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IRRITABLE BOWEL SYNDROME AND BASAL SERUM TRYPTASE: THE CORRELATION BETWEEN SUBTYPE, SEVERITY AND COMORBIDITIES. A PILOT STUDY

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ABSTRACT

Introduction

Activation of mast cells causes alteration in epithelial and neuromuscular function, and is involved in visceral hypersensitivity and dysmotility in gastrointestinal functional disorders.

Objectives

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Primary: Evaluate differences in basal serum tryptase (BST) between patients with irritable bowel syndrome (IBS) and healthy controls. Secondary: BST depending on IBS subtype (diarrhea: IBS-D; constipation: IBS-C), comorbidities and correlations with IBS severity and quality of life.

Material and methods

Prospective control-case study in IBS patients (Rome IV criteria). BST was determined (ImmunoCAP-Phadia, Sweden®) IBS Severity Score (IBSSS), pain, bloating and flatulence analogue scales, IBS quality of life (IBSQOL) and patient health status (PHQ-9) were performed. BST is the primary variable in achieving the primary end-point.

Results

Thirty-two patients, 21 (65.6 %) IBS-D, 11 (34.4 %) IBS-C and 32 controls were included. Mean IBSSSS: 326.6 (± 71.4), IBSQOL: 76 (± 20.3) and PHQ9: 10.2 (± 5.9). BST was 4.8 ± 2.6 in IBS and 4.7± 2.6 in controls (p=0.875). There was no difference in BST between IBS subtypes (4.7 ± 2.9 in IBS-D and 5± 1.8 in IBS-C; p =0.315) or IBS severity (p=0.662). However, BST was higher in patients with IBS and extraintestinal comorbidities compared to other patients and controls (p=0.029). This subgroup also has more severe bloating (p=0.021). There was no correlation between BST, quality of life (p=0.9260) and health status (p=0.3985).

Conclusion

BST does not discriminate between IBS patients and controls. However, BST was higher in patients with IBS with extraintestinal comorbidities which have more severe bloating. This finding is worthy of investigation.

Keywords

Basal serum tryptase. Irritable bowel syndrome. Comorbidities. IBS subtype. IBS severity.

Abbreviations

Basal serum tryptase (BST); Irritable bowel syndrome (IBS); Irritable bowel syndrome constipation (IBS-C) ; Irritable bowel syndrome diarrhea (IBS-D); Irritable Bowel Syndrome Severity Score (IBSSS); Chronic fatigue syndrome (CFS); Fibromyalgia (FMG)

INTRODUCTION

Mast cells are blood cells which have a modulating effect on inflammatory or allergic processes found in the mucosa and human epithelial tissue, as well as in all vascularized tissue except the Central Nervous System and retina[1]. Between 2-3 % of inflammatory infiltration in the intestinal mucosa of healthy subjects is made up of mast cells[2]. These cells contain secretory granules, which include biologically active molecules such as cytokines, histamine, protease and proteoglycans that are liberated when mast cells are activated. This could lead to conflicting biological effects. In fact, it is believed that the role of mast cells in physiological and pathological processes goes beyond allergies. They are involved in the processes of scarring, chronic inflammation, tumour growth, angiogenesis and are considered a component of the immune system [3, 4.] As a result, mast cell-derived mediators could contribute to pathogenesis of not only allergic, asthmatic and mast cell illnesses, but also others such as myalgic encephalomyelitis/ chronic fatigue syndrome (CFS), fibromyalgia (FMG), coronary heart disease and obesity, among others[5].

Tryptase is a serine protease that is primarily produced and stored in mast cells, being the most abundant protein component of human mast cell secretory granules. It is less abundantly in blood basophils. In tissue mast cells, tryptase is produced and released in a constitutive manner this is regardless of the organ location of mast cells, maturation stage or subtype of mast cells. Mature tissue mast cells also store larger quantities of the enzyme in their metachromatic granules. Two major forms of tryptase are produced in mast cells, alpha and beta. Whereas the alpha form is produced and released constantly in mast cells, the beta-form is primarily stored in mast cells granules[6]. Determination of human serum tryptase by immunoassay measures the total concentration of both alpha and beta forms. As there is no distinction between tryptase subtype, current tryptase determination depends on both the size and the activation status of an individual's mast cell population though is

not directly informative on the contribution of any of these factors. Despite that, serum tryptase is being considered as a biomarker in daily practice for different diseases[6].

