FR 1713

FINAL REPORT

FR 1713.

Section1
Information regarding Project

Contract number- RG/2005/HS/13
Title of the Project-"Irritable Bowel Syndrome (IBS):an inflammatory disease."
Principal Investigator-Dr.A.P.de Silva
Co-Investigators-Dr.A.S.Dassanayake
Prof.H.J.de Silva
Institution-Faculty of Medicine, University of Kelaniya
Date of award-27.7.2005
Date of completion of project-15.12 2009
Total allocation of funds Rs.400000
Total spent- Rs 637789.00
Number of research students employed –One student
Post graduate degree completed with dates -None
Number of Technical Assistants employed-One
Publications during the reporting period-Three

Section 2
Executive Summary of the Project

Background
There is evidence for potential roles for gut flora and the host immune response in the pathophysiology of IBS, and especially, for low grade colonic mucosal inflammation in the pathophysiology of post-infectious IBS.
Objectives
To determine whether mucosal inflammatory cytokines; IL1, TNF alpha,IL10 play a role in the pathogenesis of IBS
To investigate for evidence of sub-clinical intestinal mucosal inflammation in diarrhea-predominant IBS (IBS-D) in a tropical setting.
Methodology
In a prospective study we investigated 49 patients with IBS-D based on Rome III criteria. All patients had normal ESR, CRP, TSH and stools reports. 14 individuals with a family history of colon cancer were selected as controls. Stools of patients and controls were tested for calprotectin. During colonoscopy, serial biopsies were obtained from the ileum, caecum, ascending, transverse and descending colon, and rectum. In addition to histology, tissue expression of IL-8 and IL-10 were assessed in biopsy specimens using semi-quantitative RT-PCR.

Major findings
- Colono-ileoscopy was macroscopically normal and faecal calprotectin was undetectable in cases and controls.
- Tissue expression of IL-8 was significantly higher and IL-10 significantly lower in cases compared to controls. There was a significant inverse correlation between IL-8 and IL-10 expression.

There is an evidence that inflammatory cytokines; IL1, TNF alpha, IL10 play a role in the pathogenesis of IBS and sub-clinical intestinal mucosal inflammation in patients with IBS-D in a tropical setting.

Section 3
Report in detail
Background

Irritable bowel syndrome (IBS) is one of the commonest diseases affecting man, second only to the common cold [1]. The diagnostic criteria commonly used are the Rome III criteria [2]. The exact pathogenesis of IBS is not known; however, there are various theories. These include hypersensitivity to visceral pain, abnormal brain activation and the presence of chronic inflammation, especially in post-infective IBS [3].

The diagnosis of IBS is clinical. Several studies have shown that coeliac disease and lactose intolerance are wrongly diagnosed as IBS [4, 5]. Although coeliac disease is uncommon among Asians, 60% have lactose intolerance [6].

The best evidence for an inflammatory aetiology comes from the studies done on post-infective IBS (PIIBS) [7]. PIIBS usually gives rise to diarrhoea predominant IBS [8]. Some studies have shown an increase in mast cells in the colonic mucosa of patients with PIIBS [9], while others studies have shown an increase in rectal mucosal lymphocytes after a bout of enteritis [10]. An increase in small intestinal permeability has also been demonstrated in this condition [10]. Inflammation is associated with the production of mediators including, prostaglandins, bradykinins, nerve growth factors, adenosine and 5-hydroxytriptamine [11]. These mediators induce visceral hypersensitivity, exaggerated motor response and increased intestinal secretions [12], which could contribute to episodic diarrhoea. Inflammation can also increase 5-hydroxytriptamine by increasing the number of enterochromafin cells, which could also contribute to diarrhoea [12]; serotonin type 3 (5HT3) receptor antagonist are effective in symptom alleviation in diarrhoea predominate IBS [12]. Although inflammatory markers such as CRP and ESR are normal, it is possible that low-grade inflammation occurs in the colon and small bowel in patients with IBS. Some studies have shown that there is activation of the mucosal immune system in IBS, with an increase in intraepithelial lymphocytes on immunohistology despite normal conventional histology [13].

Cytokines are substances that help immune cells to communicate and coordinate the immune cascade [14]. Various cytokines including, IL 10 and transforming growth factor beta, have been shown to be involved in the pathogenesis of IBS [14]. However, there are no large studies on cytokine expression in IBS.

IBS is common in Sri Lanka. However, the exact prevalence has not been established. We have previously found that 60% of patients with IBS (diagnosed using the Rome III criteria) had microscopic colitis not otherwise specified (MCNOS), (unpublished data). MCNOS is a histological entity distinct from microscopic colitis [19]. However, the significance of the mild mucosal inflammation remains unknown. Whether, MCNOS is a feature of PIIBS is also unclear. As mucosal inflammation seems to be common in our IBS patients and since cytokines are pivotal in the regulation of mucosal inflammation. We have decided to investigate whether pro

inflammatory cytokines are increased in patients with IBS. This would give us a unique insight to the pathogenesis of IBS.

