A STUDY ON CYTOKINE RESPONSE IN DIARRHEA PREDOMINANT IRRITABLE BOWEL SYNDROME

Dissertation submitted in partial fulfillment of the requirements for the Degree of

D.M. (MEDICAL GASTROENTEROLOGY)

BRANCH - IV

THE TAMILNADU Dr .M.G.R MEDICAL UNIVERSITY
CHENNAI-600 032.

FEBRUARY 2015

CERTIFICATE

This is to certify that this dissertation entitled **"A STUDY ON CYTOKINE RESPONSE IN DIARRHEA PREDOMINANT IRRITABLE BOWEL SYNDROME"** submitted by **Dr.M.Tarakeshwari** to the faculty of Medical Gastroenterology, The Tamilnadu Dr.M.G.R. Medical University, Guindy, Chennai – 600 032, in partial fulfilment of the requirement for the award of DM Degree, Branch IV (Medical Gastroenterology) is a bonafide work carried out by her under my direct supervision and guidance.

Dr.P.Ganesh M.D.,D.M.
Guide, Professor and HOD,
Department of Medical Gastroenterology,
(DDHD@GPH, Annanagar),
Kilpauk Medical College
Chennai

Dr.N.Gunasekaran, M.D., DTCD
Dean,
Kilpauk Medical College,
Chennai

DECLARATION

I, **Dr.M.Tarakeshwari,** solemnly declare that this dissertation **"A study on Cytokine Response in Diarrhea Predominant Irritable Bowel Syndrome"** was done by me at the Department of Medical Gastroenterology, (DDHD@GPH, Annanagar), Kilpauk Medical College, Chennai-10 under the guidance and supervision of the Professor and HOD of Medical Gastroenterology, Kilpauk Medical College, Chennai-10 between 2011 and 2015.

This dissertation is submitted to the Tamil Nadu Dr.M.G.R. Medical University, Chennai-600032 in partial fulfillment of the University requirements for the award of the DM Degree in Medical Gastroenterology.

(Dr.M.TARAKESHWARI)

Place: Chennai
Date: 15.10.2014

ACKNOWLEDGEMENT

I am extremely grateful to **Prof.Dr.N.Gunasekaran, M.D., DTCD.,** Dean, Kilpauk Medical College, Chennai.

It is with great pleasure that, I record my indebtedness to my academic guide, **Prof. P.Ganesh, M.D., D.M.,** Professor of Medical Gastroenterology, Department of Digestive Health and Diseases, Kilpauk Medical College, Chennai, for giving me the chance to undertake this dissertation work under his guidance. Also I express my sincere gratitude for his constant encouragement, directions, periodical discussions, rigorous reviews and precious suggestions that helped in the shaping of my dissertation.

I express my sincere gratitude to **Dr.S.Jeevankumar, M.D., D.M.,** Professor of Medical Gastroenterology, Department of Digestive Health and Diseases, Kilpauk Medical College, Chennai, for giving me the permission to do this dissertation in Govt Peripheral Hospital, AnnaNagar, Chennai-102 and also his kind encouragement and review of my work, besides providing me with all the required facilities.

I also thank **Dr.T.Rajkumar Solomon, M.D.,D.M.,** Professor of Medical Gastroenterology, Department of Digestive Health and Diseases, Kilpauk Medical College, Chennai for his constant encouragement and suggestions for my study.

I am also thankful to **Dr.Balaji**, Assistant Professor, Department of Social and Preventive Medicine, SRM College, Chennai for his help in the statistical analysis of my dissertation work.

I am also extremely thankful to **Dr.R.Balamurali, M.D., D.M., Dr.G.Ramkumar, M.D., D.M.,** and **Dr.K.Muthukumaran, M.D., D.M.**, who guided me a lot.

I am also very thankful to all my fellow residents who have helped me in this dissertation.

I thank all the patients who volunteered in this study, without whom this dissertation would not have seen the light of the day.

Last but not the least, I would like to thank my husband, **Dr.V.C.Shrinivas** who supported me in every possible way and also offered valuable tips in research methodology.

TABLE OF CONTENTS

INTRODUCTION

INTRODUCTION

Irritable bowel syndrome is a functional disorder which is characterised by recurrent abdominal pain and altered bowel habits. The prevalence ranges between 10% - 20% in the western population. However in Indian population the prevalence ranges between 4.9% - 7.2%. The pathophysiology of IBS is still elusive and advances in molecular evaluation including the likes of proteomics and genomics have only added newer hypothesis to the existing lot. Among the proposed pathophysiology visceral hypersensitivity, altered gut motility and psychosocial factors are the well known age old mechanisms. Recently numerous studies have demonstrated evidence showing inflammation and mucosal immune activation to be a part of the disease. Mucosal inflammation as demonstrated by raised fecal calprotectin levels, marker of inflammation and organic disease has been reported in a few studies as also mucosal cytokine imbalance. This is especially so in post infectious IBS. Cytokines both pro and anti-inflammatory play a vital role in immune modulation. The normal gastrointestinal immune response is under tight regulation with the balance between pro and anti-inflammatory cytokines defining the immune status of the gut.

Tumour necrosis factor alpha a polypeptide cytokine produced by monocytes and macrophages, has a crucial role in chronic inflammatory states such as inflammatory bowel disease and rheumatoid arthritis. Patients with post infectious IBS have a five-fold increase in the number of activated macrophages in the rectal lamina propria. The macrophage derived TNF-α has been shown to play a pivotal role in orchestrating the cytokine cascade in many inflammatory diseases and was once thought to be the major mediator of sepsis cascade. Cachectin (TNF) is the "master-regulator" of inflammatory cytokine production.

AIMS AND OBJECTIVES

AIMS AND OBJECTIVES

Primary

- To estimate serum levels of pro-inflammatory cytokine TNF α in adult patients with diarrheal type of irritable bowel syndrome and compare it with sex and age matched healthy volunteers.

Secondary

- To correlate TNF α levels with severity of symptoms.

REVIEW OF LITERATURE

REVIEW OF LITERATURE

Irritable Bowel Syndrome, one of the most prevalent functional gastrointestinal disorders worldwide, can trace its symptomatology to antiquity. It is an intriguing disease with varied spectrum of presentations, the understanding of which has undergone a rapid evolution with scientific advancement.

Historical perspective

The first report of the disease dates back to almost 150 years when in 1849, Cumming reported functional disorder of the bowel characterised by constipation and the same time irritable and wondered at the possible mechanism behind it.[1]

It has been variously described by Manning and Fred H. kruse, as irritable colon, spastic colon and functional colonopathy wherein they ponder for an adequately good name to describe the bowel difficulties of the common man. Through the centuries the treatment of this disease has remained elusive making patients frequent the hospitals more and has impaired quality of life[2].

Indian perspective

The bowel pattern of Indians is different from that of the Westerners. In India, 99% of normal subjects have a stool frequency of at least 1 or more

per day. This is in contrast to a normal stool frequency of three times per week to three times per day in the West.

There are some key differences between IBS in India and the West. Abdominal pain has been described as upper abdominal pain in Indians compared to lower or generalised abdominal pain seen in west. Increased frequency of stools (tachychezia) is common in Indian population.

The Asian Neurogastroenterologist and Motility Association suggest the use of a broader definition of IBS rather than the use of Rome criteria. The Indian task force defines IBS as "a condition characterized by abdominal pain, bloating or discomfort occurring in association with disturbed bowel pattern in the absence of organic causes that can be detected by routine medical tests".

However most of the clinical trials still use the ROME criteria as it is validated.

Epidemiology

It is one among the few functional gastrointestinal disorders for which patients seek health care much more frequently and the prevalence in the general population is around 5% - 11%.[3-5] The average prevalence is around 11.2% as shown in various meta analysis. The geographical region wise estimated prevalence shows considerable variation with the least reported in Asia Pacific (7.0%) while highest incidence is seen in South America (21.0%).[3] Indian population-based studies estimate the prevalence of IBS

at 10–20% and the incidence of IBS at 1–2% per year. Approximately 10–20% of patients with IBS seek medical care. Of gastroenterology referrals 20- 50% can be attributed to this symptom complex.[4]

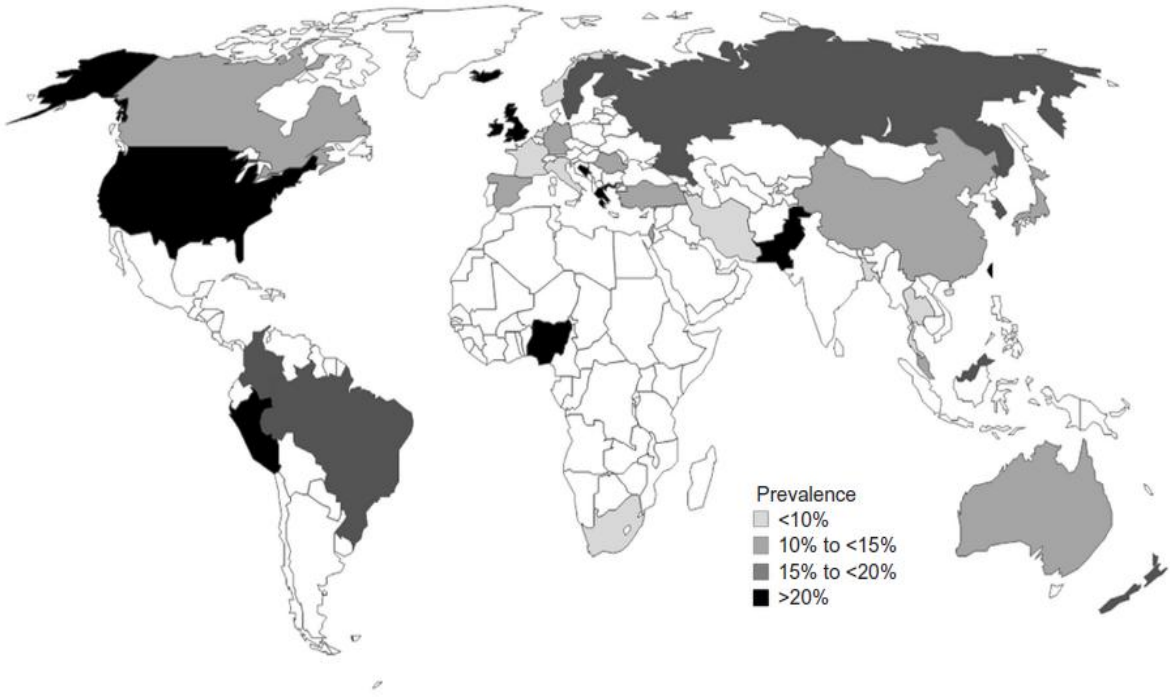


Figure 1 Worldwide prevalence of irritable bowel syndrome, as reported by country.

The epidemiology of irritable bowel syndrome
Clin Epidemiol 2014

IBS shows a gender predilection with most studies showing a male to female ratio of 1:2-3. Even among those symptomatic, women are more likely to seek medical assistance.[5] The prevalence of the disease is in the third to fourth decade and declines thereafter. Even among patients who report to the hospital in the fifth decade milder form of symptoms usually antedate. There are very few reports of disease onset after age 60 and most

of these patients have a precipitating factor in the form of infection, surgery or stress.

Diagnostic Criteria

Since 1960s, attempts were made to make a more precise diagnostic criteria for IBS without the risk of misdiagnosing an organic disease. A number of diagnostic criteria for IBS are available: Manning criteria, Kruis criteria, and the Rome I, II and III criteria. Rome III criterion has found acceptance and is the current criterion of choice.

An international working group in Rome, Italy published the first refined criteria in 1990 (Rome I), and followed it up with more simplified and practical versions in 1999 and 2006.[8] Rome Criteria has been the standard reference for most of the clinical trials. Though Rome I and II found utility limited to trials Rome III is currently used in physician clinical practice as well [6].

Rome III Criteria (2006)

Diagnostic criterion*

Recurrent abdominal pain or discomfort** for at least 3 days per month in the last 3months which is associated with two or more of the following:

1. Alleviation of symptoms with defecation.
2. Onset of symptoms associated with a change in form of stool.
3. Onset of symptoms associated with a change in frequency of stool.

* Criterion fulfilled for the last 3 months with symptom onset at least 6 months prior to diagnosis.

** "Discomfort" means an uncomfortable sensation not described as pain.

In pathophysiology research and clinical trials, a pain / discomfort frequency of at least 2 days a week during screening evaluation is recommended for subject eligibility.

Red flag symptoms which are ***not*** typical of IBS:

1. Age of 50 or more
2. Nocturnal awakenings due to any of the above symptoms
3. Significant weight loss
4. Family history of Organic disease like IBD or malignancy
5. Bleeding per rectum
6. Unexplained anemia
7. Fever
8. Loss of appetite
9. Positive physical examination findings

Subtypes of IBS are diarrhea-predominant (IBS-D), constipation predominant (IBS-C), mixed type (IBS-M), or with alternating stool pattern (IBS-A)[6].

IBS with diarrhea (IBS-D)*

- Loose stools >25% of the time and hard stools <25% of the time.
- Seen in one third of cases.
- More common in men.

IBS with constipation (IBS-C)

- Hard stools >25% of the time and loose stools <25% of the time.
- Seen in one-third of cases.
- More common in women.

IBS with mixed bowel habits or cyclic pattern (IBS-M)

- Both hard and soft stools >25% of the time.
- One-third to one-half of cases.