The basal serum tryptase level (BST) in healthy individuals results from the constant release of the enzyme from mature tissue mast cells[6]. Elevated basal serum tryptase concentration is associated with increased prevalence of multiple predominantly functional and clinical phenotypes, including recurrent cutaneous symptoms, symptoms of autonomic instability, and functional gastrointestinal disorders and connective tissue abnormalities[7].

In the intestines, mast cells are found in the lamina propria of mucosa in healthy patients and represent 3 % cellularity, regulating the intestinal barrier by acting in the blood flow, the contraction of smooth muscle, peristalsis and immune response [2, 8].

There are specific characteristics of mast cells in the bowel, different to those of mast cells in other locations, as can be seen in morphological and immunohistochemical studies[9, 10].

Mast cells are known to provoke disruption to epithelial and neuromuscular function, as well as generate visceral hypersensitivity and alter intestinal motility patterns in functional gastrointestinal disorders[4], such as irritable bowel syndrome (IBS), a functional somatic disorder characterised by abdominal pain, disrupted defecation and frequently accompanied by abdominal swelling/bloating[11].

Recent studies show an increase in small intestine inflammatory cells, particularly in the colon of some IBS patients after a previous bout of gastroenteritis [12, 13]. In cases of IBS patients with abdominal pain, and specifically in relation to severity, a larger quantity of mast cells, T-lymphocytes and degranulation of mast cells have been found around the nerve fibres of the colon giving way to tryptase release [14, 15].

Moreover, IBS is also associated with digestive and extraintestinal comorbidity such as FMG and CFS, which are also related to mast cell disruption[5,7].

Therefore, and for these reasons, the hypothesis to be considered is that basal serum tryptase (BST) levels could be increased in IBS regarding healthy patients and, if this is confirmed, BST could represent an inflammatory marker in IBS. This could also aid the identification of susceptible patients for consequent improvement in symptoms with

mast cell-stabilizing drugs, as is cromoglycate.

The main objective of this study is to evaluate whether an increase of BST exists in IBS patients compared to control. The secondary objectives are to investigate the possible differences in BST depending on the IBS subtype, constipation (IBS-C) and diarrhea (IBS-D). Also, to study BST according to patients associated comorbidities and its correlations with IBS severity and quality of life.

MATERIAL AND METHODS

Prospective control case study performed in Hospital Universitario 12 de Octubre from June 2017 to May 2018

Patients and control group

Patients were included consecutively and prospectively with a diagnosis of IBS and complying with the following inclusion criteria: 1) Patients of both sexes over 18 years of age diagnosed with IBS according to Rome IV criteria[16]. 2) Normal analysis study including hemogram, biochemistry, thyroid hormones, coeliac disease study, Protein C Reactive, faecal calprotectin range and negative parasite study. 3) Normal colonoscopy up to 2 years previous in patients over 50 years of age.

Patients were excluded from the analysis if they demonstrated:

1) Presence of organic disease, such as diverticulitis, chronic intestinal inflammatory disease, tumours, haematological and/or connective tissue disease.
2) Presence of important inmuno-allergic diseases such as systemic and cutaneous mastocytosis, asthma, atopic dermatitis, allergic rhinitis and IgE-mediated anaphylaxis.
3) Presence of active peptic ulcer disease.
4) Gastrointestinal parasite infestations.
5) Lactose malabsorption.
6) Coeliac disease.
7) Diabetes types 1 and 2
8) Kidney failure or creatinine above stage 2.

