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PJBS

ISSN 1028-8880

Pakistan Journal of Biological Sciences

ANSInet
Asian Network for Scientific Information
308 Lasani Town, Sargodha Road, Faisalabad - Pakistan

OPEN ACCESS Pakistan Journal of Biological Sciences

ISSN 1028-8880
DOI: 10.3923/pjbs.2020.898.903

Research Article
Serum Levels of Interferon Gamma INF-γ and Interleukin 10 Il-10: an Immunological Aspect among Irritable Bowel Syndrome Patients

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Abstract

Background and Objective: The pathophysiological mechanisms of irritable bowel syndrome are controversial and the exact mechanism that play role in exaggeration of symptoms is mysterious. As an altered immunological functions in IBS patients may play role to study pro and anti-inflammatory cytokines among the study population. The aim of this study is to examine the serum cytokines of IL 10 and INFγ profile among a group of IBS patients and control. **Materials and Methods:** A cross sectional prospective study was conducted among 40 participants, who were referred to gastroenterology out patients clinics at Khartoum Teaching Hospital, Khartoum State, Sudan. Five milliliters blood were collected in EDTA tubes for measuring levels of cytokines in serum. Cytokines were measured by ELISA-MSD (Meso Scale Discovery). They were measured according to the manufacturer's instructions and expressed as pg mL^{-1}. Optical density was measured at a wavelength of 450 nm and a reference wavelength of 590 nm. **Results:** Out of 16 (40%) male and 24 (60%) female, their age group range between 20-70 years old. The majority of them 21(52.5%) in age group (31-50) years old. Overall IBS patients showed significantly increased ($p = 0.0001$) of INF-γ (29.50 ± 17.98 vs. 6.9 ± 1.724 pg mL^{-1}) between patients and control, respectively. The serum levels of IL-10 was significantly higher in patients with IBS compared with control group ($p = 0.001$). **Conclusion:** There is an abnormal immune regulation, supporting the presence of immune activation in IBS.

Key words: Irritable bowel syndrome, pro-inflammatory cytokines, anti-inflammatory cytokines, immune activation

Citation: Lienda Bashier Eltayeb, Doa Shams, Nazik Babikr, Mohammed Madani, Sara Abdelghani and Hisham Ali Waggiallah, 2020. Serum levels of interferon gamma INF-γ and interleukin 10 Il-10: An immunological aspect among irritable bowel syndrome patients. Pak. J. Biol. Sci., 23: 898-903.

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

The irritable bowel syndrome is a common Functional Gastrointestinal Disorder (FGID) that considerably reduces the quality of life, affecting between 10-20% of the world populations. It is a complex, heterogeneous disorder, whose development is widely considered as multifactorial in nature[1,2].

The IBS is diagnosed on the basis on symptom assessment and Rome III criteria (A criteria established to create uniformity in reporting and enhance diagnostic accuracy) and symptoms reported by the patients as recurrent abdominal pain or discomfort at least 3 days a month in the previous 3 months)[3]. The impaired bowel function and abdomen pain cause changes in bowel habit (constipation and/or diarrhea) and is further classified according to the predominant bowel habit into diarrhea predominant IBS (IBS-D), constipation predominant IBS (IBS-C) and mixed bowel pattern IBS (IBS-M)[4].

The IBS etiopathogenesis and pathophysiological mechanism are still unknown. Several pathogenic factors proposed to play role for development of IBS, such as genetic and environmental factors, alterations in digestive motility, visceral hypersensitivity, inflammatory, post infection mechanisms, psychological morbidity, stress, bacterial overgrowth and persistent low-grade inflammation may plays a role in IBS. However, none of them seem to clearly explain the real mechanisms that trigger the syndrome[5,6].

