and the clinic.

With respect to limitations, the authors recognise that data was collected anonymously and that convenience sampling was used. As a consequence, the results should be interpreted with caution because bias due to misrepresentation cannot be entirely excluded. Another weakness of the study is that it relies on respondents reporting an IC/PBS diagnosis made by a clinician without recourse to review of their clinical records. However, the fact that members of the charity have been consulting their care providers, are very engaged with their disease management, and fulfil the criteria for a diagnosis of IC/BPS using the O'Leary Sant questionnaire serves to reduce such bias. Our study did not extract data on educational levels of participants. Thus, data on perceived causes of the disease should also be interpreted with caution due to technical nature of this domain.

5 | CONCLUSION

The outcome of this study has shown poor illness perception in all items of the BIP-Q by individuals with IC/PBS. Of these items, belief in the fact that treatments will control symptoms and concern about the disease demonstrated a positive predictive value for severity of IC/PBS symptoms. Such findings confirm the importance that cognitive and emotional factors play in those affected by IC/PBS.

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DATA AVAILABILITY STATEMENT

The data in support of the findings of this study is available on request from the corresponding author.

CONFLICT OF INTERESTS

The authors declare that there are no conflict of interests.

ETHICS STATEMENT

Ethics approval was obtained from Ethics Research Governance Online II (ERGOII) of the University of Southampton, United Kingdom. Patient fully consented to participate in the study.

AUTHOR CONTRIBUTIONS

Conception and design: Kamaluddeen Garba, Brian R. Birch, Miriam Avery, and Bashir A. Lwaleed. *Drafting of the manuscript*: Kamaluddeen Garba, Brian R. Birch, Muhammadbukhoree Yusuh, Omar Abdelwahab, Scott Harris, Bashir A. Lwaleed. *Final approval*: Kamaluddeen Garba, Brian R. Birch, Miriam Avery, Muhammadbukhoree Yusuh, Omar Abdelwahab, Scott Harris, Bashir A. Lwaleed.

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Appendix D Article No 2

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CLINICAL ARTICLE

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Real world use of oral treatments in interstitial cystitis/bladder pain syndrome in the UK: Outcome of a cross sectional study

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Abstract

Background: To describe the oral treatments people living with interstitial cystitis/bladder pain syndrome (IC/BPS) are using to treat their urologic condition in the UK.

Method: A questionnaire hyperlink encompassing current and previous medications taken for IC/BPS with other sociodemographic and diagnostic indices was available to the Bladder Health UK website. Interested and fully consented individuals accessed and completed the survey.

Results: A total of 601 accessed the questionnaire of whom 173 participants responded (response rate: 28.7%) with a mean ± *SD* O'Leary/Sant scores of 20.12 ± 9.38. A sample size of 171 was estimated to be used in the survey. A fifth of the participants were not on any treatment at all. Amitriptyline was the most prevalent medication in use both alone and in combination. A shift in the use of unapproved (for IC/BPS) antidepressant, smooth muscle relaxant, opioids, gabapentenoids, and antibiotics was observed in the sample. There were no significant differences between the mean (*SD*) O'Leary/Sant scores of cohorts currently taking oral medications and those not taking it. More than two-thirds of the participants had been diagnosed with the disease more than 5 years. Just under a half (47.4%) of participants reported a history of allergy.

Conclusion: Our study provides contemporary evidence that the treatments used for managing IC/BPS encompass a broad range of medications both recommended and not recommended by current guidelines. The latter suggests patients are willing to try novel treatments when more conventional ones are ineffective.