9) Chronic liver disease and/or elevated transaminases or bilirubin 1.5 times above normal.
10) Active consumption of alcohol and drugs.
11) AIDS
12) Patients consuming narcotics, anticoagulants, antibiotics, sodium cromoglycate.
13) Previous abdominal surgery, except appendectomy or inguinal herniorrhaphy.
14) Pregnant or lactating women.
15) Patient not signing informed consent.

The age and sex matched control group was made up of hospital workers over 18 years of age, with no prior history of digestive pathologies or other endocrine-metabolic pathologies, immunological, rheumatological, cardiovascular or allergic disorders. Those patients complying with IBS Rome IV criteria[16] were included, and were subjected to BST determination.

The study complies with the ethical principles of the Declaration of Helsinki, and the Good Medical Practice code of conduct and the legal regulations in force. All patients and subjects were fully informed and signed the informed consent containing all relevant information on the study. The study was approved by the Hospital Universitario 12 de Octubre Ethical Committee (Nº CEI 17/124).

Clinical variables and IBS study

All patients provided epidemiological data, medical history, year of IBS diagnosis and IBS subtype, bowel habits and stool type (Bristol scale), previously-performed treatments, current treatment for IBS and comorbidities. The patients according to comorbidities were classified into three groups for analysis purposes (none, psychiatric and extraintestinal; table 1). The following questionnaires and scales were autocompleted by the patient: IBS severity assessment (IBSSS), considering IBSSS moderate-severe if greater than 175 points[17], evaluation of pain and abdominal distension using the analogic scale (0-6), quality of life assessment using the IBSQOL questionnaire (validated Spanish version)[18] and health scale (Patient Health

questionnaire PHQ-9) (validated Spanish version)[19].

The primary variable is BST determination in IBS patients and controls to achieve the primary end-point of the study which is to investigate if BST discriminate between IBS patients and healthy controls as a potential biomarker. The secondary end-points to investigate is if BST varies depending on IBS subtype, associated comorbidities and correlations with IBS severity and quality of life.

Determination of basal serum tryptase

A venous blood sample is obtained from both patients and controls at 8 am. after an overnight fast and rest. None of the healthy controls were taking any medication at the time of the serum tryptase detection and the patients continued taking their regular medication for IBS if needed (table 1). The participants were asked to avoid antihistamines, ketotifen, nedocromil, cromolyn, theophylline, β2-agonists, antibiotics, angiotensin-converting enzyme inhibitors, codeine or opioid derivatives for at least 2 weeks. Diet modifications were not requested neither in patients nor in controls. Blood samples were then centrifuged for 10 min at 4000 rpm. The supernatants were decanted to get the serum, which was frozen at -20ºC until tryptase detection. The study of BST level was performed with the ImmunoCAP Tryptase (Phadia, Uppsala, Sweden®) automated technique in the Allergology Department Laboratory. Tryptase detection was carried out by using a Unicap 250 analyser (Phadia, Uppsala, Sweden®) then performed with the sandwich-type fluoroenzimoimmunoassay technique[20]. The range of normal values were 1-13.5 ng/mL and the detection limits from 1 to 100 ng/mL[20, 21]. This test measures total tryptase (alpha and beta tryptase).

STATISTICAL ANALYSIS

A sample size of 20 patients and 20 controls was estimated in order to achieve a statistical power of 80 %, with a confidence interval of 95 % based on the mean and standard deviation (SD) previously reported in which twenty-three newly diagnosed D-IBS patients and 14 healthy volunteers were examined (D-IBS: 5.52 (SD 2.01; 95 % CI 4.52 to 6.53); H: 5.40 (SD 2.15; 95 % CI 3.96 to 6.85) µg/l)[22]. Eventually, 32 patients were included along with a control group of 32 healthy subjects. Age, sex and BST

values were recorded for the control group. The qualitative values will be described with their frequency distribution, and will be summarised with mean and standard deviation (SD), as well as the confidence interval (CI) at 95 %. The normality of continuous quantitative variables study will be performed using the Kolmogorov-Smirnov and Shapiro-Wilk goodness of fit test.

