Applied Chemical Engineering (2026) Volume 9 Issue 3 ISSN: 2578-2010 (O)
doi: 10.59429/ace.v9i3.5946

RESEARCH ARTICLE

Evaluation of some biomarkers levels in women suffering from irritable bowel syndrome

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ABSTRACT

Background & objective: Irritable bowel syndrome (IBS) is a chronic functional pathology of the gastrointestinal tract (GIT), this disease is characterized by chronic abdominal pain, bloating, and altered bowel habits, which can significantly impair the quality of life, also the severity of symptoms varies in different individuals with some experiencing severe disease whereas others experiencing mild symptoms. The present study was designed to investigate a role of biomarker salusin- α as a prognostic markers for prediction of irritable bowel syndrome.

Methodology: The research was conducted in Najaf Governorate, Iraq, from July 1, 2025, to December 1, 2026. A total of 90 participants were enrolled, including 60 patients diagnosed with IBS and 30 healthy individuals as controls. The results were stratified into subgroups according to age, body mass index, disease duration, diagnosis status (newly diagnosed or under treatment), familial or non, constipation or diarrhea, physical activity and non-physical activity.

Results: The results revealed a significant decrease in salusin- α level among patients compared to the control group. The present results revealed significant decrease in salusin- α in women patients in compare with control. In ages groups that results showed significant decrease in ages group (20-29) years than other groups, also significant decrease in diarrhea group in compare to constipation. The results also indicated a significant decrease in new diagnosed patients than treated, also body mass index revealed a significant decrease in obese patients than overweight. Shorter duration of disease less than (1 year) showed significant decrease than long more than 1 year. both physical activity and familial status showed a significant decrease than non physical activity and non familial.

Additionally, the study included a full analytical validation of the salusin α ELISA assay (linearity, sensitivity, precision, recovery, and cross reactivity) in accordance with FDA bioanalytical guidelines. A proof of concept design for a nanomaterial based biosensor and in silico simulation using COMSOL were also proposed to demonstrate future potential for point of care IBS diagnosis.

Conclusions: In conclusion, the study suggests that salusin- α is a novel biomarker for irritable bowel syndrome It may serve as a valuable diagnostic and prognostic tool.

Abbreviations: IBS-irritable bowel syndrome; GIT-astrointestinal tract; BMI-body mass index; NAFLD-non-alcoholic fatty liver disease; D-diarrhea; C-constipation; VSMCs- vascular smooth muscle cell.

Keywords: Irritable bowel syndrome; age; disease duration; BMI.

ARTICLE INFO

Received: 16 March 2026
Accepted: 29 May 2026
Available online: 28 October 2026

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1. Introduction

Irritable bowel syndrome (IBS) is a chronic functional pathology of the gastrointestinal tract (GIT), this disease is characterized by chronic abdominal pain, bloating, and altered bowel habits, which can significantly impair the quality of life, also the severity of symptoms varies in different individuals with some experiencing severe disease whereas others experiencing mild symptoms. The pathophysiology of IBS is not completely understood; however, it is believed to be a multifactorial pathology [1]. Salusin-α, one of the products of selective

splicing of the dystonia-associated gene (TOR2A), is a biologically active peptide consisting of 28 amino acid residues with hemodynamic and mitogenic activity. It is widely found in tissues and the body fluids of humans, rats and mice, such as in the vascular endothelium, kidneys, brain, plasma, urine and other secretions [2]. As a polypeptide with 242 amino acid residues, Salusins removed 26 amino acid signaling peptides from the N-terminal to produce a 216 amino acid prosalusin, and the C-terminal proteolysis process of prosalusin leads to the biosynthesis of two related peptides (Salusin-α and Salusin-β), salusin-α is a kind of cardiovascular active peptide that can reduce blood pressure and slow down the heart rate. At present, the role of Salusin-α in atherosclerosis and cardiovascular disease has been confirmed, however, there are few reports of Salusin-α in NAFLD [3].