* **WGO PRACTICE GUIDELINES**

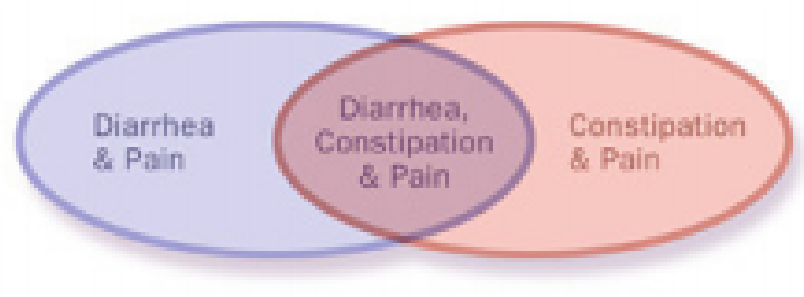
The Manning Criteria

1. Onset of abdominal pain associated to more frequent bowel movements.
2. Onset of pain associated with looser stools.
3. Pain relieved by passage of stool.
4. Noticeable abdominal bloating.
5. Sensation of incomplete evacuation more than 25% of the time.
6. Diarrhea with mucus more than 25% of the time.

The Manning's criteria has a sensitivity of 90% and a specificity of 87% if three or more items were regarded as positive[7].

The Kruis Scoring System had a sensitivity of 81% and a specificity of 91% when a score more than 44 was used as the cut off [8,9]

Score >44 = IBS **Symptoms**	**score**
Pain, flatulence, or bowel irregularity	34
Description of abdominal pain (burning to "not so bad")	23
Alternating diarrhea and constipation	14
Red flags	
Abnormal physical findings or history pathognomonic of other disease	−47
ESR >10 mm/h	−13
WBC > × 109	−50
Anemia	−98
History of blood in stool	−9
The Kruis scoring system has a sensitivity of 81% and a specificity of 91% [8,9]	



The three symptom subcategories of IBS

Extraintestinal symptoms

Symptoms unrelated to the intestine (extraintestinal symptoms) are common in patients with IBS. In varying frequency those commonly reported are headache, sleep disturbances, post-traumatic stress disorder, temporomandibular joint disorder, sicca syndrome, pelvic pain, myalgias, back pain, and chronic pelvic pain. Fibromyalgia and interstitial cystitis are also frequently encountered in patients with IBS. In fact, Fibromyalgia is seen in up to 33% of patients with IBS and almost one third of patients with fibromyalgia also have IBS.

Pathophysiology

The pathophysiology of functional gastrointestinal disorders is still a matter of research and though multiple mechanisms have been proposed no definitive etiopathogenesis is available. Various studies report various etiology and hence IBS is a multifactorial disease [10]

VISCERAL HYPERSENSITIVITY AND IBS

Altered colonic sensation and increased colonic motility leading to spastic colon has been associated with pain[11,12]. Studies have demonstrated varying motility patterns in IBS[13]. In the last decade, attention has focused on brain gut interactions. The efferent pathways which coordinate motor functions, secretory functions, and process sensations originating from the gastrointestinal tract up to the central cortex have been studied.

A reduced threshold for perception of visceral stimuli is a common finding in FGID, including non-cardiac chest pain, functional dyspepsia, and IBS. The mechanoreceptors of intestine have a heightened pain perception. Parietal nerve sensitisation due to mild mucosal inflammation is found in a subset of patients with IBS.[14]

A link between the onset of IBS and an episode of intestinal infection has led to the concept of post infectious IBS. Chaudhary and Truelove, in 1962 were able to show that gastroenteritis preceded IBS in 30% of patients. Concrete evidence in the form of brain imaging studies showed that IBS patients display enhanced activation of areas involved in pain processing (thalamus, insula, anterior circulate cortex) when subjected to visceral pain compared to healthy controls.[15]

Injury to enteric mucosa stimulates a cascade of chemical mediator release like bradykinin and prostaglandin E2. These substances further stimulate release of pain producing algogenic mediators like histamine, seratonin, and nerve growth factor by activation of afferent nerve terminals.

Visceral pain is exaggerated by these algogenic mediators. The C afferent fibres that are thus activated are silent receptors for neurokinins. It has been found that neurokinis can accentuate the pain reception of the nerve terminals and render them chronically susceptible.[15]

THE ENTERIC NERVOUS SYSTEM

The Enteric Nervous System comprises the third major division of the autonomic nervous system, the sympathetic and parasympathetic nervous systems are the other, better known parts. It comprises the submucosal plexus, (Meisner plexus) and the muscular plexus (Auerbach Plexus). An intricate network of motor, sensory and intermediate neurones and their corresponding projections together with supporting glial cells, Schwann cells and interstitial cells of Cajal forms the Enteric Nervous System. The Enteric Nervous System that can produce an adaptive functional behaviour in the gut even when isolated from the central nervous system. This property of the Enteric Nervous System was described by Bayliss and Starling already in 1899. IBS has been shown to have increased activity of sympathetic system as compared to parasympathetic system. Agarwal et al has demonstrated that constipation predominant irritable bowel disease is associated with vagal dysfunction while IBS-D is related to adrenergic sympathetic dysfunction.[16] other studies have demonstrated cortisol hyper-responsiveness which is linked to cutaneous hyperalgia. Symptoms of central hyperalgesia like migraine headache, back pain, myalgia and dyspareunia are some of the extraintestinal manifestations of IBS which prove a imbalance in enteric nerve system activation.

Intestinal Gas

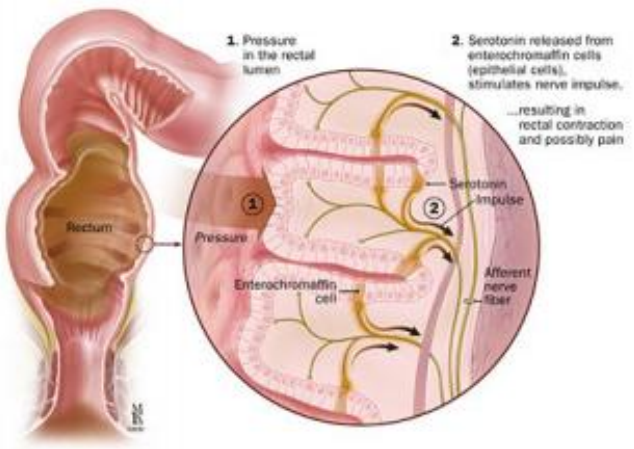
Bloating is extremely common in patients with FGID and occurs in up to 96% of patients with IBS. Most patients consider this symptom extremely distressing and about two-thirds of them consider it the worst of their symptoms. Bloating is more frequent in patients with IBS-C (75%), than in those with IBS-D (41%), and in IBS-C bloating correlated with abdominal distension.[16]

Work from the Barcelona group[17 18] has shown that metabolism and absorption of gas is altered in IBS leading to gas entrapment in bowel. It has also proposed an increased perception of gas due to stimulation of mechanoreceptors. Recently fermentable oligosacharides have also implicated as a possible mechanism for intestinal gas. In a study by Halmos et al FODMAPS elimination diet showed fecal frequency and stool form, especially in IBS-D. This diet was developed a Monas university in melbourne by Peter Gibson and Susan Sheperd. Restriction of fructans, galactans and polyols which are high in wheat, barley, pulses, beans and fruits alleviates the symptom of intestinal gas.

Psychosocial Factors

Numerous physiology studies have shown correlation between emotion and gut motility. Anxiety induces enhanced contractile activity in rectum-sigmoid, increased small bowel transit time and enhanced stool which is mediated by activation hypothalamo pituitary axis. The converse

has also been seen where depression leads to decreased peristaltic activity, colonic transit and hence certain personality types are more prone to develop IBS especially type-A. Neuroticism, perfectionism, exaggerated conscientiousness are some of the personality features detected. In a recent study, were a large sample of community population was screened for the presence of stress or anxiety and were followed for a period of 12 years showed that the presence of psychological impairment during the start had an increased risk for developing functional gastrointestinal disorder. Nevertheless not all subjects with altered stress levels developed IBS showing that this is not the sole factor in the disease.



Role of serotonin in rectal contraction and pain

Genetic Factors

Epidemiological studies of familial aggregation show a genetic predisposition in the incidence of IBS. Numerous small studies have identified polymorphisms in seratonergic expression as also single nucleotide polymorphism in susceptibility genes. Gene which is involved in

inflammation and activation of pain pathway is neuropeptide S receptor gene. Zucheli et al as demonstrated increased expression of neuropeptide receptor gene in IBS–D patients. Two cohort studies done in Sweden sho an association between IBS-D and RS 42639 which is a gene for tumour necrosis factor. The other genes that have been implicated in IBS include genes for colonic transit, pain perception, bile acid synthesis like Kloth b gene which is the principal regulator of hepatic bile syntesis[19].

Disturbed gastrointestinal motility

The disturbed bowel habit associated with IBS indicates a GI motor abnormality.

From a wide range of studies the following can be pointed out: Delayed gastric emptying[19,20], especially in patients with constipation or overlapping dyspepsia[21]. Small bowel hyper-contractility in the form of discrete clustered contractions[22] and increased frequency of the migrating motor complex and duodenal retropropagation. Exaggerated motor response to food intake both when examining the small bowel[23] and colon. Decreased transit time[24] and increased number of high-amplitude propagated colonic contractions[25] in diarrhea predominant IBS and increased transit time and decreased number of high-amplitude propagated colonic contractions in constipation predominant IBS. Finally, decreased rectal compliance[26], is a measure of a non-sensory rectal aberration seen in some IBS patients.

Manometry studies show varying pattern of colonic and small intestinal motor function. High amplitude propagated contractions are exaggerated and increased response to meal induced release of cholecystokinin. Similarly increased colonic activity and propagated contractions were seen with stress where coticotrophin releasing hormone is increase rectosigmoid contractions[26].

These findings were also emphasised by scintigrapy studies and also by colon transit studies using radio opaque markers.

Fructose and Lactose Intolerance

Common symptoms of dietary fructose and lactose intolerance like bloating, flatulence, pain, and diarrhea have been reported in functional bowel disorders such as IBS. It has been shown that approximately one third of patients with suspected IBS might also have fructose intolerance as identified by a positive fructose breath test. Although there are no data documenting the efficacy of a fructose-restricted diet, a study of 80 suspected IBS patients showed significant relief of symptoms in those who were compliant with a fructose-restricted diet[27]. Patients with IBS have subjectively reported higher incidence of lactose intolerance, but it is hard to tell whether reported symptoms are secondary to lactose intolerance or IBS in the absence of documented lactose malabsorption. A period of avoiding dairy products or requesting a test for lactose malabsorption (or both) may be beneficial in this area but no major trials or guidelines recommend this.

Moreover the addition of probiotic lactobacilli confers no benefit to symptom alleviation in IBS.

Latent or Potential Celiac Disease

The concept of latent or potential celiac disease has recently been introduced into the pathogenesis of IBS. Normal mucosa with symptoms of altered bowel habits and features of malabsorption are seen in latent celiac disease.In a study of genetic, serologic, and histologic markers of celiac disease in 102 patients with diarrhea-predominant IBS, 35% of the patients had positive findings for human leukocyte antigen (HLA)-DQ2, 23% had increased intraepithelial lymphocyte counts, and 30% had increased celiac disease–associated antibodies in the duodenal aspirates, including antibodies against gliadin, tissue transglutaminase, β-lactoglobulin, and ovalbumin. Stool frequency and the intestinal immunoglobulin A(IgA) level decreased significantly under a gluten-free diet in a subgroup of IBS patients with positive HLA-DQ2 and positive intestinal celiac disease–associated antibodies when compared with IBS patients without these markers. Celiac disease–associated IgG and HLA-DQ2 expression can identify likely responders to gluten-free diet in patients with IBS-D (diarrhea predominant IBS. The possibility of celiac disease has to be ruled before a confirmed diagnosis of IBS is made.

GUT MICROBES AND IBS

The changes in gut flora of patients with gastrointestinal disorders, including IBS have been sought for decades.[27] When advanced molecular techniques were used to determine shifts in flora between IBS and controls, a lack of lactobacillus and Collinsella species were seen in IBS. In IBS-D a decrease[28] in bifidobacterium was seen. In the largest published study of small bowel culture in IBS, aspirates of jejunal fluid in IBS were found to harbor a greater number of coliform bacteria compared with healthy controls[29]. Controlled trials in IBS demonstrate successful treatment of IBS with antibiotics with 75% improvement[30] in IBS symptoms observed with normalisation of breath test proving a role for SIBO (small intestinal bacterial overgrowth). SIBO which is presence of more than 10^5 colony forming units / ml of proximal small bowel aspirate has been documented in post infectious IBS, commonly Streptococci, Bacteroides, Escherichia and Lactobacilli.[30] Te eects of altered microbiota is mostly mediated by activation of the innate immune system as evidenced by increased expression of toll like receptor -4 and 5 in the mucosa and increase in beta-defensin 2. This could explain the pain and altered motility pattern seen in IBS. Treatment with probiotics Bifidobacterium infantis and Saccharomyces bouldari have been found to alleviate symptoms. The mechanism behind abdominal bloat could be fermentation of FODMAPS producing acetic acid, propionic acid and increased intestinal gas.