It is well established that acute gastrointestinal infections initiate the onset of symptoms in at least a subgroup of patients with IBS. Some IBS patients display persistent signs of low-grade mucosal inflammation with activated T lymphocytes, mast cells and enhanced expression of proinflammatory cytokines. Because major risk factors for the development of postinflammatory abdominal symptoms include the intensity and duration of an acute inflammation, it might be speculated that immune activation predicts the symptom pattern and severity. Recent studies have suggested that there may be genetic risk factors for altered immunologic function in patients with IBS. A single nucleotide polymorphism in the Tumor Necrosis Factor (TNF), gene encodes for a higher production of this pro-inflammatory cytokine and appears to be more frequent in patients with IBS[7,8]. Furthermore, a genetic predisposition to impaired production of the anti-inflammatory Interleukin (IL)-10 points toward an imbalance between proinflammatory and anti-inflammatory cytokines[9]. Considering the profound effects of cytokine gene polymorphisms on cytokine production, it is likely that immunologic alterations are not restricted to peripheral tissues but may lead to systemic alterations. Indeed, there is some evidence for an abnormal plasma IL-10/IL-12 ratio as an indicator for a proinflammatory type 1 helper T cells (Th1) state, but more comprehensive data on systemic cytokine production in patients with IBS are lacking[10].

With regard to their role in inflammation, pro-inflammatory cytokines IFN-γ) and anti-inflammatory cytokines (IL-10) cause activation of helper T-lymphocytes and gives rise to their differentiation into Th1 or Th2 lymphocytes[11]. There are no phenotypic markers that identify these cells, which therefore call for their *in vivo* culture and cytokine analysis in order to differentiate between both subsets. Th1 lymphocytes primarily secrete interferon γ (IFN-γ), interleukin 2 (IL-2) and the tumor necrosis factor α (TNF-α). Conversely, Th2 lymphocytes mainly secrete IL-4, IL-5, IL-6, IL-10 and IL-13. The differentiation of Th0 lymphocytes from Th1 lymphocytes is induced, essentially, by IL-2 and IFN-γ that, in turn, inhibit the differentiation of Th2 lymphocytes. Similarly, the differentiation of Th0 lymphocytes from Th2 lymphocytes is induced by IL-4, which together with IL-10, inhibit the differentiation of Th1 lymphocytes[12,13]. A low frequency of-1082*G allele has been observed in patients with IBS, responsible for coding a high production of IL-10, although this difference was not significant. On the other hand, in these patients, a high incidence of A allele positive subjects, both homozygous (-1082*A/A) and heterozygotes (-1082*G/A) has been observed which code a low and intermediate production of IL-10, respectively[14]. Van der Veek *et al.*[9], however, did not confirm this data, having observed regular levels of IL-10 genotypes and alleles at position-1082. In patients with IBS, particularly in the D-IBS subset, a high incidence of patients with a TNF-α high-production genotype together with a IL-10 low-production genotype[15] has been described.

So, the current study aimed to determine the serum level of IL 10 and INF-γ of IBS among Sudanese patients comparing to control group.

MATERIALS AND METHODS

This was analytical cross sectional study was conducted from February, 2018 to February, 2019, a total of 40 IBS patients their age range between 20-70 years old, who were referred to gastroenterology out patients clinics at Khartoum teaching hospital/Khartoum State Sudan and 40 apparently healthy individuals, their age range from 19-68 years old were included in the study as control. Patients with following

exclusion criteria were excluded from the study: Severe organic diseases, history of major abdominal surgery, severe psychiatric disorders, organic lesions at colonoscopy or abdominal ultrasonography, lactose intolerance or a history of anti-inflammatory, analgesic or immunosuppressive medication (nonsteroidal anti-inflammatory drugs, steroids and so forth) within the past 3 months, this achieve by a complete blood count, renal and liver function, fibrinogen, C-reactive protein level and stool. For perfect quality, control all IBS patients were checked by professional gastroenterologists. Informed consent was obtained from the subjects enrolled in the study.

Enzyme-linked immunosorbent assay: For collection of blood samples a 5 mL were taken for measuring levels of cytokines in serum. The EDTA tubes were used to take blood samples for cytokine analysis in serum. The samples were centrifuged 15 min within 2 h after phlebotomy and serum were collected, diluted 1:2 in RPMI for later analyses was frozen at -80°C degrees. Cytokines were measured by ELISA-MSD (Meso scale Discovery) The Cytokine Human Ultrasensitive Magnetic 10-Plex Panel for the Luminex® platform (Thermo Fisher Scientific, Oslo, Norway) was used for quantification of cytokines IL-10 and INF-in serum. They were measured according to the manufacturer's instructions and expressed as pg mL^{-1}. Optical density was measured at a wavelength of 450 nm and a reference wavelength of 590 nm. Density values were correlated linearly with the concentrations of cytokine standards. The limit of sensitivity of the assays was 5 pg mL^{-1}.