KEYWORDS
amitriptyline, comorbidity, interstitial cystitis/pain bladder syndrome, oral treatments

1 | INTRODUCTION

Interstitial cystitis/bladder pain syndrome (IC/BPS) is a poorly understood disorder of the urinary bladder. Despite affecting millions of people around the world, there is no known cure.[1,2] The difficulty in both understanding and diagnosing the disease is partly due to the varying symptoms under which it may present. These, in turn, are a direct consequence of the different types of aetiologies held to be responsible for the disease.[3]

As a consequence, pharmacotherapy is often broad; mostly consisting of different drug classes, each addressing a particular symptom(s) or possible aetiology(ies). Glycosaminoglycan (GAG) replacement therapy, mast cell modulators, antidepressants, and immunosuppressant therapies have been used with varied outcomes, but overall treatment outcomes remain unsatisfactory.[4,5] Nevertheless, pharmacotherapy is an important component in the treatment algorithm for IC/BPS; being used when more conservative interventions are ineffective.[6]

The role of pharmacotherapy in the management of IC/BPS is emphasised in the management guidelines for most professional bodies in urology with slight variations in the grades of evidence and recommendations on how and when each member drug is to be used.[7,8] Rovner et al.[9] reported in the Interstitial Cystitis Database (ICDB) Study the existence of 183 treatments for IC/BPS. However, little is known regarding oral medications which occupy a central position in the treatment algorithm.

Cross sectional surveys are used to monitor treatment programs inter alia.[10] In particular, patient reported outcomes have been used to provide insight into the quality of life, dietary habits, and other experiential domains of IC/BPS sufferers.[11] In view of this, it is reasonable to identify the proportions of IC/BPS cohorts who are on oral therapy and in particular the specific treatments they are using with a view to give a clear description of this population.

In this study, we aimed to collect and describe the use of oral treatments for IC/BPS in the UK with the view to providing baseline data that could generate plausible hypotheses for the role of IC/BPS oral pharmacotherapy.

2 | METHODS

2.1 | Questionnaire content and development

The questionnaire consisted of items pertaining to sociodemographic variables, diagnostic procedures used in establishing diagnosis, medication history and dietary consumption pattern of the participants. Participants were presented with a list of guideline-recommended IC/BPS oral treatments and asked to indicate in a multiple response manner whether they have used/were using any of these medications. In addition, an open-ended item was included for them to include any other treatment they had used/were using for their bladder condition. Moreover, enquiries relating to length of time since IC/BPS was diagnosed and disease type in the cohort were made. Participants who had received a clinical diagnosis of IC/BPS also completed the O'Leary/Sant questionnaire to help confirm that they fulfilled the criteria for a diagnosis of IC/BPS thereby justifying their inclusion in the study.

2.2 | Sample and questionnaire administration

Data were collected from a convenience sample of members of Bladder Health UK—a bladder focused charity in the UK. Approval for the study was obtained from the Faculty of Medicine ethics committee at the University of Southampton, United Kingdom. Those members with a prior clinical diagnosis of IC/BPS were approached. The only inclusion criterion was age between 18 and 80 years. The questionnaire hyperlink was made available at the charity's website between February 2019 and March 2019. Interested and consented members accessed and completed the survey. Based on the confidence interval (CI) width of 0.15, CI of 0.5% and a planning proportion of 0.5, a sample size of 171 was deemed suitable for statistical purposes (precision of findings).

2.3 | Statistical analysis

Data were collected using isurvey software and result transformed into IBM SPSS statistics for windows version 26.0 (IBM Corp.) for analysis. Data were presented as means (*SD*) and percentages. Responses to questions were structured in multiple response pattern. Thus, multiple response analysis was used as applicable. A student *t* test was used to compare means between two numerical groups and the value of *p* was set at 0.05 (two-tailed).

3 | RESULTS

A total of 173 participants completed the survey out of the 601 members that accessed the survey hyperlink producing a response rate of 28.7%. The mean (*SD*) age of the participants was 56.13 (15.39) years and 93.1% of the participants were females as shown in Table 1.

