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Advances in Bioresearch

ORIGINAL ARTICLE

Measurement of some aspects and related with Irritable Bowel Syndrome

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ABSTRACT
Sixty three subjects were diagnosed in this study, 33(52%) had Irritable bowel syndrome (IBS) compared to subjects without IBS 30(48%). The percentage of infection with IBS in female is highest and IBS patients infection showed older frequency in age26-57years.The results revealed highest percentage of 19% to be obese in IBS patients. Also the ALT /GPT levels was (62.28 ± 11.73) U/L in male and (53.27 ± 11.50) U/L in female IBS patients, found that there was a increase compared with the control male and female (non patients) values (28.37 ± 6.14, 28.94 ± 5.79) U/L respectively. But CRP levels that showed no significant differences in IBS subjects and controls non patients, based upon these results found that CRP was biomarker could distinguish patients with inflammation bowel disease (IBD) or IBS from healthy controls. On the other hand decrease of vitamin-D levels in IBS infections from those non infections with IBS than normal concentration (N: 30-100 ng/ml.). In addition to the male and female positive IBS patients were decrease amylase concentration at (16.12 ± 4.45, 18.45 ± 5.05) U/l. respectively compared to normal concentration (N: 25-110U/l.), these most important factors that may be explanation associated for the amplified occurrence of IBS.
Keywords: *Irritable bowel syndrome, enzymes of liver; C-reactive protein; amylase.*

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INTRODUCTION

Irritable bowel syndrome is the most common functional gastrointestinal disorder (FGID), IBS is not a disease. It's a functional disorder, which means that the bowel doesn't work as it should. Manifesting as abdominal pain/discomfort and altered bowel function. Most people with IBS have either constipation or diarrhea. Extra symptoms are: Bloated or swollen abdomen and mucus in the stool. The sense that you have not complete a bowel movement[1].The IBS pathophysiology is multifactorial with sex-gender factors apparent to represent vital roles, the same asbefore studies have suggested there to be gender-associated alter in the incidence of IBS[2]. In addition to , most IBS in general demonstrate female predominance[3].On the other hand, some researches exposed that certain factors such as dietary habits, psychological factors and exercise level were associated to IBS. There might be also a genetic role in the etiology of IBS [4].There are a number of biomarkers for IBS diagnosis, to distinguish from other organic diseases and to separate between non-IBS from IBS patients [5]. Liver enzymes, especially Aspartate Aminotransferase AST (GOT) and Alanine Aminotransferase ALT (GPT), are common test in the general population for the discovery of nonalcoholic fatty liver disease (NAFLD).We hypothesized that IBS may possibly be associated to abnormal liver function[6].Anther serum biomarker is involved in the form of c-reactive protein (CRP),CRP is one of a number of proteins generally used to evaluate the inflammation degree[7]. Many systemic disorders has been found to be related with vitamin D strongly [8],especially its deficiency. More than 80% of metabolic vitamin D is resulting from sunbeams and the rest during dietary supplementation [9].Alpha-amylase can be found in tissues human at very small levels and amylase is a digestive enzyme secreted by the salivary glands and pancreas. Main function of amylases is to hydrolyze the glycosidic bonds in starch molecules, converting complex carbohydrates to simple sugars.[10]. Therefore, the aim of this study was to examine whether IBS was associated any of the following factors: elevated hepatic enzymes(ALT and AST), CRP, vitamin D and alterations of amylase secretion, and the

second aim was to hypothesize biomarkers that may one day present the "gold standard" in diagnosing this complex disorder.

MATERIAL AND METHODS

Subjects

In this study, sixty three subjects which included patients (N=33, 10 male and 23 female) aged from 2-57yearswho suffered from infection with irritable bowel syndrome and diagnosis by consultant physician according to the clinical signs, endoscopist, laboratory diagnosis and colonoscopy, and healthy random subjects as control group (N=30, 13male and 17female). This work was done in Gastroenterology and Hepatology Hospital and private lab. Questionnaire sheet was completed for each patients including personal data (age, sex, weight and height**).**

Blood sample collection and process

The human whole blood/plasma samples are target. It is recommended separated the serum via centrifugation at 5000 rpm for 10 min from the clot following the gathering of whole blood to measurement hematological parameters and biochemical tests. Don't maintain in a freezer the sample, which might influence the test assessment of target.