Statistical analysis: Data were analyzed using SPSS version 20. In all procedures, $p \geq 0.05$ was considered the level of significance.

Ethical approval: The study was approved by the Ethics Committee of Al-Neelain university. All the subjects were Sudanese IBS patients and the healthy comparison had no any biological relationship. The participants gave written informed consent to participate in the research.

RESULTS

Demographic characteristics: Out of 40 participants, 16 (40%) male and 24 (60%) female, their age group range between 20-70 years old. The majority of them 52.5% in age group 31-50 years old. Regarding period of illness, about 40% of patients infected with IBS for <5 years ago. There are no significance association between socio-demographic variables including age group, gender and period of IBS between patient and control all data were summarized in (Table 1).

Table 1: Association between socio-demographic variables IBS patients and control

	Patients N = 40 (100%)		Control N = 40 (100%)	
Characteristics	Number	Percentage	Number	Percentage
Age group				
20-30	13	32.5	18	45.0
31-50	21	52.5	10	25.0
51-70	6	15.0	12	30.0
Gender				
Male	16	40.0	21	52.5
Female	24	60.0	19	47.5
Period				
1 to <5 year	16	40.0	-	
5 to <10 year	10	25.0	-	
>10 years	14	35.0	-	
IBS subgroups				
IBS D	18	45.0	-	
IBS C	8	20.0	-	
IBS M	14	35.0	-	
p-value 0.442	-	-		

Values are expressed in percentage, *Statistically significant at p<0.05

Table 2: Frequency of controls and irritable bowel syndrome patients showing increased interleukin levels

	IBS patients (n = 40)		Control (n = 40)		
Interleukins	Number	Percentage	Number	Percentage	p-value
IL 10	13	32.5	5	12.5	0.14
INFγ	21	52.5	12	30	0.003
Total	34	85	17	42.5	-

IBS: Irritable Bowel Syndrome, IL 10: Interleukins 10, INF-γ: interferon Gamma, *p-value statistically significant at $p \leq 0.05$

Cytokines serum level: Table 2 summarized the frequency of controls and irritable bowel syndrome patients showing increased interleukin levels, about 32.5% of IBS patients have increased in IL10 level, there was significant difference ($p = 0.003$) between patients and control, regarding INF-γ. The serum levels of IL 10 and INF-γ cytokines in patients with irritable bowel syndrome showed in Fig. 1 and 2.

Table 3 conclude the total number of subgroups of irritable bowel syndrome comparing with IL 10 and INF-γ in which the patients showing raised interleukin levels concern patients who have high level of IL10 about 22.5% of IBS was IBS-D, while 2.5% was IBS-C (p-value 0.041 and 0.023) for IL 10 and TNF, respectively.

Table 4 compared the overall concentration of IL-10 and INF-γ between patients and control group. The IBS patients who showed significant increased in (p-value 0.0001) baseline of INF-γ compared with control

Table 3: Frequency of subgroups of irritable bowel syndrome patients showing raised interleukin level

	IBS subgroups (n = 40)								
	IBS D		IBS C		IBS M		Total		
Interleukins	Number	Percentage	Number	Percentage	Number	Percentage	Number	Percentage	p-value
IL 10	9	22.5	1	2.5	3	7.5	13	32.5	0.041
INF-γ	14	35	3	7.5	4	10	21	52.5	0.023

Table 4: Summarize the concentration of IL 10 and INF among IBS patients and control

Respondents	INF-γ pg mL^{-1}±SD (N = 40)	IL 10 pg mL^{-1}±SD (N = 40)
IBS patients (Mean±SEM)	29.500±17.98	5.405±0.8285
Control (Mean±SEM)	6.986±1.724	4.795±0.3199
p-value	0.0001	0.001
95% confidence interval	-15.40 to 60.44	-1.220 to 2.440
R^2	0.006012	0.03013