Table 1 shows that more than 90% of the participants have been living with their condition for more than 2 years and have no family history of the disease. In terms of care providers, 59% indicated they are being managed by a urologist while only 5.8% are being seen by a gynaecologist. Almost half of the participants reported a history of allergy, although specific allergens were not

Appendix D

TABLE 1 Description of the sample ($n = 173$)

Variable	N (%)	Mean (SD)
Age		56.13 (15.39)
Gender		
Female	161 (93.1)	
Male	12 (6.9)	
IC/BPS		
Ulcer	35 (20.2)	
Non-ulcer	76 (43.9)	
Don't know	62 (35.8)	
O'Leary/Sant score		20.12 (9.38)
Period of time since diagnosis of IC/BPS		
>6months <1year	3 (1.7)	
>1year <2years	14 (8.1)	
>2years <5years	38 (22.0)	
>5years	118 (68.2)	
Healthcare professional managing IC/BPS		
Urologist	102 (59.0)	
Gynaecologist	10 (5.8)	
Nurse	17 (9.8)	
Don't know	23 (13.2)	
Others*	21 (12.1)	
Family members with IC/BPS		
Father/Mother	4 (2.3)	
Grandfather/Grandmother	1 (0.6)	
Brother(s)/Sister(s)	0 (0.0)	
Children/Grandchildren	3 (1.7)	
Uncle(s)/aunt(ies)	1 (0.6)	
Cumulative family members with IC/BPS**	9 (5.2)	
Allergy	82 (47.4)	
Cups of coffee drunk in a day		
None	106 (61.3)	
1–2	57 (32.9)	
3–4	9 (5.2)	
5–6	1 (0.6)	
Cups of tea drink in a day		
None	79 (45.7)	
1–2	47 (27.2)	
3–4	30 (17.3)	
5–6	17 (9.8)	
Units*** of alcohol consume in a week		
None	104 (60.1)	
1-5	48 (27.7)	
5-10	16 (9.2)	
>10	5 (2.9)	
Number of cigarettes smoked in a day		
None	165 (95.4)	
1–5	1 (0.6)	
5–10	3 (1.7)	
>10	4 (2.3)	
Vape e-cigarettes	3 (1.7)	

*Include pain specialist, nutritionist and nonresponse by participants.
**Represent total family members with the disease.
***Unit of alcohol = 1 small glass of wine; or = 1 shot of spirit; or = half a pint of beer or lager.

recorded. There was no significant difference between the O'Leary/Sant scores of those with allergy 19.41 (10.02) and those without it 20.76 (8.76).

Regarding beverages, 54.3, 38.7%, and 39.9% consumed tea, coffee, and alcohol, respectively. Only 4.6% and 1.7% smoked cigarettes or vaped e-cigarette, respectively. There were no significant differences between the O'Leary/Sant scores of those drinking 18.78 (10.02) and not drinking 20.97 (8.89) coffee and of those drinking 19.24 (9.46) and not drinking tea 21.16 (9.23). However, there were significant differences in the mean O'Leary/Sant scores of cohorts smoking 27.13 (9.28) and not smoking cigarettes 19.78 (9.28); and those drinking 17.17 (8.98) and not drinking alcohol 22.13 (9.15).

From Table 2, participants mostly underwent more than one diagnostic procedure to confirm their diagnosis. Cystoscopy under anaesthesia appeared to be the commonest procedure used either alone or in combination. Only a very small number of participants had no idea as to the type of procedure they had undergone.

Endometriosis, vulvodynia and asthma were the commonest comorbidities in the sample in descending order of frequency of cases. Systemic Lupus Erythematosus was the least reported comorbid condition (Table 3). The relationship between presence/absence of specific comorbid condition and O'Leary/Sant scores was run using an independent t test. Only vulvodynia and chronic fatigue syndrome showed a significant difference. This underscores the finding that other chronic conditions often coexist with IC/BPS. The mean (SD) O'Leary/Sant cores of those having and not having vulvodynia were 24.09 (10.24)

TABLE 5 Proportions of current medications used by the participants using multiple response analysis ($n = 173$)