Body Mass Index (BMI)

BMI of all subjects was calculated by two parameters: the subject's height (in meters) and subject's weight (in kg) as the following equation:

BMI = Weight (Kg) / square of height (m2)

Each person's BMI indicates his/her weight status according to the following classification [11]:

Weight status in relation to BMI

BMI	Weight Status
Below 18.5	Underweight
18.5 – less than 25	Normal weight
25-30	Overweight
More than 30	Obese

Hematological parameters and biochemical tests

The following laboratory tests were also included for detect target: Biochemical exams enzymes liver (AST/GOT and ALT/GPT),C-reactive protein (CRP),Vitamin-D,and alpha amylase for irritable bowel syndrome infectious, measurement of targeted levels has been used a clinical tools.

Biochemical exams enzymes liver (GOT and GPT)

Quantitative determination of (Aspartate Aminotransferase AST (GOT) and Alanine Aminotransferase ALT (GPT) in serum sample using method manual procedure of kits (Giesse Diagnostics srl/Italia):

A) Aspartate Aminotransferase (GOT) - Activated Pyridoxal Phosphate:

Principle of testing: The technique in accordance with the International Federation of Clinical Chemistry (IFCC), with occurrence of α- ketoglutarate, AST/GOT in the sample converted aspartate into oxalacetate and glutamate. In occurrence of NADH and malate dehydrogenase, oxalacetate is transforms into malate and NAD.NADH Consuming per unit of time, calculated at 340 nm, is proportional to the concentration of AST/GOT in the sample andpyridoxal phosphate acts as an enzyme resource in the amino delivery reaction. Ensures full enzyme activation [12].**Procedure :**Added 1 part of reagent (B) to 4 part of reagent (A) of kit for prepared working solution (A+ B),than mix 1000µl of working solution with 100µl of sample and after that incubation 37ºc for 1 min, calculated the average absorbance value per minute at 340 nm, this method describes the procedure of AST (GOT) kit.

B) ALT alanine aminotransferase (GPT) - pyridoxal phosphate activator:

Principle of testing: The approach in step with the International Federation of Clinical Chemistry (IFCC), with pyridoxal five'-phosphate. 3, four ALT stimulates the interaction between L-alanine and 2-oxoglutarate. The pyruvate produced through NADH is reduced in a lactate dehydrogenase-stimulated (LDH) reaction to form Lactate and NAD +. Pyridoxal phosphate acts as an enzyme useful resource in the amino delivery response. Ensures complete enzyme activation[12].**Procedure :**Added 1 part of reagent (B) to 4 part of reagent (A) of kit for prepared working solution (A+ B),than mix 1000µl of working solution with 100µl of sample and after that incubation 37ºc for 1 min, calculated the average absorbance value per minute at 340 nm, this method describes the procedure of ALT (GPT)kit.

C-reactive protein (CRP) [13]

Principle: Measurement of CRP in human serum using a quantitative turbidimetry test, when mix samples containing CRP with latex particles coated with specific anti-human CRP are agglutinated. The agglutination causes absorbance alter depended upon CRP concentration of the patient sample that can be quantitative by compassion calibrator of human CRP concentration. **Procedure :**Mixed 9 ml of reagent

(A) CRP diluent with 1 ml reagent (B) latex particles coated with specific anti-human CRP (PH 7.3) of kit for prepared working solution (A+ B),than mixed 1000µl of working solution with 5µl of sample and control whatever mixed 1000µl of working solution with 5µl of calibrator after that read absorbance of the sample and calibrator at 540 nm, than calculate concentration of CRP this method describes the procedure of CRP kit (Giesse Diagnostics srl/Italia).

.**Vitamin-D (V-D) measure**[14]

Instrument use

AFIAS-1 and AFIAS-6 (AFIAS-automated fluorescent immunoassay system/boditech) are automatic immunoassay systems for the measurement amount of targeted in a whole blood of human, urine, and other samples using quantitative or semi-quantitative methods. The AFIAS reader is has a simple composition and easy to carry. Besides, AFIAS uses all-in-one cartridges that user loads samples only in cartridges which automates the whole procedures from preparation of sample to examination. The AFIAS-1 analyzer it is optimized for mid and small-sized clinics has a single channel testing up to six irritable bowel syndrome samples per hour while the AFIAS-6 comes with six channels with the capacity to process more 36 samples per hour. Via an applicable C-tip (capillary tip) for a quantitative tests, such as vitamin-D of irritable bowel syndrome patient, can be determinant using a small sample from a finger at (10uL or 50uL) of whole blood.