The association between qualitative variables will be evaluated using the chi-square test or Fisher's exact test. Prior study of the characteristics of the distribution of variables and whether they comply with the conditions of normal distribution, the differences in variables studied between groups will be analysed using: Student's t test, variance analysis (ANOVA), and intergroup analysis with contrast studies (Scheffé test). If distribution is not normal, non-parametric tests will be resorted to (Kruskal-Wallis, Wilcoxon). Correlations will be analysed with the appropriate test according to normal distribution or not. In order to calculate the agreement between variables, the Cohen´s Kappa index was used.

RESULTS

Thirty-two patients were included, 30 (93.7 %) of those women; the mean age being 48 years (± 15.3). The control group was formed by 32 healthy subjects, of whom 29 (90.6 %) were women. Mean age of the control group was 46.5 years (± 15.3). No differences were found between patients and control group regarding distribution by age and sex (p=0.667 and p=0.644 respectively).

The clinical characteristics of the patients' group are expressed in Table 1. The mean time for IBS diagnosis was 61.7 months (± 61.4). Mean severity according to the IBSSS questionnaire was 326.6 (± 71.4). Mean days with pain was 12.2 days (± 2.77) and with bloating 13.1 (± 1.90). Pain severity was 3 (±1.15) and that of bloating 3.7 (± 1.3). Mean score in quality of life was 76 (± 20.3) and PHQ9 scale was 10.2 (± 5.9).

A statistically significant positive correlation was observed, although not pronounced, between BST and age (p=0.022).

BST values were similar in both patients and control (4.8 ± 2.6 and 4.7 ±2.6 respectively; p = 0.875). Moreover, there were no differences seen in IBS subtype function, being 4.7 ± 2.9 in IBS-D and 5 ± 1.8 in IBS-C, respectively; p=0.315. Nor

between BST and IBS severity (p=0.662), degree of abdominal pain (p=0.769) or abdominal bloating (p=0.066). However, in subdividing the group of patients depending on any kind of comorbidity, it was observed that BST was greater in those patients with IBS and extraintestinal comorbidities compared with other groups (p=0.029) (Fig. 1). When analysing whether differences existed between clinical characteristics depending on comorbidity, it was observed that this group presented greater severity of bloating (p=0.021), and no differences with other characteristics (Fig. 2). The increase in BST did not correlate with worse quality of life (p=0.9260) (Fig. 3), nor with the patient's state of health (p=0.3985), either in general or considering IBS subgroups according to comorbidity (p=0.863 and p=0.206, respectively).

DISCUSSION

Our study shows that BST is similar between IBS patients and healthy controls and therefore is not a useful marker for IBS. BST was not significantly different depending on the IBS subtype but we found higher levels in IBS patients that also have extraintestinal comorbidities and more severe abdominal distension. BST did not correlate neither with the severity of the disease nor with the patient's quality of life and health status. IBS is a complex heterogenic disorder with a wide variety of symptoms of varying severity and frequency, as well as the potential influence of other both somatic and psychological comorbidities. The diagnosis of IBS is currently performed by means of symptom-based diagnostic criteria, but there has been interest in developing biomarkers which could simplify the diagnosis and severity of the disease. Biomarkers would be useful to identify the presence of IBS pathophysiological mechanisms. Among them, local and systemic immune activation is significant, which leads to a state of microinflammation. In some IBS patients, an increase has been recorded of colonic inflammatory cells, such as mast cells[23, 24]. This mainly low-grade inflammation, could be responsible for peripheral sensitization and visceral hypersensitivity associated with the pain suffered by IBS patients[25]. In fact, degranulation of mast cells, close to colonic nerve fibres with histamine and tryptase release, could be correlated with the severity of abdominal pain [(12)], due to the fact that it could activate the Protease Activated Receptor 2 which is related to visceral pain in