Medications status	Frequency	% responses	% of cases
Pentosan polysulphate	16	7.3	9.4
Amitriptyline	45	20.5	26.5
Cimetidine	11	5.0	6.5
L-arginine	2	0.9	1.2
Hydroxyzine	13	5.9	7.6
Prednisolone	3	1.4	1.8
None	62	28.3	36.5
Others*	63	28.8	37.1
I don't know	4	1.8	2.4
Total	219	100.0	128.8

Note: Missing cases: 3.
*Others currently in use viz: Antibiotics (cefradine, flucloxacillin, cephalexin, and nitrofurantoin); Smooth antispasmodics (solifenacin, oxybutynin, and tamsulosin); Proton pump inhibitors (ranitidine, lansoprazole); Antidepressants (nortriptyline, imipramine, fluoxetine, duloxetine); Opioids (morphine, paracetamol and codeine, phenazopyridine, naltrexone, oxycodone, hydromorphone, and tramadol); NSAIDs (celecoxib) Gabapentenoids (pregabalin and gabapentin); heparin, hexamethylenetetramine, cannabis oil, and herbal tea.

addition to its known antidepressant action. Thus, it also has a smooth muscle relaxant effect, mast cell stabilising actions, provides relief of neurogenic pain and is a sedative;[12] all actions which help aid pain relief and improve sleep in IC/BPS. It is to be noted that amitriptyline is the only recommended (by guidelines) antidepressant for IC/BPS use due to a supportive evidence base.[13] Although the use of the antidepressant's nortriptyline, fluoxetine, duloxetine, and imipramine were also noted in the cohort, these were based on "off-label" use. The reason for this statement being that the studies in support of their use in IC/BPS were inadequately powered, flawed, nonrandomised or performed on non-IC/BPS patients. For instance, van Ophoven and Hertle[14] reported a nonsignificant change in global response assessment from baseline when using duloxetine in IC/BPS patients in a nonrandomised–noncontrolled prospective study. Likewise, a randomised control trial of nortriptyline showed a significant ($p \leq 0.05$) reduction in mean pain scores in the nortriptyline arm. However, the study population were chronic pelvic pain (CPP) sufferers rather those with a diagnosis of IC/BPS.[15]

With respect to gabapentenoids, the rationale for their use in IC/BPS stems from modest improvements in pain seen when they are used to treat neuropathic pain, vulvodynia and related conditions where there is plausible central nervous system involvement.[3,16] Gabapentin is thought to act via increasing the inhibitory neurotransmitter (Gamma-aminobutyric acid) at postsynaptic fibres and also inhibiting calcium currents.[17] Gabapentin had shown significant reductions in visual analogue scale pain scores at both 12 and 24 weeks from baseline, when compared to placebo in a randomised double blind control trial of CPP patients.[18] The fact that it has not been trialled in IC/BPS makes the evidence anecdotal.

Regarding opioids, a paradigm shift in the increased consumption of opioids was observed in the cohort (Tables 4 and 5). Recently, Zillioux et al.[19] reported an upsurge in opioid consumption among IC/BPS patients. This, together with our findings, suggests an unmet need in pain management in this patient group. While the use of opioids in noncancer patients especially those with IC/BPS is still open to debate,[20] there is a clear desire to optimise nonopioid pain management in IC/BPS using existing or novel (e.g., cannabinoids) drugs. Although our study is limited by the fact that participants were not questioned further on how long they had been on opioids, given that IC/BPS is a chronic disease, the possibility of chronic usage cannot be excluded. This raise concerns relating to tolerance, addiction, and misuse.

While smooth muscle relaxants (anticholinergic) such as solifenacin have been tried in overactive bladder with modest outcomes,[21] there is no good evidence to justify their use in IC/BPS. Similarly, antibiotic use in the cohort cannot be supported because they are neither indicated for IC/BPS nor any of the comorbid condition the participants have identified to be suffering from. Nevertheless, such drugs are not uncommonly used for symptom control in IC/BPS.