Using C-tip for collect of capillary blood sample

1) Using a pre-injection swab clean the area.
(2) With a sterile lancet puncture.
(3) First blood drop wipe away.
(4) A second drop softly massage the adjacent area towards a C-tip.
(5) Contact the tip of C-tip to the drop of blood with hold a C-tip horizontally.
(6) Enter automatically the sample blood to C-tip by capillary action
(7) Clean at all overload blood in the region of the tip.
(8) AFIAS reader is prepared on the 'C-tip mode' for a examination samples.
9) Depression the screen the 'START' icon.
10) The tests results were displayed following at 12min.for vitamin-Don the screen.

Detection alpha amylase using (α-Amylase -SL) kit bio lab[15]

Principle: Alpha amylase hydrolyzes 2 chloro-4 nitrophenyl- α Dmaltotrioside (CNPG3) into 2-chloro-4-nitrophenyle α D maltoside (CNPG2), maltotriose (G3), glucose and chloronitrophenol. The absorbance change in unit time measured at 405nm in proportional in the enzyme activity in the sample. **Procedure**: This method describes the procedure of α- Amylase kit (Giesse Diagnostic's srl/Italia). Take 1ml reagent (A) plus 20µl of serum sample was added to test tube. Mix well, perform 3reading at 60 sec. intervals .Calculate the average of absorbance wavelength at 405nm.

- Calculations

  ΔE = Initial absorbance - Absorbance after 1st /2nd min.
  Calculations determine ΔA/min. for every reading
  Find the mean value of ΔA/min.
  ΔA = (Avg ΔA/min) x 3517 = activity U/L of **α-**amylase

Statistical analysis

IBM SPSS computer program version 25.0 was used to calculate the mean ± standard deviation (SD), probability (two tailed) by using ANOVA table.

RESULT AND DISCUSSION

Distribution of subjects

Sixty three blood subjects were collected from Gastroenterology and Hepatology Hospital in Baghdad province, They were 30 (48%) negative IBS as control group (13 male and 17female) and 33 (52%) patients with positive IBS (10males and 23females)(Table 1). The positive and negative IBS patients were basically-characterized according to their gender, age, Body mass index (BMI), and fallowing measure biochemical exams enzymes liver (AST/GOT and ALT/GPT),(CRP),Vitamin-D, and alpha amylase, in specific laboratory.

Table 1: Study subjects distribution

Subjects(63N)	Number/%
Negative IBS	N=30(48%)
Positive IBS	N=33(52%)

Distribution of subjects according to gender

The gender of33 patients with positive IBS are included10(30%) males and 23(70%) females, the results of collected specimens revealed that female's patients' percentage is highest than male's patients(Table 2).As well as the gender of **30**negative IBS group males are 13(43%) while females are 17(57%),as shown in figure (1).Consequences of the study showed that gender and IBS had a physically powerful association. According to Pradhan and Olsson's Biology of Sex Variations, sex-related differences, counting variances in hormone composition, inheritance, and other physiological features between males and females bodies. Anther studies have exposed that IBS is more widespread in women than men. Gender variation and sex hormones might play essential roles in the IBS pathophysiology [16].Also,the stress and symptoms during the menstrual cycle might, difference in social characters and health seeking behaviors cause to more females exposure IBS-associated symptoms[17].

Table 2: Distribution of subjects according to gender

Subjects		Number (%)
Negative IBS	Male	13 (43%)
	Female	17(57%)
Positive IBS	Male	10(30%)
	Female	23(70%)
Total	Male Female	23(37%) 40(63%)

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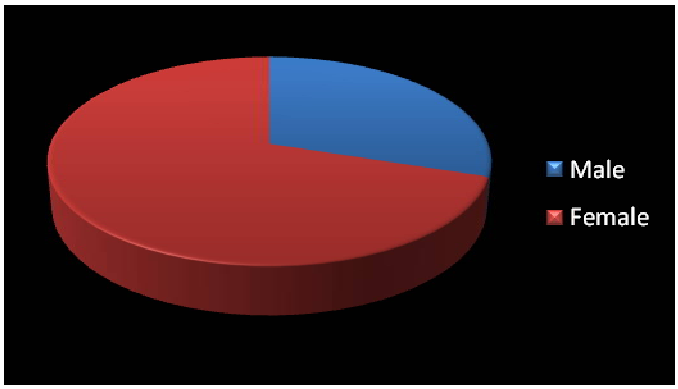