the colon, and which favours the recruitment of inflammatory cells. Under nonanaphylactic conditions, tryptase levels reflect the total body mast cells burden, which is used to diagnose and monitor mast cell diseases. Serum levels generally reflect the extent of mast cell activation either by IgE- or non-IgE-mediated mechanisms. High levels of BST have also been recorded in 4-6 % of the general population, which also increases with age [7, 26] and is confirmed in our study. However, differences have not been found between IBS patients and control subjects, or IBS-C or IBS-D subtypes. This last aspect recorded more alteration to mast cells in IBS-D patients, findings which are unconfirmed by other authors who have found a greater expression of tryptase mRNA in colon biopsy, in both IBS-D and IBS-C patients, although with no differences between either subtype regarding healthy subjects[27]. Guillarte et al[20], reported similar serum tryptase concentration, within normal ranges, in both IBS-D patients and controls. There was no correlation found in our study between BST, greater IBS severity or abdominal pain intensity. However, it was observed that with greater BST values, there was a tendency to present more severe abdominal bloating. On evaluating BST according to comorbidity, it was observed that IBS patients with extraintestinal comorbidities, presented higher values than those subgroups of patients with no comorbidity, or only psychiatric comorbidities. Also, this subgroup presented abdominal bloating, which was believed to be more severe. The majority of extraintestinal comorbidities were FMG, CFS and unspecific arthralgia-myalgias not complying with connective tissue disease criteria, with frequent overlapping of different somatic functional disorders, such as IBS, FMG and CFS, in the general population[28]. Many of these disorders have stress as a precipitating factor, which favours the release of the corticotropin-releasing hormone causing the activation of mast cells[29]. Moreover, these and many other entities, such as chronic prostatitis, interstitial cystitis, migraine, cardiovascular disease, etc. are considered neuroinflammatory diseases with mast cell implication[26, 29]. This could explain why increased BST is found in this subgroup of patients.

On the other hand, we found no correlation between BST and a greater involvement in quality of life or general state of health, or by subgroups according to comorbidity. The quality of life or state of health perceived by the patient most likely involves other

factors such as comorbidities of a psychiatric matter, or other parameters such as degree of anxiety and sleep disorders not specifically analysed in our study.

The greatest limitation to this study was in regards to the sample size, which could be considered insufficient to observe clear differences in the data obtained. The "activation" of mast cells is usually inferred by the release of its mediators, centering mainly on the increase of tryptase serum levels. However, these levels could be inadequate or deceiving and could lead to false positive results as they are affected by various conditions. Such is the case of the presence of rheumatoid factor or delay in measuring serum tryptase 0.2–4 hours after the appearance of symptoms[5], and affected the recording of the presence or not of rheumatoid factor in our group of patients. The ideal situation would be to have a BST and serial tryptase measurements available for when there is a greater intensity of symptoms. However, the data available for BST and IBS are scarce with only studies with a small number of patients. Different variables that can affect BST such as circadian rhythms or diet were not taken into account either[20]. Therefore, we performed an initial study into this topic as there is a lack of information published in real clinical practice but it seems that BST is not a useful biomarker for inflammation in IBS. Therefore this coincides with another study in which it was reported that serum tryptase levels were similar between patients with Cronh's disease and controls[30]. Nonetheless, the authors considered that the measurement of serum tryptase is a reliable, non-invasive diagnostic approach to estimate the burden of mast cells in patients with mastocytosis, which may suffer similarly from diarrhea and abdominal, and to distinguish between categories of disease.

In summary, from the data obtained from this pilot study it cannot be confirmed that BST discriminates between IBS patients and healthy subjects and therefore, BST cannot be considered a useful inflammatory marker for IBS. BST is not different according to the IBS subtype and could not predict IBS severity either. However, BST is higher in IBS associated with other functional somatic comorbidities and more severe abdominal distension. This would imply that the concurrence or overlapping of various functional somatic comorbidities could be exerting an added effect in its physiopathology, which would suggest greater participation of mast cell activity. These differences observed in

IBS patients which have extraintestinal comorbidities might be worthy of further investigation.

More studies are necessary, with a larger sample size, in order to distinguish between the possible uses of BST as a potential marker particularly in patients with IBS associated with other functional somatic comorbidities. These findings appear to reinforce the hypothesis that a significant group of functional somatic comorbidities possess an inflammatory basis involving mast cells.