Overall, there was a trend towards the use of guidelines-not-recommended medications to treat this frustrating urologic condition. This suggests a dissatisfaction on the part of caregivers with recommended drugs who need to address the pain experienced by the patients under their care. The minimum we should be doing is to try to provide an evidence base for the use of such treatments using randomised-controlled trials where this is possible or well-designed observational studies where this is not so.

Tea, coffee, and alcoholic drinks have been long identified as triggers for the worsening of IC/BPS symptoms.[22] Whether these items should be entirely avoided is still a subject of debate due to the individual nature of their impact on IC/BPS patients. However, in this sample, the nonsignificant difference in the O'Leary/Sant scores of those drinking and not drinking tea and coffee does not support any correlation between their use and IC/BPS symptoms. Conversely, a significant decrease in O'Leary Sant scores of those drinking alcohol compared to nonalcoholic drinkers was observed. This is highly

TABLE 2 Diagnostic procedures undertaken by participants for IC/BPS diagnosis by multiple response analysis ($n = 173$)

Diagnostic procedure	Frequency	% responses	% of cases
Cystoscopy under AE	114	35.6	65.9
Cystoscopy awake	47	14.7	27.2
Hydrodistension	60	18.8	34.7
Bladder biopsy	52	16.3	30.1
Clinical history	44	13.8	25.4
Don't know	3	0.9	1.7
Total	320	100	185.0

Abbreviation: AE, general anaesthesia.

and 19.51 (9.12) $p = 0.029$, respectively. Similarly, the scores for those having and not having chronic fatigue syndrome were 26.79 (5.32) and 19.53 (9.44) $p = 0.05$, respectively.

From Table 4, (sample size is 173), it can be seen that the per cent cases is 214.7% because some participants had taken more than one drug in the past and were allowed to have multiple responses to include both past and current drug usage. A fifth of participants reported being on no treatment at the time of the survey.

Respondents previously on recommended medications by current guidelines for IC/BPS had an O'Leary Sant score of 20.48 ± 9.49, while those who had not used recommended medications had a score of 17.68 ± 8.36 ($p = 0.193$).

Similarly, Table 5 has a cases greater than the responses indicating some participants were taking more than one. Of note, amitriptyline was a widely used medication either alone or in combination while L-arginine was the least used. Participants currently on any recommended medication and those who had not use alternative medication had mean $\pm$ *SD* O'Leary/Sant scores of 20.76 ± 9.38 and 18.98 ± 9.34 ($p = 0.234$), respectively.

4 | DISCUSSION

This is, to our knowledge, the first cross-sectional study to look at oral treatments in IC/BPS sufferers. Strikingly, the majority of participants were not on any oral treatment despite elevated O'Leary/Sant scores (moderate symptoms). This serves to underline the fact that for many participants the available oral treatments lack efficacy. It is also imperative to highlight the fact that a higher attrition rate (Tables 4 and 5) was observed for participants on no-medications which further strengthened this view.

TABLE 3 Distribution of comorbidity in the sample using multiple response analysis ($n = 173$)

Comorbid disease	Frequency	% responses	% of cases
Vulvodynia	23	20.0	30.7
Endometriosis	25	21.7	33.3
Asthma	22	19.1	29.3
Fibromyalgia	21	18.3	28.0
Chronic fatigue syndrome	14	12.2	18.7
Systemic lupus erythematosus	3	2.6	4.0
Sjogren's syndrome	7	6.1	9.3
Total	115	100.0	153.3

TABLE 4 Proportions of previous medications consumed by participants using multiple response analysis ($n = 173$)