IBS AND INFLAMMATION

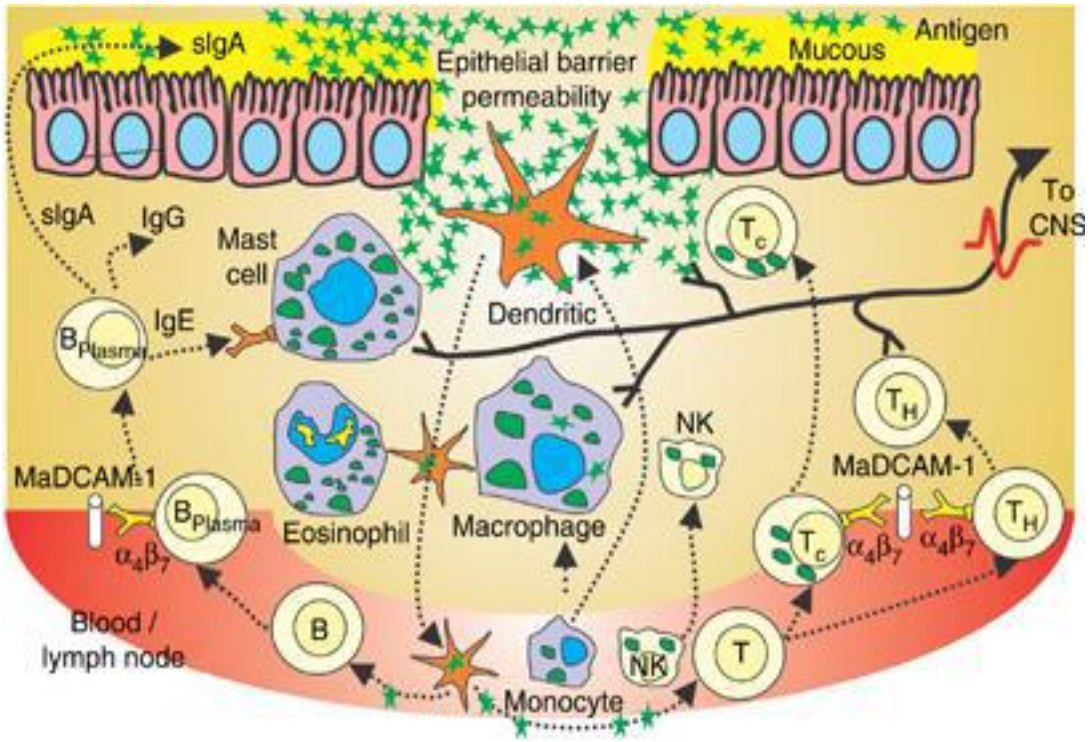
Histopathology studies

Over the past 20 years there has been a growing appreciation of gut mucosal immunology and its role in IBS, particularly the role of cytokines, mast cells and lymphocytes.

The first report of a study evaluating inflammation in IBS dates back to 1962. Hiatt and Katz were able to demonstrate elevated mast cells in IBS patients as compared to healthy population[31]. Similar findings were echoed by O´Sullivan et al when he reported mast cell densities in colonic mucosa of IBS patients [32].

In a study of IBS patients mucosal biopsies demonstrated an increase in rectal lymphocytes as compared with healthy controls that persisted for months [33].

Another finding in examining subjects with IBS is the possibility of chronic inflammation of the enteric nervous system. Strong evidence for this hypothesis comes from various studies the earliest being laparoscopic full thickness biopsy of small bowel in IBS-D patients which demonstrated evidence of excessive lymphocytes in the ganglia of myentric plexus[34]. Peripheral blood lymphocyte expression of integrin beta 7 and endothelial cell expression of receptors like MaDCAM1 was comparably elevated in patients with IBS and ulcerative colitis compared with asymptomatic controls, suggesting greater homing of lymphocytes to the gut mucosa [35].



Activated gastrointestinal immune system in Irritable Bowel Syndrome
Activated B cell and T cell homing in gut mucosa
Increased expression of immune receptors

HUMORAL IMMUNE MECHANISMS AND GI DYSMOTILITY

Not only cellular immunity, but also humoral immune mechanisms may serve as a putative pathogenetic mechanism in IBS. In severe forms of both primary and 9 secondary gut dysmotility, circulating antibodies directed toward neural structures are known to exist. Most well known are perhaps the anti-Hu[36] antibodies with binding affinity to the neural Hu-proteins that have an important function in neuron development and survival processes. Application of serum with high concentration of anti-Hu antibodies has been shown to be able to induce neuron death by apoptosis[37].

The concept of molecular mimicry with cross- reactivity between an original protein structure and a host protein is the main hypothesis for this phenomenon to happen. In cases of secondary gut dysmotility, this is easy to apply, but less so in primary cases. Even so, descriptions of this are present for anti-ganglionic[38], and anti-potassium channel[39] antibodies.

Signs of immunologic alterations in IBS

Measuring the cytokine content of colonic and rectal mucosa is a novel and objective way of assessing immune mechanisms underlying IBS. Increased expression of IL-1mRNA in the rectal mucosa was demonstrated in of PI-IBS patients, 3 months after the infection, though the sample size of the study was small[40].

Cytokines, primarily inflammatory mediators are involved in normal function of epithelial cells, gut smooth muscle and also in enteric nervous system. Changes in the cytokine profile of these tissues could promote changes in secretion, permeability and motility[41]..

Inflammation is a cascade involving both cellular and humoral immunity and a variety of soluble mediators the major ones being cytokines. Inflammatory cytokines can be sub typed as those involved in acute inflammation and those responsible for chronic inflammation, as also pro and anti- inflammatory.

Cytokines

Cytokines are a group of small soluble or cell membrane-bound protein or glycoprotein molecules that act as cell messengers conveying information from one cell to another. Numerous cytokines (>200) have been identified, and these are generally divided into subgroups of interleukins, growth factors, chemokines, interferons and colony stimulating factors. Of these the pro-inflammatory and anti-inflammatory ones are IL-1, IL-6, TNF-α, TGF-β and IL-1Ra, IL-10 respectively.

Cytokine functions are cell specifically mediated by cytokine receptors located on the surface of target cells. Cytokine mediated pro-inflammatory effector functions may be decreased by cytokine specific receptor antagonism as well as anti -inflammatory cytokines. The balance of these excitatory and inhibitory factors is essential for normal cellular function. Imbalance in cytokine production has been observed in organic diseases. These disturbances in net cytokine production may be caused at several levels.[42]

Physiological roles of TNF alpha

Tumour necrosis factor alpha was first identified in 1975 as a macrophage derived factor capable of necrotizing tumours in mice.[43] In normal serum concentrations it is of benefit with its potency to promote tissue remodelling and repair, inflammation, cytotoxic reactions and anti-tumoral immunity.[44] At the other end of the spectrum, markedly

elevated levels of TNF actually lead to shock, features of multiorgan failure like catabolic hormone response, vascular leakage, ARDS, gastrointestinal necrosis, acute renal failure, adrenal haemorrhage and DIC. In contrast chronic low dose exposure to TNF results in weight loss, anorexia, protein catabolism, lipid depletion, hepatosplenomegaly, subendocardial inflammation and insulin resistance.[45]

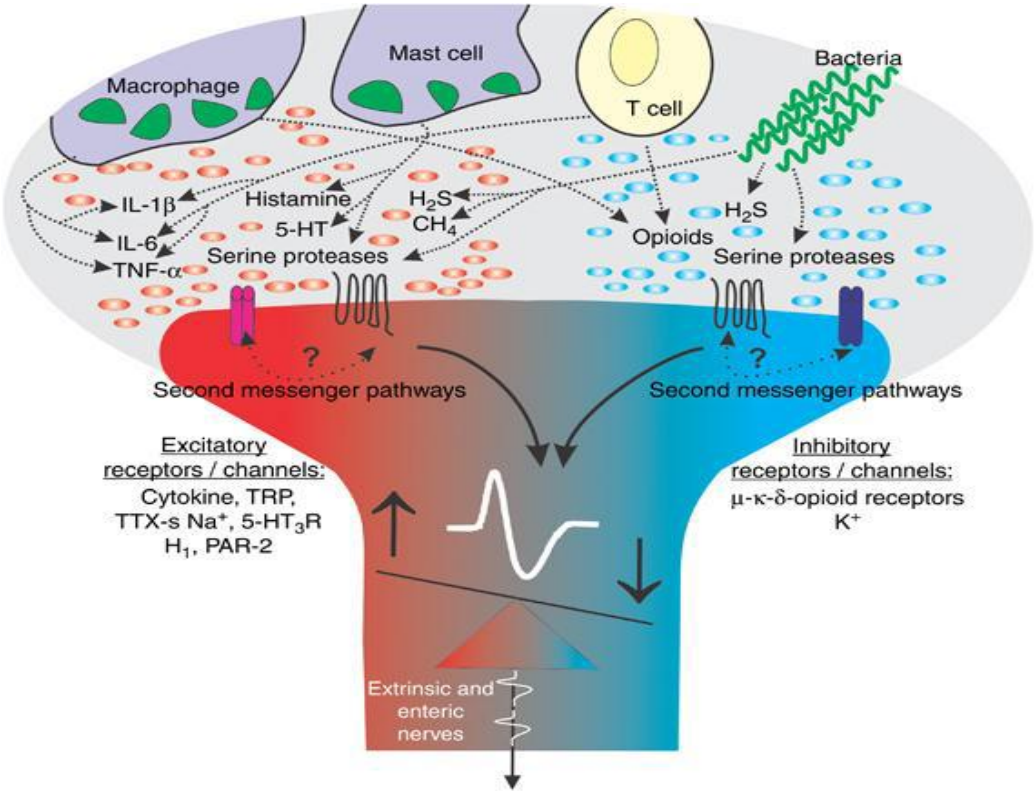
Human TNF–alpha has membrane-bound (26kDa) and secreted forms, both of which are biologically active. The membrane bound form of TNF is cleaved by a metalloprotease disintegrin to a secreted (17 kDa) monomer, three of which then associate forming biologically active secreted form of TNF. Activated monocyte- macrophage lineage cells are the major origin of local and circulating TNF[46]. The principal stimulus to its production is bacterial endotoxin and to a lesser extent phorbol esters. The serum half life of TNF ranges between 11- 30 minutes.[47]

Cytokines in IBS

The cytokines IFN-γ, IL-12, IL-2, and tumor necrosis factor-α (TNF-α) are secreted by TH1 cells. Th2 cells predominantly produce IL-4, IL-10, IL-13, and IL-6. Likewise cellular immunity is mediated by TH1 cell and humoral immunity by TH2. The counter regulatory effect and balance is essential for immune tolerance[48]. Altered equilibrium in profile of Th1/Th2 and improper immunological response may induce features of IBS[49 50]. Several studies have shown significant elevated levels of IFN-γ,

IL-2 in IBS-D patients whereas the Th2-derived cytokine IL-4 was significantly lower in D-IBS patients than in healthy persons[51]. This demonstrates that altered cytokine profile is a reflection of underlying inflammation and activated immune system[52,53]. Study by Mcsherry et al has demonstrated altered cytokine at the mucosal level.

Differential expression of various genes was confirmed by polymerasecain reaction or *ex vivo* biopsy culture for 5 out of 6 selected genes. Reduced secretion of chemokines (IL-8, CXCL-9 and MCP-1) but not pro-inflammatory cytokines (TNF-α, IL-6 and IL-1β) was established on the basis of the *ex vivo* biopsy cultures. This was in contrast to inflammatory bowel disease were the levels of cemokines were increased as well as pro imflammatory cytokines. Chemokines have an important role in mucosal defense and altered ratio suggests a propensity towards inflammation but in a milder spectrum as compared to inflammatory bowel disease.



Control of gut neural activity and cytokine

The excitatory and inhibitory nerve pathways activated by receptors for cytokine[54]

Studies show interaction between Tumour necrosis factor receptor 1 and transient receptor potential A1 produces sensitisation of colonic sensory afferents to mechanical stimuli. Hence TNF could produce varying colonic response [55]

O'Mahony conducted a case control observational study in which levels of cytokine was measured from peripheral smear of IBS patients and healthy population demonstrating increased secretion of IL12 as also evidence for activated Th1 cells.[56]

The vice versa – lowered levels of IL 10, the major anti inflammatory cytokine was also seen in the patients with IBS -D .[57]

Recent evidence from 2 large volume case control study relationship between, a single nucleotide polymorphism in the *TL1A* gene one among the several cytokine genes and increased risk of IBS-D.[58]

Non-cytokine immune modulators in IBS

Toll-like receptors are a family of pathogen-recognition molecules with a central role in mucosal innate immune responses. Altered colonic expression of Toll like receptors were demonstrated in a study conducted by Brindt et al.[61] Increased expression of TLR 4, TLR 5 and decreased TLR & TLR 8 was seen in In=IBS patients. Most of these studies showed positive results in post infectious IBS.[62 63]

Cytokines as Biomarkers

Over the years diagnosis of IBS has relied heavily upon the symptomatology of the disease, which is prone to subjective error. Considering the results of the numerous trials demonstrating increased levels of cytokines in IBS patients these could be used as a biomarker.[64]

This raises the possibility that cytokine could be used as a potential therapeutic target and also as a measure of disease activity and response to treatment. There is not enough evidence for immune biomarkers described so far in IBS studies to be used as diagnostic or disease monitoring tools.

Lack of consensus among the various studies might be attributed to small number of patients included, disease heterogeneity, presence of comorbidities, focus on intestinal versus fecal versus blood biomarkers, and variations in tissue sampling and experimental design. Immune biomarkers have greatly contributed to our understanding of various aspects of IBS pathophysiology. Moreover, certain immune biomarkers correlate with disease subgroups, raising the intriguing possibility of distinct mechanisms underlying the different IBS subtypes.

Management of IBS

Patients suffering from IBS often present for medical care only after frustrating self-diagnostic attempts to determine symptom causation and resolution. It is very important, therefore, that the treating physician foster a positive relationship with the patient in order to aid in successful clinical management. A positive, confident diagnosis, accompanied by a clear explanation of possible mechanisms and an honest account of probable disease course, can be critical in achieving desired management goals**.**

Non pharmacological recommendations

Cognitive / behavioral therapy, in group, or individual sessions which include:

- Behavioral techniques aimed at modifying dysfunctional behaviors through:

- Contingency management (by rewarding healthy behavior)
- Assertion training
- Hypnotherapy

Diet

Though evidence is lacking regarding whether food allergy testing or exclusion diets are effective in treating IBS and guidelines do not recommend any elimination recent studies have shown clear alleviation of symptoms with these changes.