Objectives

To determine if inflammatory cytokines; IL1, TNF alpha, IL10 play a role in the pathogenesis of IBS

To investigate for evidence of sub-clinical intestinal mucosal inflammation in diarrhea-predominant IBS (IBS-D) in a tropical setting.

Materials and Methods

1. Cases (patients)
All patients with IBS diagnosed using ROME III criteria were offered colonoscopy. Those who volunteer to undergo the procedure was recruited after obtaining written informed consent .The investigators ensured that inclusion criteria were met.
A. Inclusion criteria

- All patients with IBS diagnosed using the ROME III criteria.
- 18 to 60 years of age.

B.Exclusion criteria

- Those patients with increased CRP.
- Patients with weight loss, bleeding per rectum and melena.
- Patients with thyrotoxicosis.
- Patients with lactose intolerance.
- Patients with amoebiasis and giadiasis or intestinal helminthic infections.
- Patients with motility drugs or any drugs known to cause diarrhea.
- Patients with inflammatory bowel disease.
- Patients with celiac disease.
- Patients who decline colonoscopy.

2.Controls
Age and sex matched controls will be recruited from among individuals who do not fulfill ROME III criteria, who do not have IBS or infective colitis, but who require colonoscopy for:

- Colonic polyp surveillance, or
- History of a first degree relative having colon cancer, or
- A positive faecal occult blood test ,and diagnosed to have telengiectasia.

In a prospective study over five years, we investigated 49 patients with IBS-D [median age 34 years (range 18-59; M:F 36:13], based on Rome III criteria. None had alarm symptoms: unintentional significant loss of weight, bleeding per rectum or malaena. None were on NSAIDS or proton pump inhibitors. All patients had normal ESR, CRP, TSH and stools reports. 14 individuals with a family history of colon cancer [median age 46.5 years (range 23-56); median 46.5, M:F 6:8] were selected as controls. Stools of patients and controls were tested for calprotectin. During colonoscopy, serial biopsies were obtained from the ileum, caecum, ascending, transverse and descending colon, and rectum. In

addition to histology, tissue expression of IL-8 and IL-10 were assessed in biopsy specimens using semi-quantitative RT-PCR.
An internal standard RNA was incorporated with template RNA in the RT-PCR reaction. reporter and target RNA molecules will have same primer binding sites. However, the reporter produces a different sized amplicon using the same set of primers. Target RNA is amplified with increasing known amounts of reporter RNA ,and was analysed by gel electrophoresis. Equal amounts of competitor and target amplicons were produced when their target concentrations are equal and the amount of target RNA can be estimated. The results were analyzed using the Statistical Package for the Social Sciences . The statistical method of comparing and controls was the chi square test, Fisher's exact test.
Ethical approval for this study was obtained from the Ethics Committee of
The Faculty of Medicine, University of Kelaniya, Sri Lanka

Results

Colono-ileoscopy was macroscopically normal and faecal calprotectin was undetectable in cases and controls. Microscopic colitis not otherwise specified (MNOS) was seen in 10/49 cases and 1/14 controls ($p=0.43$, Fisher's Exact test). Histology was normal in others. A history suggestive of an episode of infectious diarrhoea (ID) was seen in 16/49 cases and 0/14 controls ($p=0.013$). There was no significant association between ID and the presence of MNOS. Tissue expression of IL-8 was significantly higher and IL-10 significantly lower in cases compared to controls (target/standard cDNA ratio, median (range) IL-8: 1.25 (0.75-2) Vs 0.85 (0.63-1.3), $p<0.0001$, Mann-Whitney U test; IL-10: 0.33 (0-0.63) Vs 0.55 (0.5-0.7), $p<0.0001$). There was a significant inverse correlation between IL-8 and IL-10 expression (Pearson Correlation, (-) 0.509; $p<0.01$). In patients with IBS-D, cytokine abnormalities were not significantly different in those with or without a history of ID or the presence or absence of MNOS.

Discussion

The results of this study demonstrate a significant association between IL-10 genotypes and IBS, with fewer patients having the high producer genotype compared with healthy controls. The lower prevalence of the high producer genotype in IBS suggests that high production of IL-10 may have some protective role or, conversely, that individuals predisposed to produce lower amounts of this cytokine might be more likely to develop the condition. A genetic predisposition to lower anti inflammatory
cytokine production could mean that control of the inflammatory response may be compromised in some individuals and may help to explain why gastrointestinal infections, for example, can sometimes lead to continuing problems. It is possible that an inflammatory process is perpetuated by failure of down regulation secondary to an inadequate anti-inflammatory cytokine response. There are a number of points however that need to be considered in the interpretation of these findings. IL-10 is only one of the anti-inflammatory cytokines involved in regulation of immune and inflammatory responses, and the possible involvement of other cytokines in the inflammatory process cannot be ruled out.Mucosal inflammatory cytokines; IL1, TNF alpha,IL10 play a role in the pathogenesis of IBS.