Figure 1: Rate of infection according to gender

Distribution of subjects according to age

The distributing positive IBS patients and negative IBS patients into two age groups (2-25 and 26-57 years) revealed that patients at the age group 26-57 years positive IBS patients male represented (n=8(13%)) and negative IBS patients male represented (n=9(14%)) than<26 years represented(n=2 (3%);4 (6%)) respectively, while positive and negative IBS patients female patients at the age group<26 years represented(n=5(8%)), the age group26-57years positive IBS patients female represented (n=18 (29%)),while negative IBS patients female patients at the age group 26-57 years represented (n=10 (16%))than<26 years represented(n=7 (11%) (Table3). The IBS patients infection showed older frequency in age26-57years than those the ages of <26 years, as shown in figure (2,3).People in these ages believe they are extra at risk to IBS severity infections may be for the reason that they have weak health with the human body immune system. Furthermore, individual factors counting gender and age may effect the occurrence of IBS in females are more susceptible and those have the highest risk [18].

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Table 3: Distribution of subjects according to age

IBS results	Males No. (%)		Females No. (%)	
Age period years	2 - 25	26 - 57	2 - 25	26 - 57
Negative	4 (6%)	9(14%)	7 (11%)	10 (16%)
Positive	2 (3%)	8(13%)	5(8%)	18 (29%)

Distribution according to Body Mass Index (BMI)

The calculations of BMI according to[11], current study revealed a majority of positive IBS male who were found to be obese (BMI: ≥30.0) comprising up to (6(10%)) followed by over-weight (BMI range: 25.0–29.9) and normal weight (BMI between18.5–24.9)were found to be of (2(3%)).And positive IBS female found to be obese (BMI: ≥30.0) comprising up to (12(19%)) followed by over-weight (BMI range: 25.0–29.9) comprising (8(13%)) while the remaining (3(5%)) were found to be of normal weight (BMI between18.5–24.9).However, when calculating patients with IBS, results revealed highest percentage of 19% to be obese(Table 4).The current findings of BMI distribution among subjects agreed with [19] who reported that obesity is very common in the Iraqi community in which two out of every three individuals are obese or overweight with risk factors including being female or middle-aged, living in urban areas and having low physical activity. People having obesity put them at risk of severe illness IBS and increases the risk for a lot of other serious chronic diseases. Obesity is a severe, widespread and costly chronic disease. Also,[20] have revealed that 30.3% of patients with IBS were overweight or obese, and 10.7% were underweight. The obesity and symptom severity were significant factors that influencing physical health and raise the risk of IBS. Also, a current reading a likely mechanism via which the gut microbial community be able to contribute to obesity. Bacterial lipopolysaccharides (LPS) derivative from gram-negative bacteria residing in the intestinal tract could act as a triggering factor, connecting to high-fat diet-induced metabolic syndrome (MS)[21].

Table 4: Distribution of subjects' percentage according to weight categories obtained from BMI calculations

	Positive IBS male N.(%)	**Positive IBS Female N.(%)**	**Negative IBS Male N.(%)**	**Negative IBS female N.(%)**
Underweight BMI ≤18.5	0%	0%	1(2%)	1(2%)
Normal weight BMI 18.5–24.9	2(3%)	3(5%)	5(8%)	8(13%)
Overweight BMI: 25.0–29.9	2(3%)	8(13%)	4(6%)	5(8%)
Obese BMI: ≥30.0	6(10%)	12(19%)	3(5%)	3(5%)

Hematological parameters and biochemical tests

Detect enzymes liver (AST /GOT and ALT/ GPT)