IBS-D

Elimination diet

- Lactose free
- FODMaPS free

IBS-C

- High fibre

Drug Therapy

- Tricyclic antidepressants:
  1. Amitriptyline, starting dose 10 mg/day, target dose 10–75 mg/day, at bedtime.
  2. Desipramine, starting dose 10 mg/day, target dose 10–75 mg/day, at bedtime. These tend to be constipating and should be avoided among constipated patients.

- Selective serotonin reuptake inhibitors (SSRIs):

  1. Paroxetine, 10–60 mg/day.
  2. Citalopram, 5–20 mg/day

- Alosetron, a 5-hydroxytryptamine-3 (5-HT3) receptor antagonist
- Lubiprostone:
- c-2 chloride channel activators
  - twice a day in 8-µg doses

Loperamide is effective in reducing stool frequency

Probiotics

- Bifidobacterium infantis
- Bifidobacterium lactis

Indication	Drug target	Physiological effect	Drugs
IBS-M	Serotonergic and adrenergic receptors	↑ compliance, ↔ motility	Venlafaxine, fluoxetine
	Intestinal flora	↔ motility, ↓ bloating, ↓ pain	Probiotics
	Cholinergic receptor antagonists	↓ intestinal motility, ↓ pain	Cimetropium, pinaverium, hyoscine, otilonium, mebeverine
IBS-D	5-HT3 receptor antagonists	↓ intestinal motility, ↓ pain	Ondansetron, alosetron, cilansetron
	Selective M3 receptor antagonists	↓ intestinal motility	Zamifenacin, darifenacin
	α2-agonist	↓ intestinal motility, ↓ pain sensation	Clonidine
	µ-opioid receptor agonist	↓ intestinal motility, ↓ peripheral pain	Loperamide
IBS-C	Chloride channel modulator	↑ intestinal motility, ↑ water secretion	Lubiprostone
	5-HT4 agonists	↑ intestinal motility, ↑ water secretion	Metoclopramide, domperidone, cinitapride

Emerging therapies

There are trials wherein calcium channel blockers, opioid receptor ligands, and motilin receptor ligands, which act peripherally and corticotropin-releasing hormone antagonists, autonomic modulators which act centrally are being explored for therapeutic avenues

1. **Opiod receptor agonist : Asimadoline**

   It is a selective potent kappa receptor agonist with low permeability through blood brain barrier .in a dose of 0.5 mg per day it reduces abdominal pain perception. A randomised control trial evaluating the efficacy of asimadoline in doses of 0.5, 1.0 mg given over a period of 12 weeks achieved the primary end point of reduction in abdominal pain. This was especially so in patients with IBS- D who showed improvement in pain scores, decrease in stool frequency, and increased pain free days.

2. **Oral carcarbon adsorbent**

   It adsorbs ammonia, histamine, bacterial products, bile acids, seronin, and other luminal mediators within the gastrointestinal tract. Though a few trials have reported significant improvement in abdominal bloat they are limited by small sample size, hence larger clinical trials are needed.

3. Crofelemer

It is a novel proanthrocyanidin oligomer which has an anti secretory action that reduces excess intestinal chloride ion secretion. It was developed from plant Croton lecheri. Chaturvedi et al used it in doses of 500 mg twice daily I a randomised control trial and found significany decrease in stool frequency and form.

4. Dextofisopam

It is an autonomic modulator and R enantiomer of tofisopam. It decreases colonic motility by blinding to benzodiazipne receptors in sub cortical regions of the brain. Though numerous studies have reported impovedsymptoms, it is limited by side effects of nausea, abdominal pain and flu like symptoms.

5. Others

Corticotrophin releasing factor recptor type I antagonist Tryptophan hydroxylase inhibitor Glucagon like peptide analogue.

Cytokines are not only used as a potential therapeutic target but also as a measure of disease activity and response to treatment.

MATERIALS AND METHODS

MATERIALS AND METHODS

Study Group

Patients aged 18–75 years and fulfilling Rome III criteria for IBS-D with at least 25% of the stools having a stool form score of 6 or 7 on the Bristol Stool Form Score (BSFS) and no more than 25% with a stool form score of 1 or 2 were included. Patients were excluded if they had inflammatory bowel disease or microscopic colitis as evidenced by colonoscopy and biopsy. The evaluation included a detailed history, examination, sigmoidoscopy and biopsy, full blood count, and assay of pro inflammatory cytokine TNF-a.

Control Group

- Age matched Healthy controls were taken as the control group.
- They were subjected to detailed history and clinical examination.
- Informed consent was obtained. Complete blood count including Total WBC count, ESR were obtained and peripheral blood samples were assayed for TNF α levels.

Inclusion criteria

- age: 18–75 years
- patients fulfilling Rome III criteria for IBS-D with at least 25% of the stools having a stool form score of 6 or 7 on the Bristol Stool Form Score (BSFS) and no more than 25% with a stool form score of 1 or 2 were included.

Exclusion criteria

- Pregnant women
- individuals diagnosed with lactose intolerance
- immunodeficiency
- individuals who had undergone any abdominal surgery, with the exception of hernia repair and appendectomy
- those with a psychiatric illness

Methodology

- The evaluation includes a detailed history, clinical examination, sigmoidoscopy and biopsy, full blood count, hematinics and assay of pro inflammatory cytokine TNF-α.
- **VERBAL NUMERIC ANALOG SCALE**

Subject is asked:

On a scale of 0 to 10,

0 being no pain

10 being the worst pain imaginable

What do you rate your current pain?

- Patients with features of inflammatory bowel disease on scopy, microscopic colitis in biopsies are excluded
- Levels of TNF α in peripheral blood sample done using ELISA.

Cytokine assay

Peripheral venous blood samples were collected from all participants. Five ml blood samples were collected in plain vials. Samples were centrifuged after proper clotting to prevent hemolysis. Serum was separated and stored at -80°C until further analysis. Serum TNF- α was measured by a commercially available enzyme-linked immunosorbent assay (Diaclone). IBS-D patients and controls samples were processed in same run for proper comparison. The assays were performed according to the manufacturer's protocols. The minimal detectable concentration were <3.16 pg/ml for TNF-α. Inter and intra assay assessments of the kit reliability were also conducted.

ELISA KIT:

STATISTICAL ANALYSIS

STATISTICAL ANALYSIS

Among the 44 patients enrolled in the study group, 1 excluded because of presence of polyps at colonoscopy and two were excluded as stool analysis revealed presence of cyst and ova. A total of 41 patients were included in study group and an equal number of age and sex matched population were taken as control group. One of the healthy subjects turned out to be positive pulmonary tuberculosis and hence was dropped from the final analysis.

Statistics

Statistical significance for serum cytokine levels between IBS-D patients and healthy controls was evaluated using an unpaired two-tailed Student's t-test and Annova. Values have been reported as means+SD. Any statistical difference was considered significant at $p < 0.05$.

- H0= null hypothesis
- M1=1.7
- M2=2.2
- Sd1=1.2
- Sd2=1.2
- N2/n1=1
- Sample size= 40
- Power of study=90%

RESULTS

RESULTS

Table 1: AGE DISTRIBUTION OF CASES AND CONTROLS

Age group	Cases	Controls	Chi square	P value
18 – 29	13	12	2.56	0.633
30 – 39	13	17		
40 – 49	12	8		
50 – 59	2	3		
60 – 69	1	0		
Total	41	40		

Mean Age of IBS Prevalence in the study population is 35 years

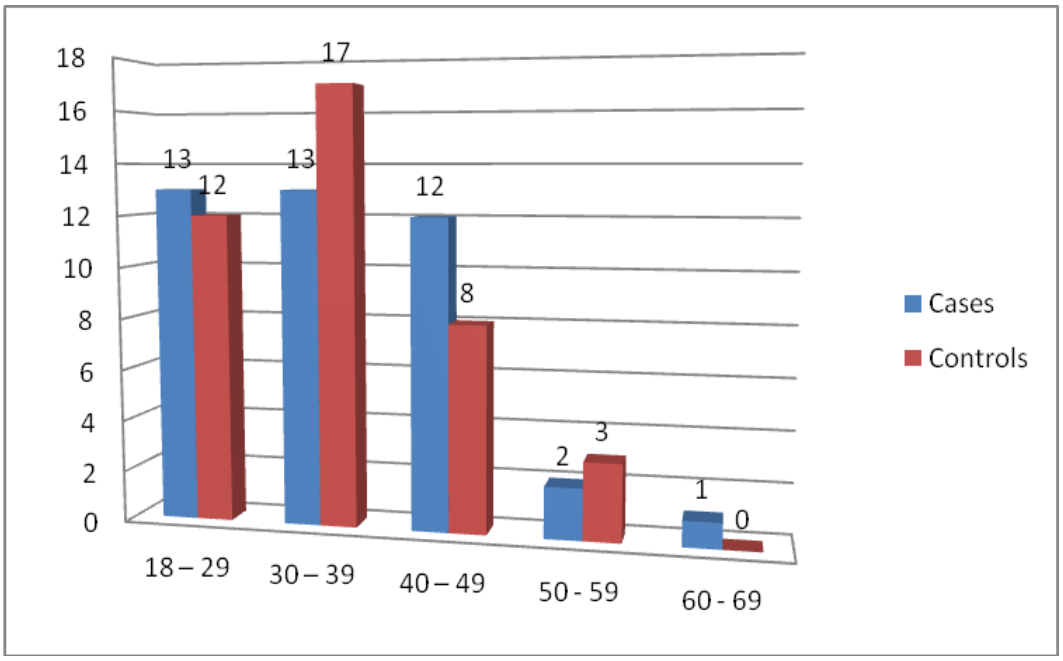


Figure: Age group in decades

IBS D prevalence is equally distributed across second and third decade

Table 2: GENDER DISTRIBUTION

Gender	Cases	Controls	Chi square	P value
Male	**23**	**23**	**0.02**	**0.898**
Female	**18**	**17**		
Total	**41**	**40**		

The incidence of IBS D is more common in male than females

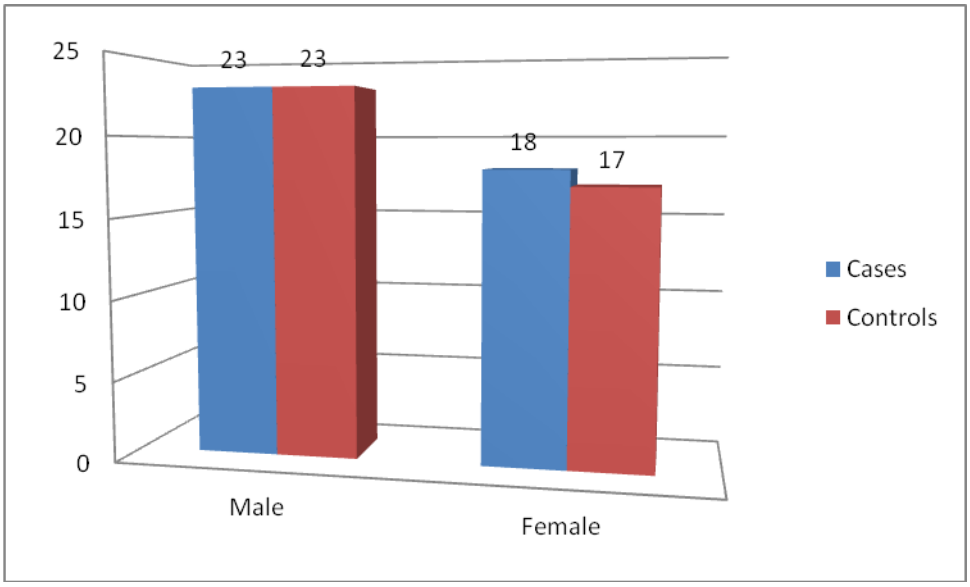


Figure: Gender Distribution

Age and sex matched controls were taken for the study as is evidenced by the lack of statistical significance in the two groups.

Symptoms of IBS and TNF-α

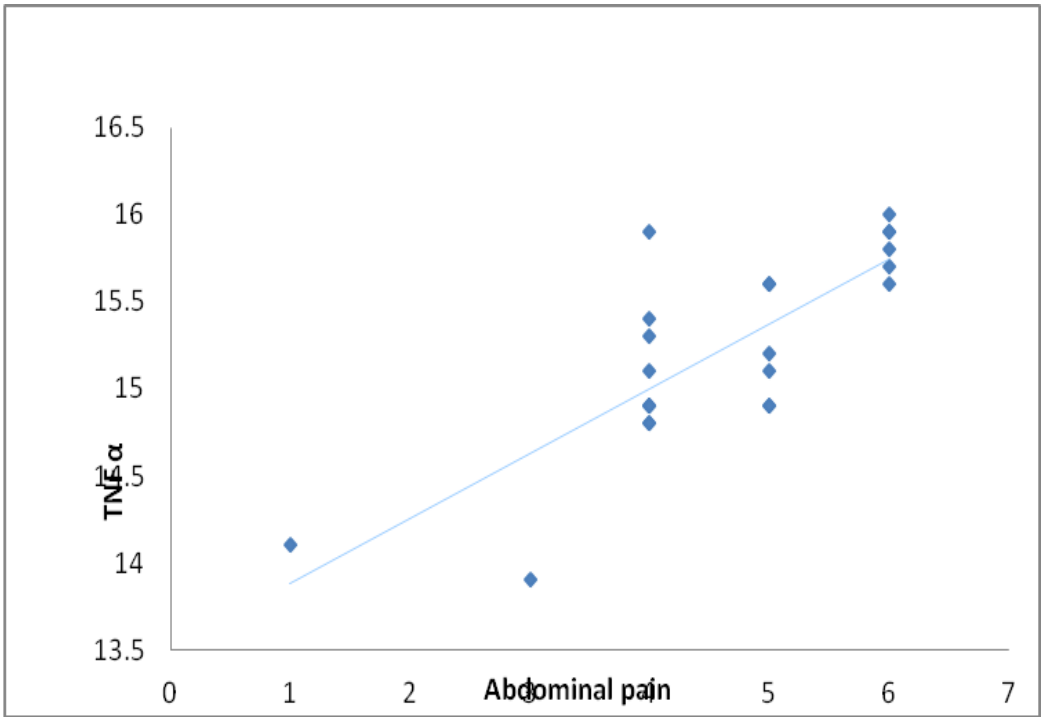
Table 3: ABDOMINAL PAIN

Abdominal pain	TNF α			P value
	Mean	S.E	ANOVA (F value)	
1	14.10	-	6.171	0.001
2	14.50	0.30		
3	14.38	0.28		
4	15.05	0.15		
5	15.11	0.23		
6	15.82	0.05		
7	16.00	-		

- ❖ 30% of the study population had a pain severity of 4 on the verbal numeric scale.
- ❖ 22% of the study population had a pain severity of 5 on the verbal numeric scale.
- ❖ 25% of the study population had a pain severity of 6 on the verbal numeric scale.