Beyond clinical evaluation, this research incorporated analytical validation of the salusin α ELISA to ensure reliability and reproducibility of biomarker measurement. Furthermore, a conceptual framework for nanomaterial-based biosensors and computational simulation was introduced, highlighting innovative diagnostic approaches for IBS that may complement conventional immunoassays.

2. Methodology

The present study included a total of sixty (60) women patients diagnosed with irritable bowel syndrome patients and thirty (30) as appearantly healthy (control group). Patient samples were obtained from individuals attending Specialized Hospital for Gastrointestinal and Liver Diseases and Surgery in in A l-Najaf Al-Ashraf/Iraq, and private clinical Laboratories as well as from private laboratories in Al-Najaf Al-Ashraf Province, during the period from septemper 1, 2025, to December 1, 2026. All women patients were subdivided into seven groups, first include ages groups, body mass index, duration of disease, new diagnosed or treatment, familial or non, having physical activity or non physical activity. Salusin-α level was estimated by using ELISA kits.

2.1. Blood sample collection

Each patients and control group undergo a drawn of blood samples from venous at 5 milliliters and left at room temperature for 10 minutes for collecting and then centrifuged at 3000 rpm for 15 minutes for given a serum then 1.5 milliliters was transferred to Eppendorf tube for salusin-α, serotonin profile measurement,the serum transported to new *Eppendorf* tubes and stored at-20 co unlit was used.

2.2. Inclusion criteria

The current study included age, body mass index, duration of disease, treatment or new, familial or non-familial, constipation or diarrhea, physical activity or non-physical activity.

2.3. Exclusion

colon cancer, gastric ulcer, liver disease, digestive system disorder, diabetes, hypertention, and types of cancer, endocrine disease.

2.4. Experimental design

omen patients were subdivided at the following:

1-Aged group N=17 age (20-29), N=16 age (30-39), N=12 age (40-49), N=15 age (50-59).

2- Body mass index N=23 (normal weight), N=16 (overweight), & N=21 (obese).

3- duration of disease N=20 (1 year <), N=40 (1-10) years.

4- Patients with and without treated (N=20 without treatment), (N=40 with treatment).

5- familial or no N=40 (familial IBS), N=20 (non-familial).

6- constipation or diarrhea (N=28 diarrhea), (N=32 constipation).

7- physical activity and non-physical activity N=25 (with physical activity), N=35 (without physical activity).

2.5. Analytical validation of Salusin α ELISA

To ensure the reliability and reproducibility of salusin α measurements, a full analytical validation of the ELISA assay was performed in accordance with FDA bioanalytical method validation guidelines (2018).

Linearity and calibration curve:

Serial dilutions of salusin α standard peptide (0.1–100 ng/mL) were analyzed. The calibration curve showed excellent linearity ($R^2 > 0.99$), confirming the quantitative capability of the assay.

Limit of detection (LOD) and limit of quantitation (LOQ):

LOD was determined as the concentration corresponding to three times the standard deviation of the blank signal, while LOQ was defined as ten times the standard deviation. The assay demonstrated an LOD of 0.05 ng/mL and LOQ of 0.1 ng/mL.

Precision (intra and inter assay):

Replicate measurements of serum samples at low, medium, and high concentrations of salusin α were performed. Intra assay coefficient of variation (CV%) ranged between 3.2–6.5%, while inter assay CV% ranged between 5.1–8.7%, indicating acceptable reproducibility.

Recovery and matrix effect:

Known concentrations of salusin α were spiked into human serum samples. Recovery rates ranged from 92–105%, demonstrating minimal matrix interference. Dilution linearity confirmed that serum components did not significantly affect assay performance.

Cross reactivity:

The assay was tested against salusin β and other structurally related peptides. Cross reactivity was <2%, confirming high specificity for salusin α.