IBS-Irritable bowel syndrome, *p value statistically significant at p≤0.05

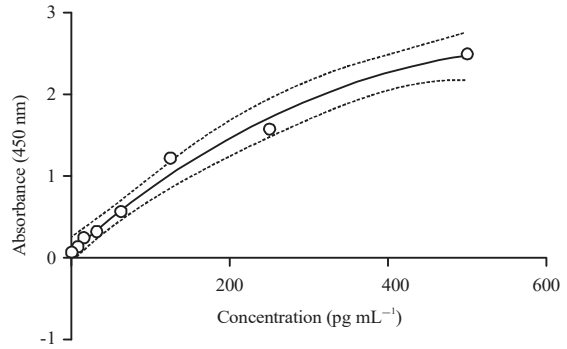

Fig. 1: Serum levels of cytokines (INF-γ) in patients with irritable bowel syndrome

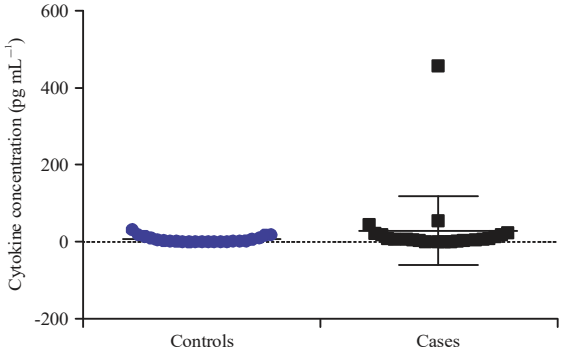

Fig. 3: Serum levels of cytokines INF-γ in patients with irritable bowel syndrome and controls

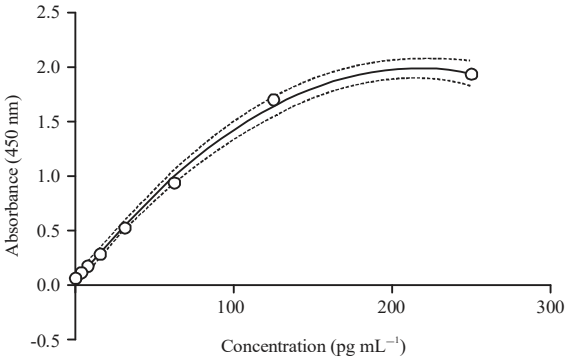

Fig. 2: Serum levels of cytokines (IL10) in patients with irritable bowel syndrome

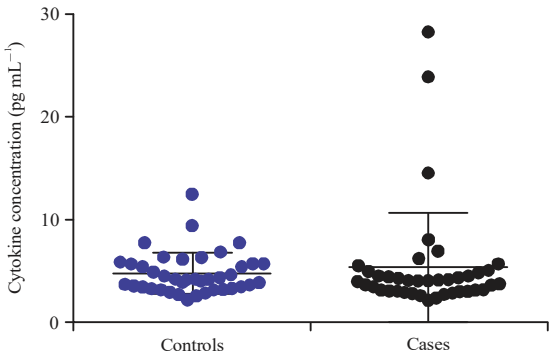

Fig. 4: Serum levels of cytokines IL 10 in patients with irritable bowel syndrome and controls

(29.50±17.98 vs. 6.9±1.724 pg mL^{-1}). The serum levels of IL-10, was significantly higher in patients with IBS compared with a healthy control group (p = 0.001). Figure 3 and 4 illustrates the spread of IL-10 and INF-γ concentration in the patient and among controls.

DISCUSSION

As immune activation is one of the proposed mechanisms that may play role in the pathophysiology of exaggeration of IBS symptoms, present study endeavored to find a correlation of possible role of cytokine profiles in the development of IBS.