Hence, 77% of the study population who had a severity of abdominal pain ranging from 4-6 in the verbal numeric scale had statistically significant correlation with increasing TNF values. This is supported by anova test scatter diagram showing statistically significant correlation between abdominal pain and TNF value.

Scatter diagram showing linear correlation with abdominal pain

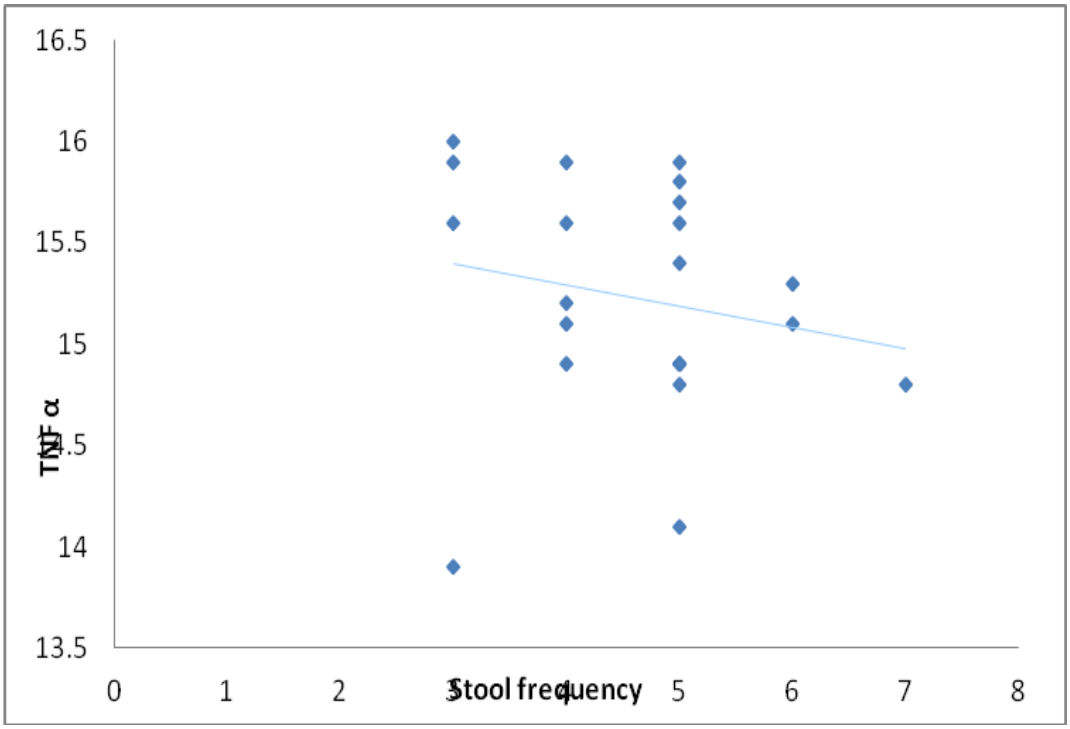


r – 0.68, p – 0.001

Stool Frequency

- 25% of the study population had an average stool frequency of four per day.
- 52% of the study population had a stool frequency of 5 or more per day. Hence 77% of the study population who had increased stool frequency of more than 4 per day has a statistical correlation with increased TNF values as shown in the following scatter diagram.

Scatter diagram : Stool Frequency

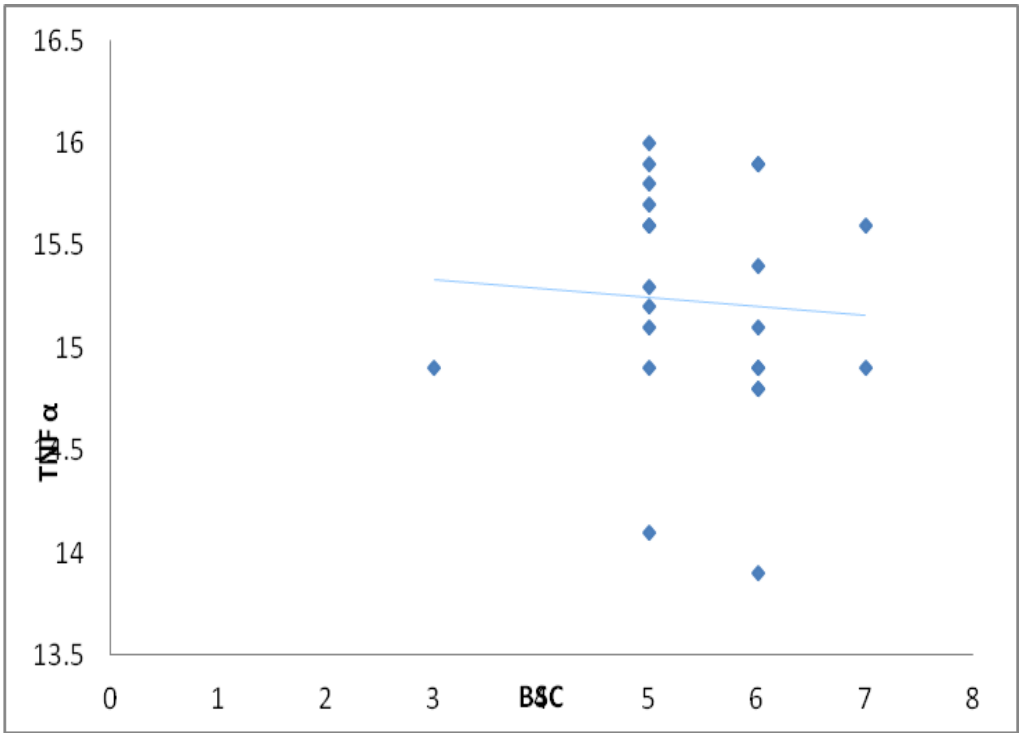


r – -0.31, p – 0.049

Table 4 : STOOL FORM

BSC	TNF α		ANOVA (F value)	P value
	Mean	S.E		
3	14.90	-	0.545	0.655
5	15.27	0.17		
6	15.18	0.16		
7	14.80	0.35		

Figure : Scatter Diagram



r – -0.116, p – 0.469

Table 5 : ABDOMINAL BLOAT

Abdominal bloat	TNF α		T test	P value
	Mean	S.E		
Present	15.12	0.13	0.833	0.414
Absent	15.31	0.19		

There is no significant correlation between abdominal bloat and TNF values.

Table 6 : MUCOUS IN STOOL

Mucous in stool	TNF α		T test	P value
	Mean	S.E		
Present	15.12	0.18	0.358	0.723
Absent	15.20	0.14		

There is no significant correlation between serum levels of TNF and mucous in stool. Of the 40 cases only 4 reported presence of mucous in stool.

Table 7 : DIABETES MELLITUS

Diabetes mellitus	TNF α		T test	P value
	Mean	S.E		
Yes	15.68	0.17	2.728	0.031
No	15.12	0.12		

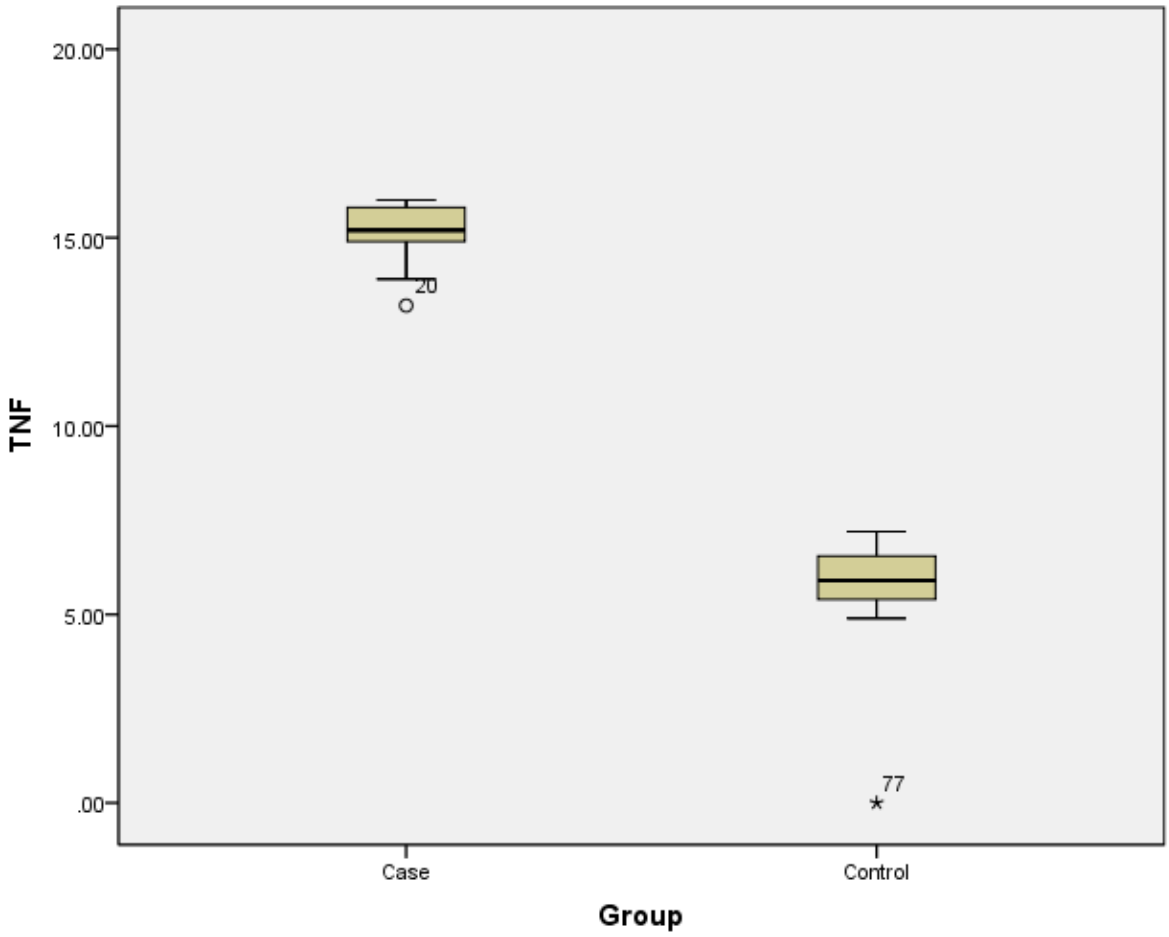
Of the cases 5 reported to be on oral hypoglycemic agents for diabetes mellitus they were found to have euglycemic values during evaluation. Both among cases and control population those with diabetes mellitus were found to have elevated TNF levels.

TNF –ALPHA Case Vs Control Group

Group	TNF α		T test	P value
	Mean	S.E		
Cases	15.18	0.11	44.128	0.001
Controls	5.81	0.18		

Table shows the TNF profile of study group and control group. The mean serum level of TNF –α in patients and control group were 15.18±S.E 0.11pg/ml and 5.9±0.18 pg/ml respectively with a p value of 0.001.

Box Plot showing raised TNF Levels in cases as compared to controls



DISCUSSION

DISCUSSION

IBS is a chronic functional disorder and patients have repeated hospital visits translating as lowered quality of life with very few therapeutic modalities available. Explaining the pathophysiology of the disease is the crucial step towards a definitive treatment modality. This study was started as a pathway to find newer diagnostic and therapeutic modalities.

Age

Mean age of patients with IBS-D in our study population is 35. Among these nearly 25% had symptoms for more than five years. Two of the patients who were in the sixth decade had been completely evaluated over the years with colonoscopy including biopsy and imaging of the abdomen. Though IBS has been reported across all age groups[65] Lin et al and Maxwell et al have shown that most patients who seek medical care for IBS are in the third decade of life.[66, 67]

Gender

The incidence of IBS-D in our study, surprisingly, is twice as common in males as in females though previous studies have shown IBS to be more common in females. This might be a reflection of the changing lifestyle of the present generation. It could also be due to underreporting and lack of healthcare awareness among the females of our society.

Some of the studies have shown IBS to be 1.5 to 3 fold[68,69] more prevalent in female than male while others have shown equal distribution between both genders seeking primary care for IBS[70,71]

Symptomatology and TNF values

In our study TNF levels correlated with the severity of symptoms especially abdominal pain and stool consistency.