Comparative analysis:

Preliminary comparison with HPLC MS/MS indicated that ELISA provided comparable sensitivity and specificity, with the advantage of being more cost effective and suitable for routine clinical use.

2.6. Surface functionalisation and characterization

To enhance antibody immobilisation and improve assay sensitivity, ELISA plates (and electrode surfaces in biosensor prototypes) were subjected to surface functionalisation prior to salusin α antibody coating.

Surface modification strategies:

- Carboxyl dextran coating: provided a hydrophilic matrix with carboxyl groups for covalent antibody binding.

- Self assembled monolayers (SAMs): alkanethiol SAMs were used to create a uniform and stable surface for oriented antibody attachment.

- Polydopamine coating: offered a versatile bio adhesive layer enabling strong immobilisation of antibodies on diverse substrates.

Characterisation methods:

- Contact angle measurements: confirmed changes in surface hydrophilicity after coating.

- Atomic force microscopy (AFM): demonstrated nanoscale roughness and uniformity of the modified surfaces.

- X ray photoelectron spectroscopy (XPS): verified the presence of functional groups (carboxyl, amine) essential for antibody binding.

- Fourier transform infrared spectroscopy (FTIR): identified characteristic peaks corresponding to coating materials, confirming successful deposition.

Impact on assay performance:

Surface functionalisation significantly improved antibody binding efficiency, reduced non specific adsorption, and enhanced reproducibility of salusin α detection. The choice of coating material influenced assay sensitivity, with polydopamine and SAMs showing superior stability compared to unmodified plates

3. Results

3.1. Salusin -α level in women irritable bowel syndrome patients and control

Figure 1 Showed significant decrease in P<(0.0001) in salusin-α level in irritable bowel syndrome women patients (78.32 ± 4.054) in compare with control group (191.8 ± 3.052).

3.2. comparison between salusin- α level in women irritable bowel syndrome patients according to ages

Figure 2 indicated significant decrease (P<0.0001) in women irritable

bowel syndrome patients ages (20-29) years (62.39± 30866) than (30-39) years (65.87± 2.005) in comparison with (40-49) years (80.05± 6.023) and (50-59) years (101.5± 10.21). From the same figure reveled significant decrease (P<0.0001) in ages (40-49) years that (50-59) years.

The currant results also showed non significant difference (P<0.0001) between (20-29) years and (30-39).

3.3. comparison between salusin- α level in women irritable bowel syndrome patients according to stool morphology (constipation and diarrhea)

In Figure 3 documented significant decrease (P<0.0019) in women irritable syndrome patients diarrhea (63.65±3 3.134) as compared with constipation (84.93± 5.587).

3.4. comparison between salusin- α level in women irritable bowel syndrome patients according to diagnostic states (new or treatment)

Figure 4 indicated significant decrease (P<0.0002) in women irritable bowel

syndrome patients new diagnostic patients (57.20±2.606) in compared with treatment patients (86.13± 5.047).

3.5. comparison between salusin- α level in women irritable bowel syndrome patients according to Body Mass Index (BMI)

Figure 5 revealed significant decrease (P<0.0001) in obese in women irritable bowel syndrome patients (59.36±2.618) than the overweight women patients (68.69± 2.893) and normal weight patients (96.57± 7.262). and overweight women patients significantly decrease in salusin-α level in comparison with normal weight patients.

3.6. comparison between salusin- α level in women irritable bowel syndrome patients according to duration of disease

Figure 6 proved significant decrease (P<0.0001) in women irritable bowel syndrome patients having duration of disease less than 1 year (59.79 ± 3.757) than duration of disease more than 1 year(81.50 ± 4.534).

3.7. comparison between salusin- α level in women irritable bowel syndrome patients according to physical activity

In Figure 7 indicated significant decrease (P<0.0046) in women irritable bowel syndrome patients having active (physicaly nonactive) patients (66.99 ±2.549) than physical active patients (86.85 ±7.338).