The related of Aspartate Aminotransferase (AST/GOT) and Alanine Amino transferase (ALT /GPT) of liver enzyme with IBS, has been studied in patients as compared with control values. Results showed that the two enzymes were significantly elevated in IBS patients compared to the control (nonpatients). The (AST/GOT) levels of male and female patients (62.08 ± 10.44, 60.10 ± 10.61) U/L respectively, we found that there was a increase compared with the control male and female non patient values (28.51 ± 6.18, 25.72 ± 19.55) U/L respectively (Table 5)**(**Figure 5). Also the ALT /GPT levels was (62.28 ± 11.73)U/L in male and (53.27 ± 11.50)U/L in female IBS patients, found that there was a increase compared with the control male and female (non-patients)values (28.37 ± 6.14, 28.94 ± 5.79) U/L respectively (Table 6) 6).There are a number of likely explanations for the increased ALT level in IBS patients. Before studies demonstrated higher altered occurrence of small intestinal bacterial overgrowth (SIBO) and gut microbiota in IBS patients, establishing a association between changed gut microbiota, SIBO, and IBS [22].Changed in both SIBO and gut microbiotaare linked with amplified gut permeability and distorted tight junction, and these abnormalities are related to hepatic function[23].In fact, a new study demonstrated that intestinal permeability is larger in IBS[24].therefore, it is likely that IBS itself could reason high liver enzymes, and this present study supports the positive association between liver enzymes and IBS.

Table 5: Detect enzyme liver (GOT) liver enzymes level

Sample	G0T (N: 10-40 U/L)	
Male	**(13 N) Negative**	**(10N)Positive**
Mean ± SD	28.51 ± 6.18	62.08 ± 10.44
Probability	3.68×10^{-9}	
Female	**(17N)Negative**	**(23N) Positive**
Mean ± SD	25.72 ± 19.55	60.10 ± 10.61
Probability	4.93×10^{-13}	

Table 6: Detect enzyme liver (GPT) liver enzymes level

Sample	GPT (N: 10-50 U/L)	
Male	**(13 N) Negative**	**(10N)Positive**
Mean ± SD	28.37 ± 6.14	62.28 ± 11.73
Probability	1.23×10^{-8}	
Female	**(17N)Negative**	**(23N) Positive**
Mean ± SD	28.94 ± 5.79	53.27 ± 11.50
Probability	1.18×10^{-9}	

C-reactive protein (CRP)

The results study that have showed there were no significant differences in the serum amount of CRP levels in IBS subjects and controls non patients than normal concentration (N:0.0-5 mg/dl.).The mean amount of CRP was non significantly (p=0.235)in positive IBS infection male was (2.48 ± 0.76 p) mg/dl and negative IBS patients male was (2.83 ± 0.95) mg/dl. Also, CRP was showed no significantly (p=0.919) in the positive and negative IBS female at (2.97 ± 0.88, 2.99 ± 0.90) mg/dl respectively (Table 7).Based upon these results and the researchers found that CRP was biomarker could distinguish patients with inflammation bowel disease (IBD) or IBS from healthy controls. High CRP was predictive of IBD, CRP is generally used to evaluate the grade of inflammation [25].

Table 7: C-reactive protein levels

Sample	C-reactive Protein (CRP) (N: 0.0-5 mg/dl)	
Male	**(13 N) Negative**	**(10N)Positive**
Mean ± SD	2.83 ± 0.95	2.48 ± 0.76
Probability	0.235	
Female	**(17N)Negative**	**(23N) Positive**
Mean ± SD	2.99 ± 0.90	2.97 ± 0.88
Probability	0.919	

Vitamin-D levels

The mean concentration of vitamin-D of positive IBS patients males were (18.33 ± 4.65)ng/ml, (52.08 ± 11.75) ng/ml for negative IBS patients males, and(18.58 ± 6.28) ng/ml for positive IBS female than negative IBS female at(48.55 ± 17.25) ng/ml. The results study, as shown in table (8), determined a decrease significant of vitamin-D levels in IBS infections from those non infections with IBS than normal concentration (N: 30-100) ng/ml.The role for vitamin D in the management of IBS, which was supported by another study demonstrated the elevated incidence deficiency of vitamin D in IBS patients [26].[27] have been demonstrated that little vitamin D levels are related with amplified central sensitivity. In addition, generally increased pain [28], depression [29] and anxiety[30] which all are associated with the risk of IBS progress. Hence, can recover the IBS symptoms during decrease in all identified threat factors that are involved in the pathogenesis of IBS.