Conclusions

There is an evidence that inflammatory cytokines; IL1, TNF alpha,IL10 play a role in the pathogenesis of IBS and sub-clinical intestinal mucosal inflammation in patients with IBS-D in a tropical setting, whether or not a history of ID or MNOS was present or absent.

References

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Problems encountered during implementation of the project

Inadequacy of funds was the major problem encountered during implementation of the project and it was overcome by using personal funds.

Section 4

Impact of Research

Findings of the research were disseminated in the form of research papers in national and international conferences.

Section 5

Publications

Three abstracts were selected for publication from this study

1.Abstract presented in SLMA session

Title: Colonoscopy with ileoscopy and serial biopsies should be done to differentiate diarrhea predominant irritable bowel syndrome (IBS) from microscopic colitis not otherwise specified (NOS) and low grade inflammatory bowel disease (IBD)

Introduction: Colonoscopy is not routinely recommended in IBS unless there are alarm symptoms. However; studies have shown that microscopic colitis and low grade IBD can mimic IBS.

Aims: To establish if colonoscopy and ileoscopy with serial biopsies will help to differentiate diarrhea predominant IBS from microscopic colitis (NOS) and low grade IBD.

Methods: In a prospective study over one year, we selected 20 patients aged between 18 and 60 years with diarrhea predominant IBS (using Rome II) and no alarm symptoms. Alarm symptoms were defined as significant loss of weight, bleeding per rectum or melaena. All patients had normal ESR, CRP, TSH and stools reports. None of the patients were on NSAIDS. There were four controls (patients with a family history of colon cancer). We performed colonoscopy on them with serial biopsies from the ileum, caecum, right colon, transverse colon, descending, and rectum. The entire study was conducted at the Professorial Unit University of Kelaniya Ragama.

Results: Colonoscopy was macroscopically normal in 95 % of cases and 100% of controls. Histology was normal in 100% of controls. Caecum was the commonest site of inflammation (46.7%). Microscopic colitis NOS was present of cases 70%. Crohn's in 5% and normal in 25%. There were no complications due to colonoscopy.

Conclusion: Colonoscopy with ileoscopy seems effective in differentiating diarrhoea predominate IBS from microscopic colitis NOS and low grade IBD. Since the inflammation is patchy and mainly in the right side serial biopsies should be done even though the mucosa is macroscopically normal.

2.Abstract selected for Digestive Disease week 2010 USA (DDW 2010)

Title: Sub clinical intestinal mucosal inflammation in diarrhea predominant irritable bowel syndrome (IBS) in a tropical setting

A P De Silva[1], A Manamperi[2], J Hewavisenthi[3], Ariyasinghe MP[1], AS Dassanayake[4], DP Jewell[5], HJ de Silva[1]

Departments of [1]Medicine, [2]Molecular Medicine Unit, [3]Pathology and [4]Pharmacology, and Faculty of Medicine, University of Kelaniya, Ragama, Sri Lanka, and [5]Nuffield Department of Medicine, University of Oxford, Oxford, UK.

Background:
There is evidence for potential roles for gut flora and the host immune response in the pathophysiology of IBS, and especially, for low grade colonic mucosal inflammation in the pathophysiology of post-infectious IBS.
Aim:
To investigate for evidence of sub-clinical intestinal mucosal inflammation in diarrhea-predominant IBS (IBS-D) in a tropical setting.
Methods:
In a prospective study over one year, we investigated 49 patients with IBS-D [median age 34 years (range 18-59; M:F 36:13], based on Rome III criteria. None had alarm symptoms: unintentional significant loss of weight, bleeding per rectum or malaena. None were on NSAIDS or proton pump inhibitors. All patients had normal ESR, CRP, TSH and stools reports. 14 individuals with a family history of colon cancer [median age 46.5 years (range 23-56); median 46.5, M:F 6:8] were selected as controls. Stools of patients and controls were tested for calprotectin. During colonoscopy, serial biopsies were obtained from the ileum, caecum, ascending, transverse and descending colon, and rectum. In addition to histology, tissue expression of IL-8 and IL-10 were assessed in biopsy specimens using semi-quantitative RT-PCR.
Results:
Colono-ileoscopy was macroscopically normal and faecal calprotectin was undetectable in cases and controls. Microscopic colitis not otherwise specified (MNOS) was seen in 10/49 cases and 1/14 controls ($p=0.43$, Fisher's Exact test). Histology was normal in others. A history suggestive of an episode of infectious diarrhoea (ID) was seen in 16/49 cases and 0/14 controls ($p=0.013$). There was no significant association between ID and the presence of MNOS. Tissue expression of IL-8 was significantly higher and IL-10 significantly lower in cases compared to controls (target/standard cDNA ratio, median (range) IL-8: 1.25 (0.75-2) Vs 0.85 (0.63-1.3), $p<0.0001$, Mann-Whitney U test; IL-10: 0.33 (0-0.63) Vs 0.55 (0.5-0.7), $p<0.0001$). There was a significant inverse correlation between IL-8 and IL-10 expression (Pearson Correlation, (-) 0.509; $p<0.01$). In patients with IBS-D, cytokine abnormalities were not significantly different in those with or without a history of ID or the presence or absence of MNOS.
Conclusion:
There is evidence for sub-clinical intestinal mucosal inflammation in patients with IBS-D in a tropical setting, whether or not a history of ID or MNOS was present or absent.