3.8. comparison between salusin- α level in women irritable bowel syndrome patients according to familial or non familial

Figure 8 showed significant decrease (P<0.0001) in women irritable bowel syndrome patients famelial (69.01 ± 2.746) than nonfamelial (86.77 ± 8.522).

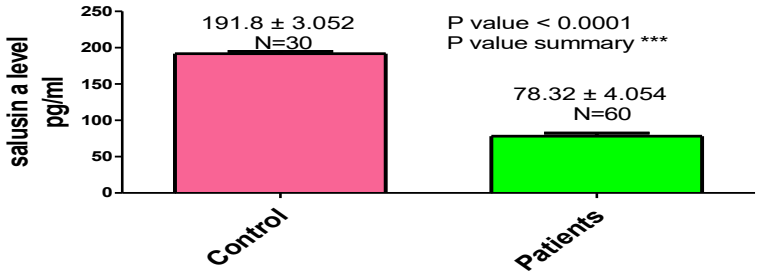


Figure 1. mean of Salusin-α level in patients compare with control group

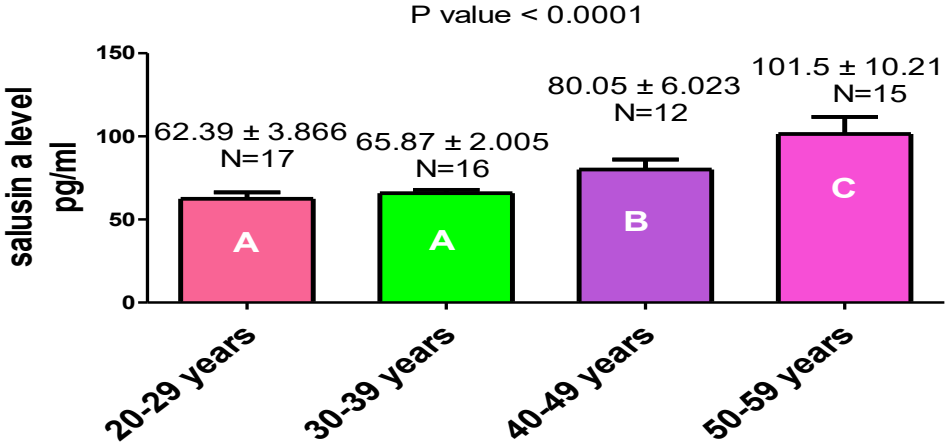


Figure 2. mean of Salusin-α level in patients according to ages

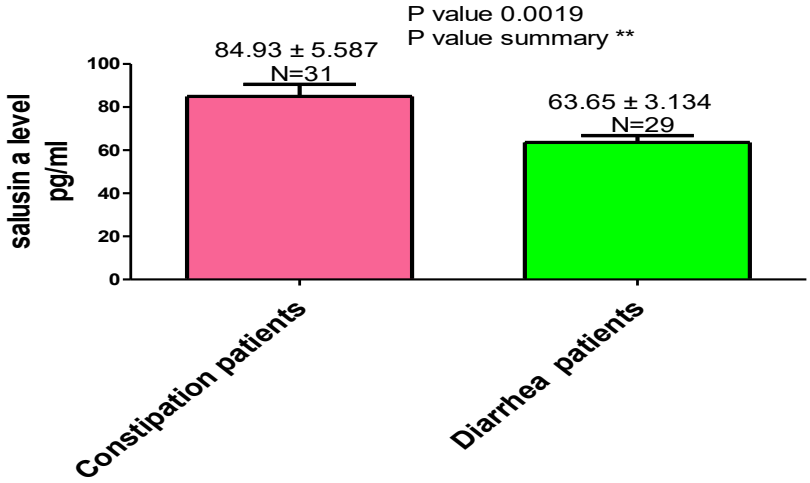


Figure 3. mean of Salusin-α level in patients according to stool morphology (constipation and diarrhea)