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AUTHOR CONTRIBUTION

Constanza Ciriza de los Rios: Development of the study protocol, patient recruitment, data analysis and interpretation, manuscript preparation; Isabel Castel and Fernando Canga: Patient recruitment, data analysis; M. Carmen Diéguez: Development of the study protocol, control group recruitment and basal serum tryptase analysis. Natividad de las Cuevas: basal serum tryptase analysis; Enrique Rey: data interpretation and support for manuscript preparation.

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Table 1. Clinical Characteristics of patients with IBS

Characteristics	N	%
Subtype		
Constipation	11	34.4
Diarrhea	21	65.6
Previous Treatment		
None	6	18.8
Fibre only	3	9,9
Laxatives only	8	25
Antispasmodics only	3	9,4
Probiotics only	1	3.1
Laxative + Antispasmodics	1	3.1
Three or more drugs	10	21.4

Current Treatment (Last 7 days)		
Constipation		
– No	19	59.4
– Fibre	1	3.1
– Laxatives	7	21.9
– Linaclotide	2	6.3
– Two or more drugs	3	9.4
Diarrhoea		
– No	20	62.5
– Rifaximin	7	21.9
– Probiotics	3	9.4
– Xyloglucan	2	6.3
Pain		
– No		
– Antispasmodic calcium antagonist	14	43.8
– Antispasmodic smooth muscle relaxant	4	12.5
– Antispasmodic anticholinergics	6	18.7
– Two or more treatments	7	21.9
	1	3.1
Bloating		
– No		
– Probiotics		
– Antibiotics	21	65.6
– Activated carbon	9	28.2
	1	3.1
	1	3.1
Comorbidity		
– None	13	40.6
– Psychiatric	7	21.9

– Extraintestinal	12	37.6
- Systemic (FM, CFS, arthralgias)	10	31.3
- Localized (cephalea, interstitial cystitis)	2	6.3