77% of the study population who had a severity of abdominal pain ranging from 4-6 in the verbal numeric scale had statistically significant correlation with increasing pain associated with increasing TNF levels. This is supported by anova test.

Stool frequency also showed statistical association with TNF levels in the scatter chart.

Most of the trials which show altered cytokine profile in IBS patients have compared it to the sub type of IBS and found it to be significant in IBS-D and Post infectious IBS. Few studies have shown (Rana et al, Mckerman et al) have demonstrated a direct correlation with the symptoms.

The other symptoms like abdominal bloat and stool form show no statistical correlation with TNF values suggesting a different complimentary pathophysiology probably a role for gut microbiota .

Patients with diabetes had elevated cytokine levels but this cannot be taken as a confounding factor in the result analysis for less than 25% of the population had diabetes.

TNF – α Case Vs Control groups

There is presence of statistically significant elevated serum TNF levels in the serum of diarrhoea predominant IBS patients as compared to controls by the student t test analysis. This is further demonstrated explicitly in the Box Plot.

This finding echoes the result of numerous studies. Hughes et al conducted a case control study of 35 IBS patients in which several cytokines were elevated in IBS supernatants, and their levels correlated with pain frequency and intensity.

Although we excluded patients with co-existent inflammation, metabolic disorder was included. Surprisingly, TNF levels were elevated in diabetic patients as well.

Scully et al[72] conducted an analysis of pro-inflammatory cytokines in female IBS patients with extra intestinal co-morbidities which showed increased levels of IL-6, IL-8, and TNF- α.

McKerman et[73] demonstrated elevated cytokine levels in patients with the irritable bowel syndrome. Of the various cytokines TNF α has been demonstrated to be consistently elevated in IBS D patients and also correlating with the intensity of pain.[73 74]

TNF-α have been shown to control the release of corticotrophin releasing hormone, the main hypothalamic regulatory peptide of the hypothalamic pituitary adrenal (HPA) axis.[74 75 76] Genotyping studies of IBS patients indicate they are more likely to have alleles associated with excessive production of IL-6, IL-2,TNF-α whereas a recent meta-analysis indicated high IL-10 producers are less likely to develop IBS[77-81]. It has further been seen that TNF-α cytokine gene polymorphisms could change an individual's susceptibility to IBS and these might have pathophysiological role.[82] George et al demonstrated polymorphism differences in cytokine genes between patients with IBS and healthy controls from a pilot study conducted in India.[83] Similar results were reported by Lee et al and Vander veek et al.[77 84] Studies have documented the onset of IBS following bacteriologically confirmed gastroenteritis, while others have provided evidence of low-grade mucosal inflammation[85] and immune activation in IBS.[86,87]

Cytokines are not only important signalling messengers in the immune system, but also modulate nerve function. Several studies have shown that TNF- α sensitizes colonic afferent and enteric nerves. Increased

sensitisation of colonic nerves could lead to increased pain perception as evidenced in our study where there is a linear correlation between TNF levels and abdominal pain .[88-90]

A chronic state of low grade subclinical inflammation characterizes IBS. This might be the result of an imbalanced host response to the gut microbiome, triggered by a pathogen, as is the case of PI-IBS, and accentuated by genetic factors.

This opens up a new approach where in cytokines can be used as a biomarker of disease activity in IBS. Newer and better therapeutic possibilities will materialise in the future as the understanding of the disease process becomes lucid. This requires further interventional trials and cytokines can be both used as the target for therapy as well as marker of treatment response.

CONCLUSION

CONCLUSION

- The results of this case control study indicate that mild inflammation is involved in IBS-D patients as the major pro-inflammatory cytokine TNF-α value is raised in the serum.

- There appears to be a significant correlation between serum TNF values and symptoms of abdominal pain and stool frequency.

BIBLIOGRAPHY

BIBILIOGRAPHY

1. Horwitz B J, Fisher R S. The irritable bowel syndrome. *N Engl J Med*. 2001;344(24):1846–1850.
2. Scarlett EP. et al Functional Disturbances of the Colon: the Irritable (Spastic) Colon. *Can Med Assoc J*. May 1937; 36(5): 484–489.
3. Lovell RM, Ford AC. Global prevalence of and risk factors for irritable bowel syndrome: a meta-analysis. *Clin Gastroenterol Hepatol*. 2012;10(7):712–721.
4. Ghoshal UC,Abraham P,Bhatt C,et al. Epidemiological and clinical profile of irritable bowel syndrome in India: report of the Indian Society of Gastroenterology Task Force. *Indian J Gastroenterol* 2008;27:22-24.
5. Payne S (2004). "Sex, gender, and irritable bowel syndrome: Making the connections". *Gender medicine* **1** (1): 18–28.
6. Videlock EJ, Chang L. Irritable bowel syndrome: current approach to symptoms, evaluation, and treatment. *Gastroenterol Clin North Am*. 2007;36:665–685.
7. Manning AP, Thompson WG, Heaton KW, Morris AF (1978). "Towards positive diagnosis of the irritable bowel.". *Br Med J* **2** (6138): 653–4.

8. Holten KB, Wetherington A, Bankston L (2003). "Diagnosing the patient with abdominal pain and altered bowel habits: is it irritable bowel syndrome?". *Am Fam Physician* 67 (10): 2157–62.
9. Longstreth GF, Thompson WG, Chey WD, et al. Functional bowel disorders. *Gastroenterology* 2006;130:1489–1491.
10. N.J.Talley S F Philips L J Melton Diagnostic value of the Manning criteria in irritable bowel syndrome. *Gut.* Jan 1990; 31(1): 77–81.
11. Drossman DA, Whitehead WE, Camilleri M. Irritable bowel syndrome: a technical review for practice guideline development. *Gastroenterology*1997;112:2120–37.
12. Kellow JE, Miller LJ, Phillips SF, et al. Altered small bowel motility in irritable bowel syndrome is correlated with symptoms. *Gastroenterology*1987;92:1885–93.
13. Bueno L, Fioramonti J, Ruckebusch Y, et al. Evaluation of colonic myoelectrical activity in health and functional disorders. *Gut*1980;21:480–5.
14. Kellow JE Delvaux M Azpiroz F et al. Principles of applied neuro gastroenterology: physiology/motility-sensation. *Gut* 1999; 45:II17–24.
15. Collins SM, Vallance B, Barbara G, et al. Putative inflammatory and immunological mechanisms in functional bowel disorders. Baillieres *Clin Gastroenterol*1999;13:429–36.

16. Mertz H et al. Regional cerebral activation in irritable bowel syndrome and control subjects with painful and non painful rectal distension. *Gastroenterology*. 2000;118:842-8.
17. Tremolaterra F et al. Intestinal tone and gas motion. Neurogastroenterol Motil. 2006;18:905-10
18. Serra J. Azpiroz F. and Malagelada, J.R. Impaired transit and tolerance of intestinal gas in the irritable bowel syndrome. *Gut*. 2001; 48: 14–19.
19. Caldarella, M., Serra, J., Azpiroz, F. et al. Prokinetic effects in patients with intestinal gas retention. *Gastroenterology*. 2002; 122: 1748–1755.
20. Van Wijk HJ, Smout AJ, Akkermans LM, Roelofs JM, ten Thije OJ. Gastric emptying and dyspeptic symptoms in the irritable bowel syndrome. Scand J Gastroenterol 1992;27:99-102.
21. Evans PR, Bak YT, Shuter B, Hoschl R, Kellow JE. Gastroparesis and small bowel dysmotility in irritable bowel syndrome. Dig Dis Sci 1997;42:2087-93.
22. Stanghellini V, Tosetti C, Barbara G, De Giorgio R, Cogliandro L, Cogliandro R, Corinaldesi R. Dyspeptic symptoms and gastric emptying in the irritable bowel syndrome. Am J Gastroenterol 2002;97:2738-43.

23. Kellow JE, Gill RC, Wingate DL. Prolonged ambulant recordings of small bowel motility demonstrate abnormalities in the irritable bowel syndrome. *Gastroenterology* 1990;98:1208-18.

24. Simren M, Castedal M, Svedlund J, Abrahamsson H, Bjornsson E. Abnormal propagation pattern of duodenal pressure waves in the irritable bowel syndrome (IBS) [correction of (IBD)]. Dig Dis Sci 2000;45:2151-61.

25. Cann PA, Read NW, Brown C, Hobson N, Holdsworth CD. Irritable bowel syndrome: relationship of disorders in the transit of a single solid meal to symptom patterns. *Gut* 1983;24:405-11.

26. Whitehead WE, Engel BT, Schuster MM. Irritable bowel syndrome: physiological and psychological differences between diarrhea-predominant and constipation-predominant patients. Dig Dis Sci 1980;25:404-13.

27. Steens J, Van Der Schaar PJ, Penning C, Brussee J, Masclee AA. Compliance, tone and sensitivity of the rectum in different subtypes of irritable bowel syndrome. Neurogastroenterol Motil 2002;14: 241-7.

28. Hooper LV,Gordon JI. Commensal host-bacterial relationships in the gut. *Science* 2001;292:1115-8.

29. Kassinen A,Kogius Kurikka L,Makkivuoko H ,et al. The fecal microbiota of IBS patients differs significantly from that of healthy subjects. *Gastroenterology* 2007 ;133(1):24- 33.

30. Posserud I, Stotzer PO,Bornsson ES , et al. Small intestinal bacterial overgrowth in patients with irritable bowel syndrome. *Gut* 2007;56(6):802-8.

31. Pimentel M, Chow EJ, Lin HC. Normalisation of lactulose breath testing correlates with symptom improvement in irritable bowel syndrome, A double blind, randomised, placebo-controlled study. *Am J Gastroenterol* 2003 ; 98(2):412-9.

32. Hiatt R. Mast cells in inflammatory conditions of the gastrointestinal tract. *The American Journal of Gastroenterology* 1962.

33. O'Sullivan M, Clayton N, Breslin NP, Harman I, Bountra C, McLaren A, O'Morain CA. Increased mast cells in the irritable bowel syndrome. Neurogastroenterol Motil 2000;12:449-57.

34. Spiller RC, Jenkins D, Thornley JP, et al Increased rectal mucosal enteroendocrine cells, T lymphocytes, and increased gut permeability following acute campylobacter enteritisand in post infectious IBS-D. *Gut* 2004;47(6):804-11.

35. Tornblom H,Lindberg G,Nyberg Bet al .Full Thicknessbiopsy of the jeunum reveals inflammation and enteric neuropathy in irritable bowel syndrome. *Gastroenterology* 2002;123(6):1972-9.
36. Ohman L, Isaksson S, Lungren Aetal. A controlled study of colonic immune activity and beta blood T lymphocytes in patients with irritable bowel syndrome. *Clin Gastroenterol Hepatol* 2005;3(10):980-6
37. Wakamatsu Y, Weston JA. Sequential expression and role of Hu RNA-binding proteins during neurogenesis. Development 1997;124:3449-60.
38. De Giorgio R, Bovara M, Barbara G, Canossa M, Sarnelli G, De Ponti F, Stanghellini V, Tonini M, Cappello S, Pagnotta E, Nobile-Orazio E, Corinaldesi R. Anti-HuD-induced neuronal apoptosis underlying paraneoplastic gut dysmotility. *Gastroenterology* 2003;125:70-9.
39. Vernino S, Low PA, Fealey RD, Stewart JD, Farrugia G, Lennon VA. Autoantibodies to ganglionic acetylcholine receptors in autoimmune autonomic neuropathies. *N Engl J Med* 2000;343:847-55.
40. Knowles CH, Lang B, Clover L, Scott SM, Gotti C, Vincent A, Martin JE. A role for autoantibodies in some cases of acquired non-paraneoplastic gut dysmotility. *Scand J Gastroenterol* 2002;37:166-70.

41. Barbara G, De Giorgio R, Deng Y, Vallance B, Blennerhassett P, Collins SM. Role of immunologic factors and cyclooxygenase 2 in persistent postinfective enteric muscle dysfunction in mice. *Gastroenterology* 2001;120:1729-36.
42. Collins SM. The immunomodulation of enteric neuromuscular function: Implications for motility and inflammatory disorders. *Gastroenterology* 1996;111:1683–99.
43. Callard R,George AJ and Stark J(1999): Cytokines,chaos,and complexity. *Immunity* 11;507-13.
44. Tracey(1997).Tumour necrosis factor in cytokines in health and disease.Eds.Remick D,Friedland J, Marcel Dekker inc., New York.
45. SindhuRS and Bollon AP(1993): Tumour necrosis factor activities and canacer therapy, a perspective. *Pharmacol Ther* 57: 79-128.
46. Pappadikas KA and Tragan SR(2000): Tumour necrosis factor: Biology and therapeutic inhibitors. *Gastroenterolgy* 119: 1148-57.
47. Ruulus SR and sedwick D (1999) Unlinking tumour necrosis factor biology from the maor histocompatibility complex:lessons from human genetics and animal models. *Am j hum Genet* 65: 294-301.
48. Sidhu RS nd Bollon AP (1993): Tumour necrosis factor activities and cancer therapy, a perspective. *Pharmacol Ther* 57: 79-128.

49. Elsenbruch S: Abdominal pain in Irritable Bowel Syndrome: a review of putative psychological, neural and neuro-immune mechanisms. *Brain Behav Immun* 2011, 25**:**386-394.

50. Longstreth GF, Thompson WG, Chey WD, Houghton LA, Mearin F, Spiller RC: Functional bowel disorders. *Gastroenterology* 2006, 130**:**1480-1491.