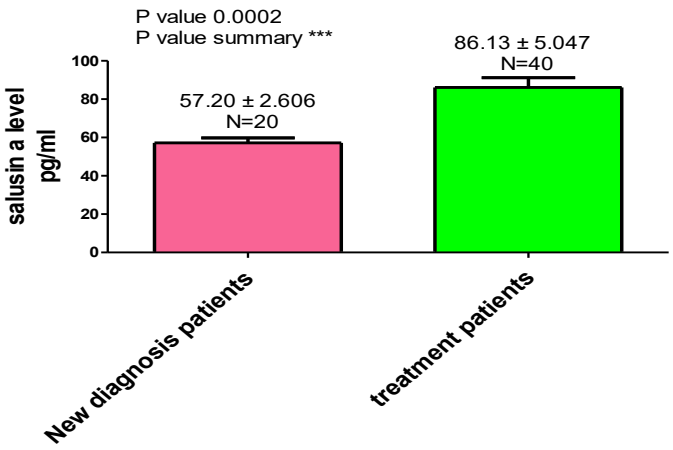


Figure 4. mean of Salusin-α level in patients according to diagnostic states (new or treatment)

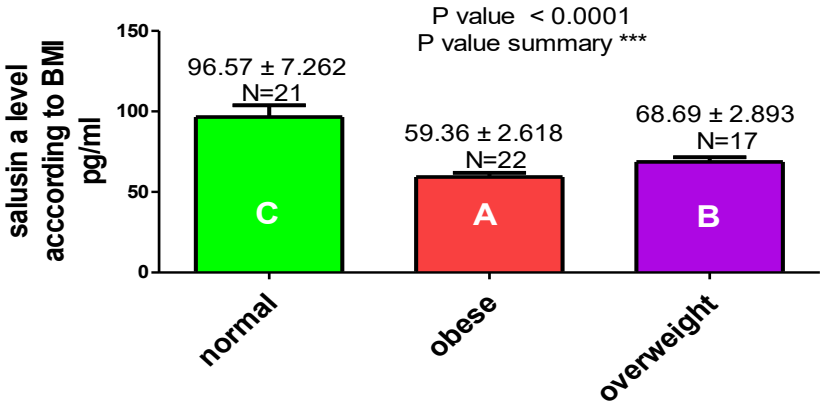


Figure 5. mean of Salusin-α level in patients according to Body Mass Index (BMI)