FM: Fibromyalgia; CFS: Chronic Fatigue Syndrome

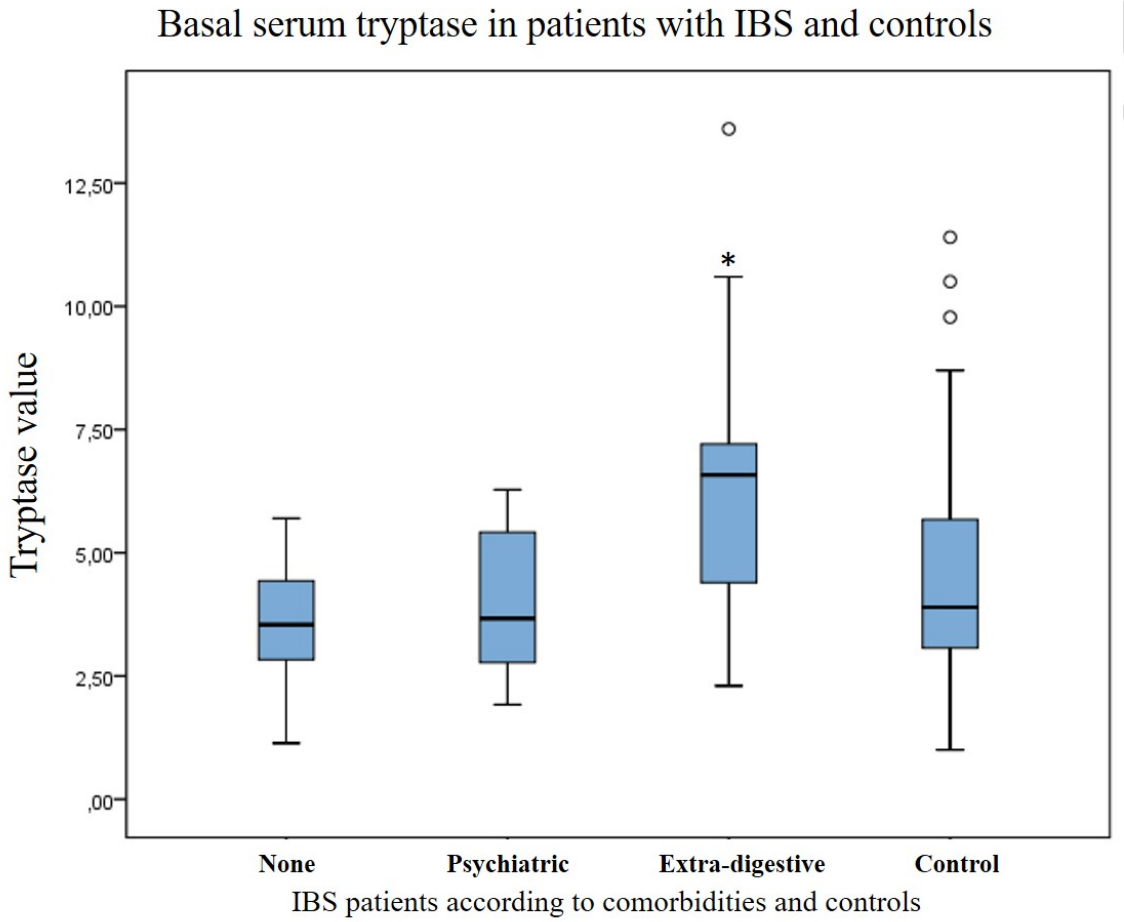

Figure 1. Basal Serum Tryptase in patients with IBS according to comorbidity and control.

***p <0.05; Kruskal-Wallis test.**

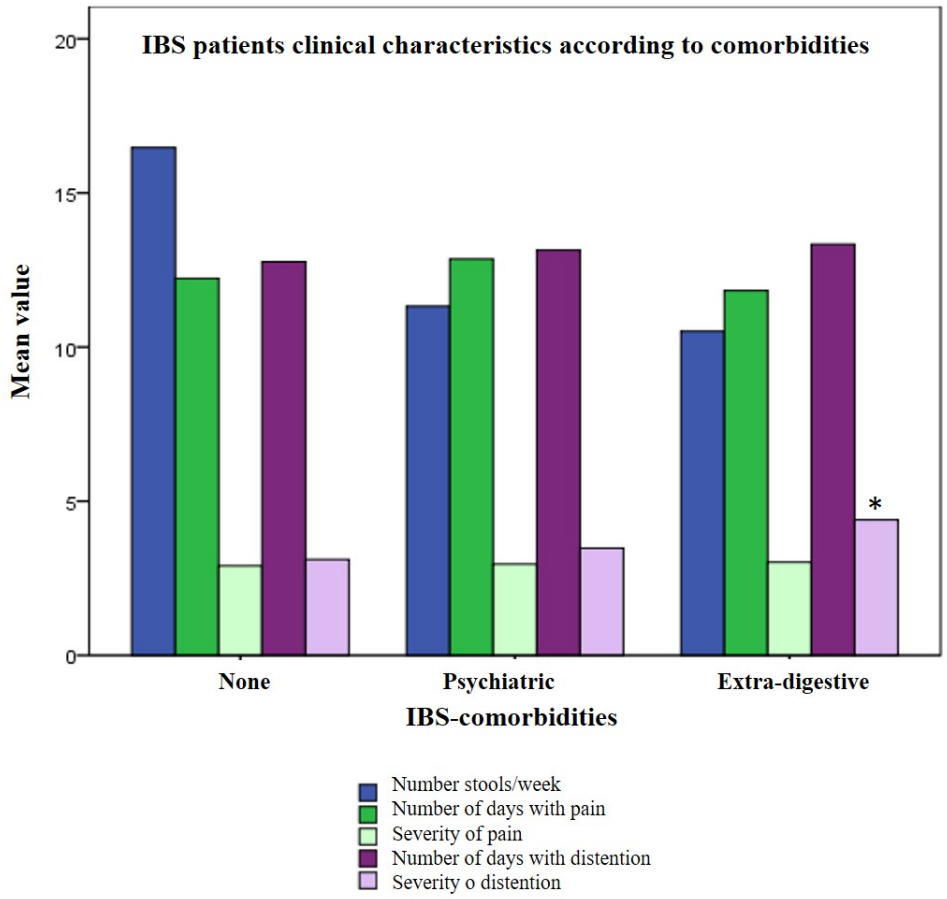

Figure 2. Clinical characteristics of IBS patients according to comorbidity.