Medication status	Frequency	% responses	% of cases
Pentosan polysulphate	33	9.0	19.4
Amitriptyline	100	27.4	58.8
Cimetidine	57	15.6	33.5
L-arginine	10	2.7	5.9
Cyclosporine	9	2.5	5.3
Hydroxyzine	34	9.3	20.0
Prednisolone	15	4.1	8.8
None	22	6.0	12.9
Others*	75	20.5	44.1
Don't know	10	2.7	5.9
Total	365	100.0	214.7

Note: Missing cases: 3.
*Other drugs used previously include: antibiotics (Cefradine, flucloxacillin, trimethoprim, doxycycline, and fluroquinolones) nortriptyline, solifenacin, mirabregon, duloxetine, tamsulosin, diclofenac, gabapentin, pregabalin, paracetamol, naproxen, uribel, ranitidine, tramadol, hydroxychloroquine, desmopressin, and anticholinergics.

From the survey, amitriptyline was the most frequent drug used for symptom control in the participants. Our finding is consistent with the outcome of the Interstitial Cystitis Database Study that also reported amitriptyline to be the most widely prescribed medication for IC/BPS.[9] The frequency of usage in the latter study was 16%, while in the current study it was 20.5%, figures that are in broad agreement. This widespread use of amitriptyline can be explained by the fact that the drug has a multiplicity of actions in

unexpected considering the diuretic effect of alcohol could mean frequent urination with attendant elevation of the problem domain of the O'Leary/Sant scale. Alternatively, IC/BPS individuals in the cohort could be aware of the adverse effects of alcohol and choose to avoid it, this being most marked in those with worse symptoms.

The fact that only 4.6% of our cohort smoked cigarettes compared to 87.9% in the snapshot study reported by Kleier[23] suggests good lifestyle measures are being adopted in this patient sample. Tobacco smoke and its constituents have deleterious effects in most bodily systems and organs with well-known adverse effects on the cardiovascular system, respiratory system, and the bladder. Given this, it is hard to conclude that the cumulative benefits of smoking cessation would have any effect other than to improve bladder function.

One of the limitations of our study is that data were collected directly from the participants and not retrieved from their clinical records which could be a source of recall bias. However, given that the option was provided for participants to choose from a list of drugs in the questionnaire minimises this risk. In addition, there was a free text option to describe any other medication they might be using, thereby providing a more complete capture of their treatments.

Another possible limitation is that the sample population was pooled from an IC/BPS charity rather than directly from a medical setting which could lead to concern regarding the accuracy of their IC/BPS diagnosis. However, participants were all originally diagnosed by medical personnel and the use of O'Leary/Sant scores to screen patients offers an additional layer of diagnostic security. The geographically diverse nature of the participants from the charity helps to strengthen the validity of our findings and counteract bias arising from the convenience sampling design.

5 | CONCLUSION

We have in this study provided a snapshot of the oral treatments currently being offered to and used by individuals with IC/BPS in the UK. The complexity of the disease is reflected in the diversity of drugs classes being used in attempt to treat this difficult condition. A substantial proportion of the sample are still on medications not commonly used to treat the disease, as reflected by frequency of "others."

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CONFLICT OF INTERESTS

The authors declare that there are no conflict of interests.

ETHICS OF APPROVAL STATEMENT

Ethics approval was obtained from Ethics Research Governance Online II (ERGOII) of the University of Southampton, United Kingdom.

AUTHOR CONTRIBUTIONS

Conception and design: Kamaluddeen Garba, Brian R. Birch, Miriam Avery, and Bashir A. Lwaleed. *Drafting of the manuscript*: Kamaluddeen Garba, Brian R. Birch, Muhammadbukhoree Yusuh, Omar Abdelwahab, Scott Harris, and Bashir A. Lwaleed. *Final approval*: Kamaluddeen Garba, Brian R. Birch, Miriam Avery, Muhammadbukhoree Yusuh, Omar Abdelwahab, Scott Harris, and Bashir A. Lwaleed.

DATA AVAILABILITY STATEMENT

The data in support of the findings of this workstudy is available on request from the corresponding author.

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