Table 8:Vitamin-Dlevels

Sample	(Vit D) (N: 30-100 ng/ml)	
Male	**(13 N) Negative**	**(10N)Positive**
Mean ± SD	52.08 ± 11.75	18.33 ± 4.65
Probability	2.83×10^{-8}	
Female	(17N)Negative	(23N) Positive
Mean ± SD	48.55 ± 17.25	18.58 ± 6.28
Probability	3.18×10^{-13}	

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Amylase levels

Male and female positive IBS patients were decrease amylase concentration at(16.12 ± 4.45, 18.45 ± 5.05) U/l. respectively compared to normal concentration (N: 25-110U/l.). Also, the amylase levels demonstrated significant variations between positive and negative subjects, the male and female negative IBS patients at (59.19 ± 20.58, 43.53 ± 16.81) U/l. respectively (Table 9). Abnormally low amylase levels are not common, therefore, we may be consider the present patients is often associated with other conditions: chronic stress may be associated with reduced alpha-amylase output, obesity or pancreatic exocrine insufficiency causing permanent damage to the cells that make amylase[31].

Table 9: Amylase levels

Sample	**Amylase (N: 25-110U/l)**	
Male	**Negative**	**Positive**
Mean ± SD	59.19 ± 20.58	16.12 ± 4.45
Probability	0.000002	
Female	**Negative**	**Positive**
Mean ± SD	43.53 ± 16.81	18.45 ± 5.05
Probability	4.86×10^{-8}	

CONCLUSIONS

Current study demonstrated a likely related between increase enzymes liver (AST /GOT and ALT/ GPT)levels, decrease vitamin–D and an autonomic dysfunction amylase with IBS, and most IBS patients in general is female predominance. Therefore, gender is assumed to be a vital factor in the pathogenesis. In addition to anxiety, depression, stress and obesity factors can account that increased incidence of IBS.

REFERENCES

1. Gregory S Sayuk and C PrakashGyawali.(2015).Irritable Bowel Syndrome: Modern Concepts and Management Options, Am J Med. ;128(8):817-827.
2. Houghton LA, Heitkemper M, Crowell M, et al.(2016). Age, gender and women's health and the patient. Gastroenterology;150:1332-1343, e4.
3. Kim, Y.S.; Kim,N.and Kim, G.H.(2016). Sex and gender differences in gastroesophageal reflux disease. J Neurogastroenterol Motil;22:575-588.
4. Kim,Y.J.and Ban, D.J.(2005). Prevalence of irritable bowel syndrome, influence of lifestyle factors and bowel habits in Korean college students. Int J Nurs Stud.; 42(3):247–54
5. Kim, J.H. and Lin, E.(2017). Pimentel M. Biomarkers of irritable bowel syndrome. The Korean society of neurogastroenterology and motility. J Neurogastroenterol Motil.;23:20–26.
6. Seung-Hwa Lee; Kyu-Nam Kim; Kwang-Min Kim and Nam-SeokJoo.(2016).Irritable Bowel Syndrome May Be Associated with Elevated Alanine Aminotransferase and Metabolic SyndromeYonsei Med J. Jan 1; 57(1): 146–152.
7. Nicola R. Sproston and Jason J. Ashworth.(2018).Role of C-Reactive Protein at Sites of Inflammation and InfectionFront Immunol. ; 9: 754.
8. Pakpoor J, Pakpoor J. (2013). Vitamin d deficiency and systemic lupus erythematosus: cause or consequence? *Oman Med J* . Jul;28(4):295.
9. Attar SM andSiddiqui AM. (2013). Vitamin d deficiency in patients with systemic lupus erythematosus. *Oman Med J* . Jan;28(1):42-47.
10. Pieper-Bigelow, C.; Strocchi, A. and Levitt, M.D.(1990).Where does serum amylase come from and where does it go?GastroenterolClin North Am. Dec;19(4):793-810.
11. Flegal K. M., Graubard B. I., Williamson D. F., and Gail M. H. (2005). Excess deaths associated with underweight, overweight, and obesity. *Jama*, 293(15), 1861-1867.
12. Young, D.S. (2001) Effects of Disease on Clinical Lab Tests. 4th Edition, AACC, Washington DC.
13. Hansson, Lars-Ol of and Lindquist, Lars.(1997). C-reactive protein its role in the diagnosis and follow-up of infectious diseases. *Current Opinion in Infectious Diseases* 10(3):p 196-201.
14. Chuanyi M. Lu .(2012).Pathology Consultation on Vitamin D Testing: Clinical Indications for 25(OH) Vitamin D Measurement .American Journal of Clinical Pathology, 137, Issue 5, Pages 831–83
15. Vassault, A., D. Grafmeyer, C. Naudin, G. Dumont, M. Bailly, J. Henny, M. F. Gerhardt, and P. Georges. (1986). Protocol for the validation of methods. Ann. Biol. Clin. 44**:**686-745.
16. Young Sun Kim and NayoungKimJ .(2018).Sex-Gender Differences in Irritable Bowel Syndrome NeurogastroenterolMotil, 1;24(4):544-558.
17. Triadafilopoulos G, Finlayson M and Grellet C.(2018). Bowel dysfunction in postmenopausal women. Women Health;27(4):55–66.
18. DoaaElhosseiny ; NehalElfawy Mahmoud and Ayat F. Manzour.(2019). Factors associated with irritable bowel syndrome among medical students at Ain Shams University. *Egypt Public Health Assoc* ;94(1):23