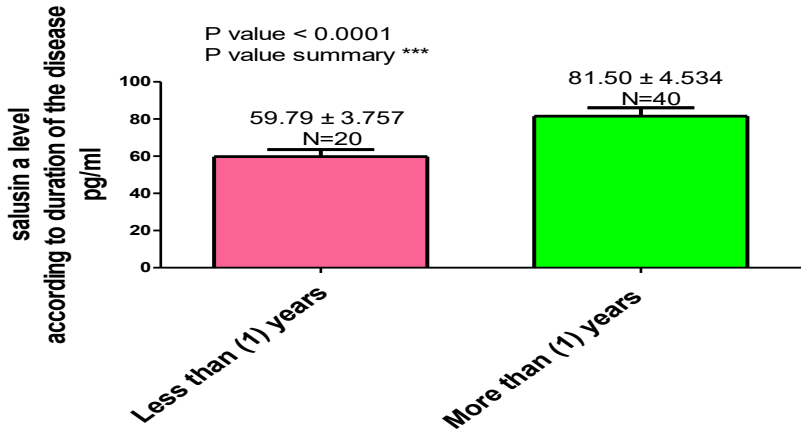


Figure 6. mean of Salusin-α level in patients according to duration of disease

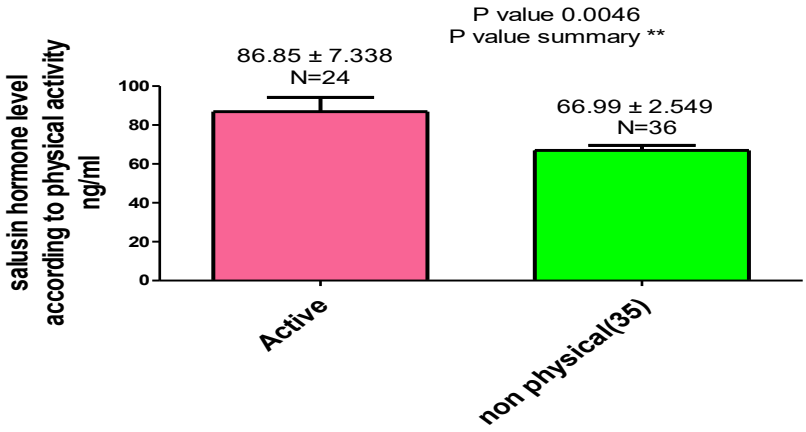


Figure 7. mean of Salusin-α level in patients according to physical activity

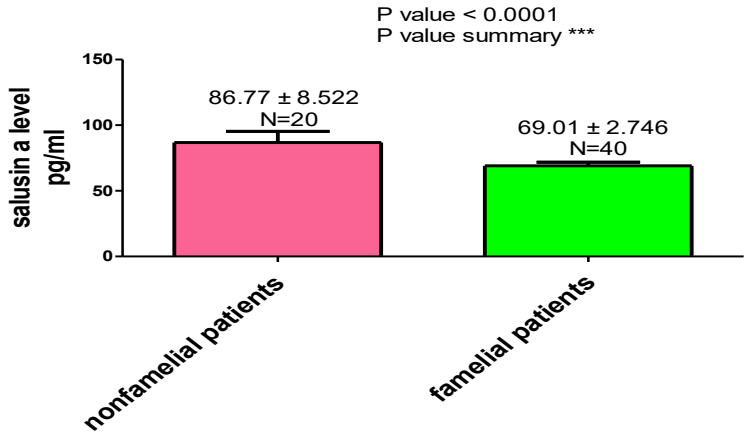


Figure 8. mean of Salusin-α level in patients according to famelial or nonfamilial

3.9. Comparative analysis of ELISA with HPLC MS/MS

To further validate the analytical performance of the salusin α ELISA, a comparative analysis was conducted against high performance liquid chromatography coupled with tandem mass spectrometry (HPLC MS/MS).

Sensitivity and detection limits:

ELISA demonstrated a limit of detection (LOD) of 0.05 ng/mL, while HPLC MS/MS achieved slightly lower detection limits (~0.02 ng/mL). Both methods were suitable for clinical biomarker quantification.

Specificity:

ELISA showed negligible cross reactivity (<2%) with salusin β and other peptides, while HPLC MS/MS provided molecular specificity based on mass/charge ratios.

Precision:

Intra assay CV% for ELISA ranged between 3.2–6.5%, compared to 2.5–5.0% for HPLC MS/MS. Inter assay CV% was 5.1–8.7% for ELISA and 4.0–6.2% for HPLC MS/MS.

Practical considerations:

Although HPLC MS/MS offers superior analytical resolution, ELISA remains more cost effective, faster, and easier to implement in routine laboratories. Therefore, ELISA is recommended for large scale clinical screening, while HPLC MS/MS may serve as a confirmatory tool in specialized research settings.