51. Cremon C, Gargano L, Morselli-Labate AM, Santini D, Cogliandro RF, De Giorgio R, Stanghellini V, Corinaldesi R, Barbara G: Mucosal immune activation in irritable bowel syndrome: gender dependence and association with digestive symptoms. *Am J Gastroenterol* 2009, 104**:**392-400.

52. Lee HF, Hsieh JC, Lu CL, Yeh TC, Tu CH, Cheng CM, Niddam DM, Lin HC, Lee FY, Chang FY: Enhanced affect/cognition related brain responses during visceral placebo analgesia in irritable bowel syndrome patients**.** *Pain* 2012, 153**:**1301-1310.

53. Spiller R, Garsed K: Postinfectious irritable bowel syndrome. *Gastroenterology* 2009, 136**:**1979-1988.

54. Ji Chen**,** Yangde Zhang and Zhansheng Deng Imbalanced shift of cytokine expression between T helper 1 and T helper 2 (Th1/Th2) in intestinal mucosa of patients with post-infectious irritable bowel syndrome *BMC Gastroenterology* 2012, 12:91.

55. Patrick A Hughes Ph.D, Heddy Zola, Irmeli A Penttila Ph.D, L Ashley Blackshaw Ph.D, Jane M Andrews MD, Ph.D and Doreen Krumbiegel Ph.D Immune Activation in Irritable Bowel Syndrome: Can Neuroimmune Interactions Explain Symptoms? *Am J Gastroenterol* 2013; 108:1066–1074.

56. Bertiaux-Vandaele N, Youmba SB, Belmonte L *et al*. The expression and the cellular distribution of the tight junction proteins are altered in irritable bowel syndrome patients with differences according to the disease subtype. *Am J Gastroenterol* 2011;106:2165–2173.

57. O'Mahony L, McCarthy J, Kelly P, et al. Lactobacillus and bifidobacterium in irritable bowel syndrome: Symptom responses and relationship to cytokine profiles. *Gastroenterology*2005;128:541–54.

58. W M Gonsalkorale, C Perrey, V Pravica, P J Whorwell, I V Hutchinson 1 Interleukin 10 genotypes in irritable bowel syndrome: evidence for an inflammatory component? *Gut 2003;52:91-93*.

59. Bashashati M, Rezaei N, Bashashati H, et al. Cytokine gene polymorphisms are associated with irritable bowel syndrome: a systematic review and meta-analysis. *Neurogastroenterol Motil*. 2012;24(12):1102.

60. Camilleri M, Katzka DA. Irritable bowel syndrome: methods, mechanisms, and pathophysiology. Genetic epidemiology and pharmacogenetics in irritable bowel syndrome. *Am J Physiol Gastrointest Liver Physiol*. 2012;302(10):G1075–G1084.
61. Zucchelli M, Camilleri M, Andreasson AN, et al. Association of TNFSF15 polymorphism with irritable bowel syndrome. *Gut* 2011;60(12):1671–1677.
62. Brint EK, MacSharry J, Fanning A, Shanahan F, Quigley EM. Differential expression of toll-like receptors in patients with irritable bowel syndrome. *Am J Gastroenterol*. 2010;106(2):329–336.
63. Belmonte L, Beutheu Youmba S, Bertiaux-Vandaele N, et al. Role of toll like receptors in irritable bowel syndrome: differential mucosal immune activation according to the disease subtype. *PLoS One*. 2012;7(8):e42777.
64. Ohman L, Lindmark AC, Isaksson S, et al. Increased TLR2 expression on blood monocytes in irritable bowel syndrome patients. *Eur J Gastroenterol Hepatol*. 2012;24(4):398–405.
65. Camilleri M, Mangel AW, Fehnel SE, Drossman DA, Mayer EA, Talley NJ. Primary endpoints for irritable bowel syndrome trials: a review of performance of endpoints. *Clin Gastroenterol Hepatol*. 2007;5(5):534–540.

66. Rasquin A, Di Lorenzo C, Forbes D, et al. Childhood functional gastrointestinal disorders: child/adolescent. *Gastroenterology*. 2006;130(5):1527–1537.

67. Tang YR, Yang WW, Liang ML, Xu XY, Wang MF, Lin L. Age related symptom and life quality changes in women with irritable bowel syndrome. *World J Gastroenterol*. 2012;18(48):7175–7183.

68. Maxwell PR, Mendall MA, Kumar D. Irritable bowel syndrome. *Lancet*. 1997;350(9092):1691–1695.

69. Kennedy TM, Jones RH, Hungin AP, O'flanagan H, Kelly P. Irritable bowel syndrome, gastro-oesophageal reflux, and bronchial hyper-responsiveness in the general population. *Gut*. 1998;43(6):770–774.

70. Drossman DA, Funch-Jensen P, Janssens J. Identification of subgroups of functional bowel disorders. *Gastroenterol Int*. 1990;(3):159–172.

71. Lee OY, Mayer EE, Schmulson M, Chang L, Naliboff B. Gender related differences in IBS symptoms. *Am J Gastroenterol*. 2001;96(7): 2184–2196.

72. Williams RE, Black CL, Kim HY, et al. Determinants of healthcare seeking behaviour among subjects with irritable bowel syndrome. *Aliment Pharmacol Ther*. 2006;23(11):1667–1675.

73. Scully P, McKernan DP, Keohane J, Groeger D, Shanahan F, Dinan TG, et al. Plasma cytokine profiles in females with irritable bowel syndrome and extra-intestinal co-morbidity. *Am J Gastroenterol* 2010;105:2235–43.

74. McKernan DP, Gaszner G, Quigley EM, Cryan JF and Dinan TG. Altered peripheral toll-like receptor responses in the irritable bowel syndrome. Aliment Pharmacol Ther. 2011;33:1045–52.

75. D inan TG, Quigley EM, Ahmed SM, Scully P, O'Brien S, O'Mahony L, et al. Hypothalamic-pituitary-gut axis dysregulation in irritable bowel syndrome: plasma cytokines as a potential bio-marker? *Gastroenterology* 2006;130:304–11.

76. Chrousos GP. The hypothalamic-pituitary-adrenal axis and immune mediated inflammation. *N Engl J Med*. 1995;332:1351–62.

77. Dunn AJ. Systemic interleukin-1 administration stimulates hypothalamic norepinephrine metabolism parallelling the increased plasma corticosterone. Life Sci. 1988;43:429–35.

78. Van der Veek PP , van den Berg M , de Kroon YE *et al.* Role of tumor necrosis factor-alpha and interleukin-10 gene polymorphisms in irritable bowel syndrome. *Am J Gastroenterol* 2005;100: 2510 – 6 .

79. Gonsalkorale WM , Perrey C , Pravica V *et al.* Interleukin 10 genotypes in irritable bowel syndrome: evidence for an infl amma-tory component? *Gut* 2003;52:91 – 3 .

80. Barkhordari E , Rezaei N , Ansaripour B *et al.* Proinfl ammatory cy-tokine gene polymorphisms in irritable bowel syndrome. *J Clin Immunol* 2010 ; 30 : 74 – 9.

81. Barkhordari E , Rezaei N , Mahmoudi M *et al.* T-helper 1, T-helper 2, and T-regulatory cytokines gene polymorphisms in irritable bowel syndrome. *Inflammation* 2010;33:281 – 6.

82. Bashashati M , Rezaei N , Bashashati H *et al.* Cytokine gene poly-morphisms are associated with irritable bowel syndrome: a system-atic review and meta-analysis . Neurogastroenterol Motil 2012;24:1102 – e566.

83. Barkhordari E, Rezaei N, Ansaripour B, Larki P, Alighardashi M, Ahmadi Ashtiani HR, et al. Proinflammatory cytokine gene polymorphisms in irritable bowel syndrome. *J Clin Immunol* 2010;30:74–9.

84. S Santhosh, AK Dutta, P Samuel, AJ Joseph, J Ashok Kumar, George Kurian Cytokine gene polymorphisms in Irritable bowel syndrome in Indian population - A pilot case control study. *Tropi-cal gastroenterol.* 2010 Jan-Mar;31(1):30-3.

85. Lee HJ, Lee SY, Choi JE, Kim JH, Sung IK, Park HS, Jin C et al G protein beta3 subunit, interleukin-10, and tumor necrosis factor-alpha gene polymorphisms in Koreans with irritable bowel syndrome Neurogastroenterol motility 2010 Jul;22(7):758-63.

86. Dunlop SP, Jenkins D, Neal KR and Spiller RC. Relative importance of enterochromaffin cell hyperplasia, anxiety, and depression in postinfectious IBS. *Gastroenterology* 2003;125:1651–9.

87. Cumberland P, Sethi D, Roderick PJ, Wheeler JG, Cowden JM, Roberts JA, et al. The infectious intestinal disease study of England: a prospective evaluation of symptoms and health care use after an acute episode. *Epidemiol Infect*. 2003;130:453–60.

88. Gwee KA, Collins SM, Read NW, Rajnakova A, Deng Y, Graham JC, et al. Increased rectal mucosal expression of interleukin 1beta in recently acquired post-infectious irritable bowel syndrome. *Gut* 2003;52:523–6.

89. Ibeakanma C , Vanner S . TNFalpha is a key mediator of the prono-ciceptive effects of mucosal supernatant from human ulcerative co-litis on colonic DRG neurons. *Gut* 2010 ;59:612 – 21 .

90. Kindt S , Vanden Berghe P , Boesmans W *e t al.* Prolonged IL-1beta exposure alters neurotransmitter and electrically induced Ca(2+) responses in the myenteric plexus. Neurogastroenterol Motil 2010; 22: 321 – e85 .

91. Xia Y , Hu HZ , Liu S *et al.* IL-1beta and IL-6 excite neurons and suppress nicotinic and noradrenergic neurotransmission in guinea pig enteric nervoussystem . *J Clin Invest* 1999;103:1309 – 16.

92. Corinaldesi R, Stanghellini V, Cremon C, et al. Effect of mesalazine on mucosal immune biomarkers in irritable bowel syndrome: a randomized controlled proof-of-concept study. *Aliment Pharmacol Ther*. 2009;30(3):245–252.

93. Foley S, Garsed K, Singh G, et al. Impaired uptake of serotonin by platelets from patients with irritable bowel syndrome correlates with duodenal immune activation. *Gastroenterology* 2011;140(5): 1434–1443. e1.

ANNEXURES

ABREVIATIONS

IBS	:	Irritable Bowel Syndrome
TNF α-	:	Tumour Necrosis Factor α
ESR	:	Erythrocyte Sedimentation Rate
WBC	:	White Blood Cell count
FGID	:	Functional Gastrointestinal Disorder
ENS	:	Enteric Nervous System
MaDCAM	:	Molecular addresin Cell Adhesion Molecule
RNA	:	Ribonucleic Acid
IL	:	Interleukin
TGF β	:	Transforming growth factor
ARDS	:	Acute Respiratory Distress Syndrome
DIC	:	Disseminated Intravascular Coagulation
TLR	:	Toll Like Receptor
ELISA	:	Enzyme Linked Immuno Sorbent Assay

PROFORMA

NAME : AGE / SEX :

DDHD No. :

HISTORY

ABDOMINAL PAIN : ABDOMINAL BLOATING :

STOOL FREQUENCY : STOOL CONSISTENCY :

BLEEDING PR : MUCOUS IN STOOL :

NOCTURNAL SYMPTOMS : TENESMUS :

LOSS OF APETITE : LOSS OF WEIGHT :

FEVER :

PAST HISTORY

DM : SHT : TB :

IHD : DRUG/ NSAID INTAKE: SIMILAR CMPLAINTS IN PAST :

PERSONAL HISTORY:

SMOKING : ALCOHOL :

SKIPING OFMEALS : TOBACCO :

CAFFINE: EMOTIONAL STRESS :

SLEEP PATTERN :

FAMILY HISTORY:

GENERAL EXAMINATION:

CONSCIOUS/ORIENTED : PALLOR :

ICTERUS: CYANOSIS : CLUBBING :

EDEMA:LYMPHADENOPATHY: PURPURA: OTHER SIGNS :

VITALS : HT : WT : BMI :

PULSE : BP : TEMP : URINE OUTPUT :

SYSTEMIC EXAMINATION:

ORAL CAVITY: P/A:

R.S: CVS: CNS:

P/R:

INVESTIGATIONS:

Hb	:	ESR	:	TC :
DC	:	P L E M		
BT	:	CT	:	Pl COUNT:
RBS	:	UREA:		
CREATININE	:			
ECG	:			
CHEST X RAY	:			
USG ABDOMEN	:			
FOBT	:			
STOOL R/E	:			
ICTC/HIV	:			
COLONOSCOPY	:			
LEVELS OF TNF α	:			
HPE	:			