3.Abstract selected for SLMA sessions 2010

Title: Subclinical intestinal mucosal inflammation in diarrhoea predominant irritable bowel syndrome in a tropical setting

Abstract:

AP de Silva, A Mannamperi, MP Ariyasinghe, ASD Nandasiri, J Hewavisenthi, AS Dassanayake, DP Jewell, HJ de Silva

Introduction: There has been increasing evidence to support an inflammatory pathology in irritable bowel syndrome (IBS), especially diarrhoea predominant type (IBS-D).

Aim: To investigate for evidence of intestinal mucosal inflammation in IBS-D in a tropical setting.

Methods: In a prospective study over one year, we investigated 49 patients with IBS-D [median age 34 years (range 18-59; M:F 36:13], based on Rome III criteria and 14 controls [median age 46.5 years (range 23-56); M:F 6:8]. None had alarm symptoms, were on NSAIDS or PPIs. All patients had normal ESR, CRP, TSH and stools reports. Stools of all subjects were tested for calprotectin. During colonoscopy, serial biopsies were obtained.Tissue expression of IL-8 and IL-10 were assessed in biopsy specimens using semi-quantitative RT-PCR.

Results: Colono-ileoscopy was macroscopically normal and faecal calprotectin was undetectable in cases and controls. Microscopic colitis not otherwise specified (MNOS) was seen in 10/49 cases and 1/14 controls ($p=0.43$, Fisher's Exact test). A history suggestive of an episode of infectious diarrhoea (ID) was seen in 16/49 cases and 0/14 controls ($p=0.013$). Tissue expression of IL-8 was significantly higher and IL-10 significantly lower in cases compared to controls (target/standard cDNA ratio, median (range) IL-8: 1.25 (0.75-2) Vs 0.85 (0.63-1.37), $p<0.0001$, Mann-Whitney U test; IL-10: 0.33 (0-0.63) Vs 0.55 (0.5-0.7), $p<0.0001$). There was a significant inverse correlation between IL-8 and IL-10 expression (Pearson Correlation, (-) 0.509; $p<0.01$).

Conclusions: There is evidence for sub-clinical intestinal mucosal inflammation in patients with IBS-D in a tropical setting, whether or not a history of ID or MNOS was present or absent.

Section6

Summary Statement of Expenditure

Type of Expenditure	Details	Amount Rs	Total Rs
Consumables	Lab consumers (POLY PROPYLENE,CULTURE BOTTLES,DISPGLOVES,CULTURE BOTTLES,CENTRIFUGE TUBES)	8305.00	453580.00
	Avon pharmo chem. (pvt) ltd (promega USA barrier tips ,sigma USA Polaroid type 667,SV total RNA isolation system50 prep,PCR tubes0.5ml,0.2m)	389655.00	
	Integrated DNA Technologies	55620.00	
Medication	Medications	860.00	14412.17
	Klean prep packets	13552.17	
Subsistence	Laboratory Investigations (ESR,CRP,TSH,FBC,LIPID PROFILE,STOOL FULL REPORT,FBS C PEPTIDE,INSULIN LEVELS,STOOLS FOR OCCULT BLOOOD)	69452.00	69452.00
Personal	One research assistant worked over a period of one year	100000.00	100000.00
Travel			
Miscellaneous	Science Direct purchase	345.00	345.00
Total			**637789.00**

Grantee's signature: Date: 27/4/2010.

Recommendation of the Head of the Department:

Head, Department of Medicine
Faculty of Medicine
University of Kelaniya.

Recommendation of the Dean of the faculty:

Recommended

14/5/2010

Dean
Faculty of Medicine
University of Kelaniya

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