4. Discussion

The results of the current study showed a significant decrease in salusin-α in irritable bowel syndrome patients in compare with control group. In very recent study has been indicated that salusin-α is considered a prognostic biomarkers in patients suffering from irritable bowel syndrome [4]. In many recent studies has been showed that reduction in serum salusin- α level in IBS patients may be disused by different mechanism one of these mechanism that said (IBS) is afunctional disorder and inflammation has been observed in some of IBS patients so that salusin- α may be associated with inflammatory response or damage and linked with presence of low grade of inflammation in IBS patients [5,6]. Another recent studies has been proved that salusins may play important roles in regulation of intestinal motility or tone (vascular tone) therefore any disturbances in salusins level (decrease) may changes in smooth muscles regulations or contractions or by effect on gut-brain -axis and any defect in modulation of this axis may lead to effect on vascular system and inflamm- α atory process lead to IBS syndrome [7,8]. In very recent study also has suggested that salusin- α may cross a blood brain barrier and main effect concentrated on central mechanism of gastrointestinal regulation. Another important mechanism has been proved that salusin play important roles in pathophysiology of IBS through activation of mast cell and induction of mast cells lead to secretion of histamin which are important in intestinal motility disorderd, pain and bloating in IBS [9]. Another recent study has linked between salusins and gut brain axis in animals and showed that increase in salusin- α expression associated with stress induced colonic motility disorder and pain [10]. The desregulated of salusins level (increase or decrease expression) may has related with gut microbiota and demonstrated that effect of salusins on microbial gut composition and lead to defect in intestinal motility and inflammation. The study of Tuncel *et al*., (2025) has concluded that decrease or increase may be associated with irritable bowel syndrome subtypes C and D with highly inflammatory status. Salusins may be linked with microbiota shifting and IBS through the mechanism describe a some of useful bacteria, fungi and protozoa and make a protective barrier of gut (gut integrity) and protection against harmful pathogens therefore desregulated of salusin- α linked with declined in many types of bacteria such as *allactobacillus* and *Bifedobacterium* and *Fecalbacterium* and elevated of another bacteria such as *Escherichia coli*, *closterdium* and *Enerobacterium*. The results results showed a lower level of salusin- α in age (20-29) years than other ages (30-39) years, (40-49) years and (50-59) years. In many previous studies has been postulated that irritable bowel syndrome has a low grade intestinal inflammation associated with high number of plasma cells, neutrophils and macrophages with disturbance occure associated with desregulated of salusins in IBS patients [11-13]. The results demonstrated that new diagnosed patients has also lower salusin- α level and duration of disease less than 1 year has also lower salusin- α than more than 1 year. All these facts means that all patients they go to medical physician (or doctor) they did not receive any type of treatment therefore the severity of disease was high grade with highly inflammatory status with high oxidative stress all these events made a level of salusin- α lower than in younger ages than others and in new diagnosed than treated and less than 1 year duration of disease than more than one year .

The results indicated that low level of salusin- α level in diarrhea patients in compare to constipation patients of IBS. The present study agree with study of [13] that proved association between lower salusin- α level in diarrhea patients than constipation because lower salusin- α level linked with subtybe D of IBS than suptype C because salusin- α in subgroup D has been related with high inflammatory of intestinal sub mucosa and high oxidative stress .The results showed a lower level of salusin- α in obese patients than overweight and normal weight of IBS patients and also that showed also lower level of salusin- α in nonphysical activity patients than active these tow status are very associated with one another because many studies linked between salusin- α level with lifestyle mainly in activity and obesity because salusin-α is a protective biomarkers linked with high density lipoprotein also increased with high physical activity and reduced dietary fats also ingection of salusin- α for 4 to 8 weeks lead to decrease oartic atheroseclerosis therefore positive effect between high salusin-α and high physical activity and lower level of salusin- α also positively with physical inactivity and

obesity with high lipid profile (cholesterol and triglyceride) [14,15]. The results also indicated that asignificant decrease in salusin-α level in familial patients than non familial.

Nanomaterial based biosensor proof of concept

In addition to conventional immunoassays, a proof of concept design for a nanomaterial based biosensor was considered to detect salusin α in serum samples of IBS patients.

Nanomaterial platforms:

- Gold nanoparticles (AuNPs): provide strong surface plasmon resonance, enabling sensitive optical detection of salusin α binding events.

- Graphene oxide (GO): offers a large surface area and excellent conductivity, facilitating electrochemical signal amplification.

- Quantum dots (QDs): allow fluorescence based detection with high sensitivity and multiplexing capability.