***p <0.05; Kruskal-Wallis test.**

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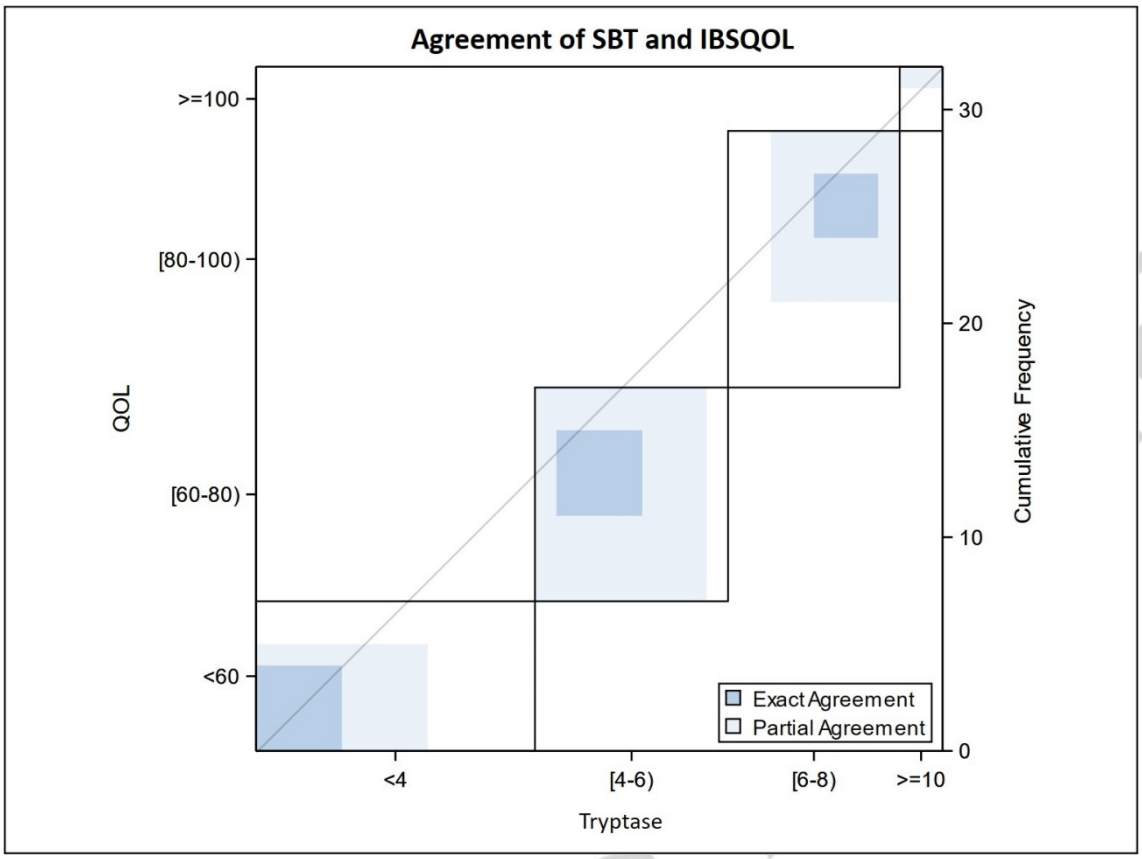

Figure 3. Correlation between Basal Serum Tryptase and Quality of Life Scale

Kappa Cohen Index: Weighted Kappa: 0.145; 95 % CI (-0.1122, 0.4026); BST: Basal Serum Tryptase