19. Pengpid, S., and Peltzer, K. (2021). Overweight and obesity among adults in Iraq: prevalence and correlates from a National Survey in. *International Journal of Environmental Research and Public Health*, 18(8), 4198.
20. Yuanjun Dong,; Sabrina Berens; Wolfgang Eich; Rainer Schaefert and Jonas Tesarz.(2018). Is body mass index associated with symptom severity and health-related quality of life in irritable bowel syndrome? A cross-sectional study . BMJ,17;8(10):e019453.
21. Cani PD, Amar J, Iglesias MA, Poggi M, Knauf C, Bastelica D, et al. (2007).Metabolic endotoxemia initiates obesity and insulin resistance. Diabetes;56:1761-1772.
22. Collins SM, Denou E, Verdu EF, Bercik P. (2009).The putative role of the intestinal microbiota in the irritable bowel syndrome. Dig Liver Dis;41:850-3.
23. Ilan Y. (2012). Leaky gut and the liver: a role for bacterial translocation in nonalcoholic steatohepatitis. World J Gastroenterol;18:2609-18.
24. Park JH, Park DI, Kim HJ, Cho YK, Sohn CI, Jeon WK, et al.(2009). The relationship between small-intestinal bacterial overgrowth and intestinal permeability in patients with irritable bowel syndrome. Gut Liver;3:174-9.
25. IvanaPlavšiT; Goran Hauser; MladenkaTkalIiT; SandaPletikosiT and NerminSalkiT. (2015).Review Article Diagnosis of Irritable Bowel Syndrome: Role of Potential Biomarkers Gastroenterology Research and Practice Volume, Article ID 490183, 12 pages
26. Khayyat Y and Attar S. (2015). Vitamin D deficiency in patients with irritable bowel syndrome: Does it exist? Oman Med J;30:115-118.
27. Abbasnezhad A, Amani R, Hajiani E, Alavinejad P, Cheraghian B, Ghadiri A, et al. (2016).Effect of vitamin D on gastrointestinal symptoms and health-related quality of life in irritable bowel syndrome patients: A randomized double-blind clinical trial. Neurogastroenterol Motil;28:1533-44.
28. McCabe PS, Pye SR, Beth JM, Lee DM, Tajar A, Bartfai G, et al.(2016). Low vitamin D and the risk of developing chronic widespread pain: Results from the european male ageing study. BMC Musculoskelet Disord;17:32.
29. Stokes CS, Grünhage F, Baus C, Volmer DA, Wagenpfeil S, Riemenschneider M, et al. (2016). Vitamin D supplementation reduces depressive symptoms in patients with chronic liver disease. *Clin Nutr*;35:950-7.
30. Bičíková M, Dušková M, Vítků J, Kalvachová B, Řípová D, Mohr P, et al. (2015). Vitamin D in anxiety and affective disorders. Physiol Res;64 Suppl 2:S101-3.
31. John S Leeds ; Andrew D Hopper; ReenaSidhu; Alison Simmonette; Narges Azadbakht; Nigel Hoggard; Stephen Morleyand David S Sanders.(2010).Some patients with irritable bowel syndrome may have exocrine pancreatic insufficiency. *Clin Gastroenterol Hepatol.* ;8(5):433-438.

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