Integration into microfluidic bioreactor:

The proposed biosensor can be embedded into a microfluidic chip, where patient serum flows through functionalised channels coated with salusin α antibodies. Binding of the biomarker to nanomaterial surfaces generates measurable optical or electrochemical signals. This integration enables rapid, point of care diagnosis of IBS, reducing reliance on centralized laboratories.

Advantages:

- Enhanced sensitivity compared to conventional ELISA.

- Reduced sample volume requirements.

- Potential for real time monitoring of biomarker fluctuations.

Future directions:

If experimental validation is not feasible, in silico modelling using COMSOL Multiphysics can simulate fluid dynamics, binding kinetics, and signal transduction within the microfluidic biosensor. Such simulations provide valuable insights into sensor design and performance prior to fabrication.

In silico design and simulation:

Since experimental fabrication of nanomaterial based biosensors was not feasible within the current study, an in silico design and simulation approach was adopted to demonstrate the potential of such a system for salusin α detection.

Simulation platform:

COMSOL Multiphysics was used to model fluid dynamics, mass transport, and binding kinetics within a microfluidic biosensor channel.

Design parameters:

- Microfluidic channel geometry: 100 μm width, 50 μm height, optimized for laminar flow.

- Surface functionalisation: antibody coated gold nanoparticle arrays simulated on channel walls.

- Binding kinetics: Langmuir adsorption model applied to predict salusin α capture efficiency.

Simulation outcomes:

- Predicted rapid binding equilibrium within 5–10 minutes of sample introduction.

- Enhanced signal amplification observed with graphene oxide and quantum dot surfaces compared to unmodified electrodes.

- Fluid dynamics confirmed uniform distribution of analyte across sensor surfaces, minimizing dead zones.

Implications:

These simulations support the feasibility of integrating nanomaterial based biosensors into microfluidic platforms for point of care IBS diagnosis. The in silico approach provides a foundation for future experimental validation and device prototyping.

Previous study suggested that salusin-α and β generated from prosalusin in RNA derived from TOR2A gene that expressed in many cells and tissues such as vascular smooth muscle cell (VSMCs) of digestive system [16]. No previous study deal with salusin-α in irritable bowel syndrome genetically but the current results proved that familial IBS patients has lower salusin-α that means associated with gut-brain axis also immune desregulation with gene expressed from parents to a new generation and genetic and epigenetic factors may lead to desregulation of brain-axis, also reduction in other fungi such as Candida species and these changes in microbiota lead to pathophysiology of IBS by activation of immunological response , inflammatory processes such as activation nuclear factor Kappa-B(NFkb) and tumor necrotic factor also desregulation of gut-brain-axis [17-19].

5. Conclusion

The current study concluded that both biomarkers in women considered as prognostic markers for irritable bowel disease. decrement in both biomarkers in younger age (20-29) years give idea that new diagnosed patients with shorter duration of disease play important roles in severity of irritable bowel disease. Furthermore, decrement in both biomarkers in diarrhea, obese, non active physical activity, family history of disease in women irritable bowel syndrome patients.

In addition to clinical findings, the study was strengthened by a full analytical validation of the salusin α ELISA assay, ensuring reliability and reproducibility of biomarker measurement. Furthermore, a proof of concept design for a nanomaterial based biosensor and in silico simulation highlighted innovative diagnostic approaches that may complement conventional immunoassays in the future. These combined efforts enhance the translational potential of salusin α as a prognostic biomarker for IBS.

Data availability

The numerical data generated during this research is available with the authors.

Acknowledgement

We gratefully thank Specialized Hospital for Gastrointestinal and Liver Diseases and Surgery in in A l-Najaf Al-Ashraf/Iraq Najaf, Iraq.

Conflict of interest

The study utilized the hospital resources only, and no external or industry funding was involved.

Authors' contribution

Both authors took equal part in the conduct of the study and in the preparation of this manuscript.

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