Among the two cytokines studied, one pro inflammatory (INF-γ) and other anti-inflammatory (IL 10), the cytokine levels did not vary significantly between genders and period of infection as there was no significant differences. Patients enrolled in this study with complain of moderate and severe IBS symptoms (severity of symptoms checked according to Rome III criteria), the serum levels of IL-10 and INF-γ were significantly higher in patients with IBS than control when they were compared. This may ensure that the increase level of cytokines in peripheral blood may indicate an immune activation or at least cytokines imbalance, so present finding come in agreement with other studies suggesting that immunological alterations or imbalances could constitute causes of inducing symptoms of IBS. Vara *et al.*[16] and Farup *et al.*[17] revealed immune activation in IBS patients in Norway populations.

They studied following anti-inflammatory cytokines (IL-5, IL-6, IL-10) and TNF-α as pro-inflammatory and summarized that they may contribute to the development of IBS. The serum levels of cytokines were not significantly different in IBS patients with fatigue compared with IBS patients without fatigue. So, the cytokine levels may be less important than expected and hypnotized in their search of common underlying mechanisms. More or less different ethnic population may give reason for difference between this study and others such as ones done by Macsharry *et al.*[18], Bashashati *et al.*[19] and Scully *et al.*[20], also this study was first study that include INF-γ as pro-inflammatory cytokines in IBS population.

In recent study among Sudanese patients done by Eltayeb *et al.*[21], authors examined cytokines (TNFα and IL 10) gene polymorphisms among IBS patients and they conclude that the high producer genotype (AA and AS) of TNF α was more prevalent in IBS patients compared to healthy controls (8.5% vs. 0).

The current finding indicted that a higher level of IL 10 and INF-γ in IBS patients with diarrhea comparing with other subgroups, this may be attributed to reasons that patients with IBS-D present with a looser stool consistency, which may result by chronic systemic and mucosal inflammation. As IL-10 plays an important role in the anti-inflammation response and is considered to be a potent suppressor of T lymphocytes or macrophages and their derived effector molecules, such as pro-inflammatory cytokines (INF-γ, tumor necrosis factor [TNF]-α). IL-10 was initially described as a T helper 2-type cytokine and was reported to be expressed in various cells of the adaptive immune system as well as the cells of the innate immune system and IFN-γ is an important activator of macrophages and inducer of Class II Major Histocompatibility Complex (MHC) molecule expression. Aberrant IFN γ expression is associated with a number of auto-inflammatory and autoimmune diseases[2].

A higher producer genotype (-1082 G/G) of IL 10 was showed a significant reduction in the high producer IL-10 genotype frequency in IBS patients compared to controls (21 vs. 32%) these data are recorded in many studies[10,21]. When comparing these finding with this data, it is come in agreement and it is important to recognize that genotype frequencies vary according to ethnicity[19,21-23].

Lastly an imbalance in pro and anti-inflammatory cytokines (INF and IL 10, respectively) was detected in the study participants attributed to abnormal immune activation among IBS patients. One of most important restrictions and limitation of this study that not any calculations was performed on the sample size according to statistical criteria, which did not allow a representation of samples for a general population to be reached, thus current findings reinforced for further cohort studies with large sample size that include additional variable that may play crucial role in pathophysiology of IBS like stress, smoking and gut hypersensitivity.

CONCLUSION

In conclusion, this study participants in Sudan, it is found that subjects fulfilling Rome III symptom criteria for IBS have significantly increase in serum IL-10 and INF than control group. In addition, female gender were more frequent than male and those with symptom criteria for IBS-D had the highest association with this serum cytokines imbalance, compared with those with criteria for the other IBS subtypes, also concentration of INF significantly higher in patients than control.

SIGNIFICANCE STATEMENT

This study discovers the an abnormal immune regulation and/or immune activation in Sudanese subjects with IBS, however, do not yet known how the immune system is activated and it is, therefore, difficult to draw any conclusion from the finding and may be useful as a biomarker in this disorder, especially for women with IBS-D. This study will help the researchers to uncover the critical area of the subgroup with symptoms criteria for IBS-D has the strongest association with the imbalance of serum cytokines levels compared with those with symptom criteria for the other IBS subtypes. Thus, a new theory on IBS sub types and possibly other combinations, may be arrived.

ACKNOWLEDGMENT

We are thankful to the Deanship of Scientific Research at Prince Sattam bin Abdulaziz University Riyadh, Saudi Arabia to support our publication.

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