MASTER CHART – CASES

Sl.No.	Name	DDHD No.	Age	Sex	Ab pain	Ab bloat	st freq	BSC	Mucous	DM	TNF α	Colonosco	Hb	TC	Stress	Smoking	st.ova	USG Ab
1	Gunasekar	797/14	35	Male	1	1	5	5	1	0	14.1	0	14	6000	1	1	1	1
2	Shanthi	47/14	37	Female	5	1	6	5	0	0	13.2	0	12	7500	1	1	1	1
3	Padmavathy	43/14	32	Female	4	1	5	6	0	0	15.8	0	14	5646	1	1	1	2
4	Balan	99/14	34	Male	4	1	4	6	0	0	15.9	0	11	6745	2	2	1	1
5	Banumathy	789/14	39	Female	6	0	5	6	1	1	15.7	0	12	4980	2	1	1	1
6	Shyam	790/14	24	Male	4	1	5	6	0	0	14.8	0	15	6700	1	1	1	2
7	Baskar	532/14	40	Male	3	0	3	6	0	0	13.9	0	11	6590	1	2	1	1
8	Nalini	6250/11	58	Female	6	1	4	6	1	1	15.9	0	9	6700	2	1	1	1
9	Manoj	817/14	23	Male	6	0	3	5	0	0	16	0	12	7500	2	1	1	1
10	Prakash	2207/14	26	Male	4	1	5	6	0	0	14.9	0	11	7634	1	2	1	1
11	Ragunath	2019/14	25	Male	4	0	6	5	0	0	15.1	0	12	6700	1	2	1	1
12	Jayanthi	2768/12	43	Female	5	1	4	5	0	1	15.2	0	11	6312	1	1	1	2
13	Venkatesan	2578/13	40	Male	6	1	5	5	0	0	15.8	1	14	8000	1	2	1	1
14	Vasantha	2703/13	58	Female	6	0	5	5	0	0	16	0	10	6500	1	1	1	1
15	Munusamy	5152/11	40	Male	5	0	5	7	0	0	14.9	0	12	6790	2	1	1	1
16	Navamani	1690/06	44	Male	4	1	5	5	1	0	14.9	0	12	6750	1	1	1	1
17	Hemanath	97/14	32	Male	5	1	4	6	1	0	15.1	1	14	6600	1	2	1	1
18	Immanuel	3518/14	20	Male	6	1	3	6	1	0	15.9	0	14	6800	1	2	2	1
19	Dhamyanthi	6250/07	64	Female	5	1	4	5	0	0	15.6	0	12	8500	2	1	1	1
20	Vanitha	1802/13	40	Female	2	1	5	6	0	0	14.8	1	13	5600	1	1	1	1

Sl.No.	Name	DDHD No.	Age	Sex	Ab pain	Ab bloat	st freq	BSC	Mucous	DM	TNF α	Colonoscopy	Hb	TC	Stress	Smoking	st.ova	USG Ab
21	Settu	3553/14	46	Male	6	1	5	5	0	1	15.9	0	10	6500	1	2	1	2
22	Sekar	3632/14	42	Male	5	0	4	5	1	0	15.2	1	12	8700	2	2	1	1
23	Ashok	4630/14	25	Male	4	1	5	6	0	0	14.9	0	14	7600	1	2	1	1
24	Chitra	4988/14	38	Female	7	1	5	5	0	0	16	0	11	6700	1	1	1	1
25	Kala	5689/14	22	Female	5	1	3	5	0	0	15.8	0	10	8500	1	1	1	1
26	Chandra	6590/14	33	Female	6	0	3	5	0	0	15.6	0	10	5600	1	1	1	1
27	Maran	6587/14	34	Male	6	0	5	5	1	0	15.7	1	12	6500	1	2	1	2
28	Keerthi	6241/14	43	Female	6	1	5	6	0	0	15.9	0	14	6578	1	1	1	1
29	Dinesh	6475/14	27	Male	5	1	4	3	1	0	14.9	0	15	6570	1	1	1	1
30	Malathi	5614/14	18	Female	4	1	6	5	1	0	13.9	0	10	6590	2	1	1	1
31	Rosemary	7500/14	42	Female	4	1	4	5	0	0	14.9	0	10	8000	1	1	1	1
32	Kumar	3340/14	36	Male	4	1	7	6	0	0	14.8	1	11	7600	1	2	1	1
33	Arokyam	3619/14	29	Male	5	0	5	5	1	0	15.6	0	11	4870	2	1	1	2
34	Neela	6745/14	21	Female	3	1	5	7	0	0	13.9	0	12	5000	1	1	1	1
35	Umayal	7632/14	33	Female	2	1	4	6	0	0	14.2	1	14	5342	1	1	1	1
36	Babyamma	7640/14	45	Female	3	0	5	7	1	0	14.8	0	15	7900	1	1	1	1
37	Sekar	4612/14	42	Male	5	1	3	7	0	0	15.6	0	10	5800	1	1	1	1
38	Yuvaraj	5615/14	29	Male	6	1	4	5	0	0	15.6	0	14	6700	2	2	1	1
39	Kannan	6784/14	34	Male	4	1	6	5	0	0	15.3	0	13	6900	1	2	1	1
40	Arun	4571/14	32	Male	4	1	5	6	0	0	15.4	0	12	6500	1	1	1	1
41	Saraswathi	3421/14	24	Female	3	1	5	6	1	0	14.9	0	10	6890	1	1	1	1

MASTER CHART – CONTROLS

Sl.No.	Name	DDHD No	Age	Sex	Pain	Bloat	Freq	BSC	Mocous	DM	SHT	TNF α	Colon	Hb	TC	st.ova	USG Ab	Smoker	Stress
1	Ammu	5757/14	37	Female	0	0	2	4	0	0	0	5.2	0	14	4000	1	1	2	1
2	Surendran	6466/14	47	Male	0	0	2	3	0	0	1	4.9	14	13	4500	1	1	1	1
3	Daniel	7342/14	23	Male	0	0	1	3	0	0	0	5.1	0	12	5400	1	1	1	1
4	Saravanan	3528/13	40	Male	0	0	1	2	0	1	0	6.8	0	13	5200	1	1	1	1
5	Parthasarathy	1686/14	34	Male	0	0	2	3	0	0	0	6.5	0	12	4000	1	1	1	1
6	Dhanalakshmi	6150 /14	24	Female	0	0	2	3	0	0	0	6.3	0	14	4200	1	1	1	1
7	Eswaran	6358/14	40	Male	0	0	2	4	0	0	0	6.9	0	12	5400	1	1	1	1
8	Sankar	6209/14	33	Male	0	0	3	4	0	0	0	6.7	0	11	4200	1	1	1	1
9	Malathi	5235/14	25	Female	0	0	1	2	0	0	0	5.8	0	13	5400	1	1	1	1
10	Gloria	6376/14	43	Female	0	0	2	4	0	0	1	5.9	0	15	4200	1	1	1	1
11	Chellama	6348/14	28	Female	0	0	2	3	0	0	0	7	0	12	5300	1	1	1	1
12	Sankar Ganesh	6209/14	33	Male	0	0	2	3	0	0	0	4.9	0	12	4000	1	1	2	1
13	Murugan	5187/14	37	Male	0	0	1	4	0	0	0	5.5	0	12	5000	1	1	2	1
14	Radhakrishnan	56413/14	55	Male	0	0	2	3	0	1	1	5	0	11	5100	2	1	1	1
15	Abdul	4228/14	28	Male	0	0	2	4	0	0	0	6.3	0	13	4500	1	1	1	1
16	Ranganayaki	6382/14	31/	Female	0	0	1	3	0	0	0	5.4	0	12	5600	1	1	1	2
17	Alli	2854/13	32	Female	0	0	3	3	0	0	0	5.4	0	11	4000	1	1	1	1
18	Masthan	6341/14	28	Male	0	0	2	4	0	0	0	6.2	0	14	4800	1	1	2	1
19	Erina	1119/14	45	Female	0	0	2	4	0	0	0	6.7	0	12	5200	1	1	1	1
20	China	147/13	42	Male	0	0	2	2	0	0	0	5.9	0	11	5100	1	1	2	1

21	**Uma**	**4344/13**	**23**	**Female**	**0**	**0**	**1**	**4**	**0**	**0**	**0**	**5.8**	**0**	**11**	**4900**	**1**	**1**	**1**	**1**
22	**Natarajan**	**3887/14**	**31**	**Male**	**0**	**0**	**2**	**5**	**0**	**0**	**0**	**4.9**	**0**	**13**	**4200**	**1**	**1**	**2**	**1**
23	**Rachel**	**6002/14**	**50**	**Female**	**0**	**0**	**2**	**3**	**0**	**0**	**0**	**6.1**	**0**	**14**	**5900**	**1**	**1**	**1**	**1**
24	**Sankari**	**4473/14**	**30**	**Female**	**0**	**0**	**2**	**3**	**0**	**0**	**0**	**6**	**0**	**12**	**4100**	**1**	**1**	**1**	**1**
25	**Venkatesan**	**6144/14**	**38**	**Male**	**0**	**0**	**2**	**4**	**0**	**0**	**0**	**6.9**	**0**	**11**	**5100**	**1**	**2**	**1**	**1**
26	**Senkutuvan**	**1785/14**	**32**	**Male**	**0**	**0**	**1**	**4**	**0**	**0**	**0**	**5.4**	**0**	**11**	**4900**	**2**	**2**	**2**	**1**
27	**Kumar**	**5096/16**	**32**	**Male**	**0**	**0**	**2**	**3**	**0**	**0**	**0**	**4.9**	**0**	**12**	**4200**	**1**	**1**	**2**	**1**
28	**Kavitha**	**5076/14**	**48**	**Female**	**0**	**0**	**2**	**3**	**0**	**0**	**0**	**7.2**	**0**	**11**	**5100**	**1**	**2**	**1**	**1**
29	**Anandhi**	**5069/14**	**38/**	**Female**	**0**	**0**	**3**	**4**	**0**	**0**	**0**	**5.6**	**0**	**14**	**4500**	**1**	**1**	**1**	**1**
30	**Vijay**	**5072/14**	**24**	**Male**	**0**	**0**	**2**	**4**	**0**	**0**	**0**	**5.7**	**0**	**11**	**4800**	**1**	**1**	**2**	**1**
31	**Subramani**	**5068/14**	**37**	**Male**	**0**	**0**	**1**	**2**	**0**	**0**	**0**	**5.1**	**0**	**12**	**5100**	**1**	**1**	**2**	**1**
32	**Gajalaxmi**	**5067/14**	**42**	**Female**	**0**	**0**	**1**	**3**	**0**	**1**	**0**	**6**	**0**	**12**	**5000**	**1**	**2**	**1**	**1**
33	**Asma**	**2351/14**	**21**	**Female**	**0**	**0**	**1**	**4**	**0**	**0**	**0**	**6.6**	**0**	**13**	**4500**	**1**	**1**	**1**	**1**
34	**Nivetha**	**3071/14**	**19**	**Female**	**0**	**0**	**2**	**2**	**0**	**0**	**0**	**6.9**	**0**	**12**	**5100**	**1**	**1**	**1**	**1**
35	**Ramdoss**	**5031/14**	**51**	**Male**	**0**	**0**	**2**	**3**	**0**	**1**	**1**	**7**	**0**	**11**	**4800**	**1**	**1**	**2**	**1**
36	**Manoj**	**5037/14**	**38**	**Male**	**0**	**0**	**1**	**4**	**0**	**0**	**0**	**0**	**0**	**11**	**5000**	**1**	**1**	**2**	**1**
37	**Ragni**	**5131/14**	**25**	**Female**	**0**	**0**	**2**	**3**	**0**	**0**	**0**	**5.9**	**0**	**13**	**4500**	**1**	**1**	**1**	**1**
38	**Harini**	**3124/14**	**28**	**Female**	**0**	**0**	**2**	**4**	**0**	**0**	**0**	**5.8**	**0**	**11**	**4800**	**1**	**2**	**1**	**1**
39	**Jegan**	**6225/14**	**34**	**Male**	**0**	**0**	**2**	**4**	**0**	**0**	**0**	**6.1**	**0**	**11**	**5300**	**1**	**1**	**2**	**1**
40	**Shrini**	**6165/14**	**34**	**Male**	**0**	**0**	**1**	**4**	**0**	**0**	**0**	**6.1**	**0**	**12**	**4500**	**1**	**1**	**1**	**1**

INSTITUTIONAL ETHICAL COMMITTEE
GOVT. KILPAUK MEDICAL COLLEGE
CHENNAI-10
REF.NO.18520/ME-I/Ethics/2013 Dt:05.12.2013
CERTIFICATE OF APPROVAL

The Institutional Ethical Committee of Govt. Kilpauk Medical College, Chennai-10 reviewed and discussed the application for approval "A Study on cytokine response in diarrhea predominant irritable bowel syndrome" – For Project work submitted by Dr.M.Tarakeshwari, DM (Medical Gastroenterology) PG Student, KMC / GRH, Chennai.

The Proposal is APPROVED.

The Institutional Ethical Committee expects to be informed about the progress of the study any Adverse Drug Reaction Occuring in the course of the study any change in the protocol and patient information / informed consent and asks to be provided a copy of the final report.

CHAIRMAN 28/1/14.
Ethical Committee
Govt. Kilpauk Medical College. Chennai

GOVT. KILPAUK MEDICAL COLLEGE
28 JAN 2014
Kilpauk Chennai-10.

Object

16112655.dm (mge) TARAKESHWARI M.MOHANARAJAN. User Info Messages

- Help Logout

- Peer Review
- My Grades
- Discussion
- Calendar

NOW VIEWING:

- HOME > THE TAMIL NADU DR.M.G.R.MEDICAL UTY 2014-15 EXAMINATIONS

- Usage Policy
- Privacy Pledge
- Helpdesk
- Research Resources

Turnitin

×

Welcome to your new class homepage! From the class homepage you can see all your assignments for your class, view additional assignment information, submit your work, and access feedback for your papers.
Hover on any item in the class homepage for more information.

Class Homepage

This is your class homepage. To submit to an assignment click on the "Submit" button to the right of the assignment name. If the Submit button is grayed out, no submissions can be made to the assignment. If resubmissions are allowed the submit button will read "Resubmit" after you make your frst submission to the assignment. To view the paper you have submitted, click the "View" button. Once the assignment's post date has passed, you will also be able to view the feedback left on your paper by clicking the "View" button.

Assignment Inbox: The Tamil Nadu Dr.M.G.R.Medical Uty 2014-15 Examinations

Info Dates Similarity

TNMGRMU EXAMINATIONS

Start 01-Sep-2014 11:27AM

Due 15-Aug-2015 11:59PM

Post 15-Aug-2015 12:00AM

15%