Article

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Galaninergic System Dysregulation in Long-COVID: Neural injury Associations

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Abstract

Background: Long-COVID disease, is a term that describes the persistent SARS-CoV-2 symptoms, is challenging to diagnose due to its complexity and incompleteness of pathogenesis. Although numerous factors were explored with this condition, the Galanin (GAL) and its receptor 1 (GalR-1) were not extensively studied in this context. Galanin system engaged in the pathophysiology of multiple chronic aging-related diseases, including alcoholism, inflammatory conditions of bowel and skin, and chronic pain. The presented study aimed to evaluate the relationship between tested Galanin system parameters in Long-COVID patients.

Methods: The amount of GAL and GALR-1 were measured using the ELISA technique in a group of (90) Long-COVID patients and (60) individuals who had healing and were no longer experiencing symptoms of Long-COVID.

Results: The study reported that Galanin, GalR-1, and Gal/GalR-1 ratio were significantly increased in Long-COVID patients. The study reported statistically higher Galanin levels ($p<0.001$), higher GALR1 levels ($p = 0.007$) and higher Gal/GalR1 ratios ($p=0.019$) in individuals diagnosed with Long-COVID patients and non-patients. According to the ROC analysis, Galanin showed the highest sensitivity (71.7%) followed by GALR1 (60.0%) to discriminate patients from non-patients.

Conclusion: The findings suggest a direct relationship between Galanin and GALR1 with Long-COVID disease. However, further studies are needed to clarify the precise role of plasma Galanin(GAL) and its receptors(GalR-1) in Long-COVID pathology.

Keywords: Galaninergic system, Galanin, Galanin receptor-1, chronic illness, Galanin/Galanin receptor-1 (Gal/GalR1).

Introduction

COVID-19 is a multifaceted disease that impacts various systems in the body, including the renal, cardiovascular, gastrointestinal, hematologic, and central neurological systems [1], it has a substantial impact on world health, causing major illness and death [2]. COVID-19 leads to multiple organ dysfunctions, and a considerable proportion of individuals who recover from the disease develop Long-COVID Syndrome [3]. COVID-19 leads to multiple organ dysfunctions, and a considerable proportion of individuals who recover from the disease develop Long-COVID Syndrome [4]. Long-COVID is a medical illness that affects persons who have had a probable or proven infection with the SARS-CoV-2 virus, according to the criteria provided by the World Health Organization. The condition commonly manifests approximately three months after the first beginning of COVID-19 and is

distinguished by a variety of symptoms that can persist for many weeks or even months, irrespective of the individual's viral load [5, 6]. Disease of Long-COVID has a range of symptoms that are diverse and intricate, stemming from immune system dysfunctions [7, 8]. There is a need to establish clear and consistent definitions and methods for researching, diagnosing, and treating Long-COVID in order to enhance the collection of national and international data on its occurrence, prevalence, and risk factors among different age groups [9]. Long- Long-COVID may not be attributed to the direct effects of SARS-CoV-2, but rather to the biopsychosocial ramifications of COVID-19 [10]. Long-term COVID can have a significant influence on adults, children, and adolescents, resulting in a failure to regain their previous levels of quality of life and employment [11]. Long-COVID disease be able to affect those worldwide, regardless of the severity of their acute SARS-CoV-2 infection [12, 13]. Long-COVID be able to result in a range of novel or enduring symptoms that can last for several weeks or months [14]. Following the onset of symptoms, the recovery period typically lasts for 7-10 days, although those with severe sickness may require two to 12 months to fully recuperate [5, 15-17]. The symptoms exhibit a wide range and are associated with specific organs, such as the genitourinary system hair, throat, stomach, eyes, nose, ears, chest, blood vessels, skin, and nervous system [18]. The symptoms encompass prolonged fevers, gastrointestinal problems, anomalies in blood and mucosa, and maybe increased autoantibodies targeting specific organs [11, 19, 20]. Some individuals have tachycardia, severe fatigue, and inability to carry out routine physical tasks [21]. Long-COVID exists a multifaceted condition characterized by a variety of symptoms that persist over a long period of time. It necessitates specialized treatment and ongoing support [22]. Neuronal damage biomarkers, such as the Galaninergic system, have been investigated for the purpose of identifying and tracking Long-COVID disease. Serum Galanin (GAL) and Galanin receptor-1(GALR1) have been examined in individuals with Long-COVID. There may be a

correlation between changes in plasma GAL and GALR1 and the presence of Long-COVID symptoms. There is a lack of information regarding the involvement of nervous system functions and numerous physiological actions in Long-COVID, as these have not been well explored. This study focuses on the analysis of Galanin, Galanin receptor-1, and Long-COVID illness. Galanin(GAL) is a neuropeptide synthesized from the Galanin gene [23]. Interacting with three G-protein coupled receptors regulates immune responses [36]. Galanin has the strongest attraction to GALR1 and GALR2 [24], which are produced by neurons in the enteric nervous system. These neurons link to Galanin receptors-1, 2, and 3 (GALR-1, 2, and 3) to affect pain sensitivity, pain-related behaviors, sadness, and anxiety [25, 26]. GAL suppresses the release of neurotransmitters in myenteric neurons through GalR-1 [27]. GALR1 was shown to be highly expressed in GABA neurons [28]. The levels of Galanin (GAL), Galanin receptor-1 (GalR-1), and the Gal/GalR-1 complex exhibited a reduction [29]. There is a correlation between higher levels of stigma and three factors: being a survivor of COVID-19, experiencing economic loss, and having signs of depression [30]. The study assessed neurological damage markers in people with Long-COVID evolution, used Receiver operating characteristics (ROC) analysis to forecast complicate outcomes, integrating sociodemographic factors.

Subjects and Methods

Participants

In a case-control study, 90 people with a history of acute COVID-19 who also showed signs of Long-COVID illness were included. Sixty health controls (HC) did not contract Long-COVID in the first quarter of 2023. The WHO post-COVID-19 criteria were used to identify Long-COVID patients [31], which include: (a) People who have tested positive for SARS-CoV-2; (b) COVID symptoms that persist beyond the initial phase of illness or during the recovery period from acute COVID-19 disease; (c) Disease symptoms that persist for at least 2 months and are

still present 3-10 months after the pandemic; and (d) COVID patients with at least two symptoms that significantly impair daily activities [31]. Nobody of the 60 controls met any of these requirements. Every person who was identified with acute COVID-19 had medical care at A specialized disinfection hospital found in the city of Kerbala. The hospitals mentioned are Al-Hindiyah General Hospital, Karbala Teaching Hospital for Children, Imam Al-Hassan Al-Mujtaba Teaching Hospital, Alkafeel Super Specialty Hospital, and Imam Al-Hussein Medical City of Kerbala. Experienced doctors and experts in the study of viruses identified the presence of SARS-CoV-2 infection and the onset of acute COVID-19, based on common symptoms like fever, coughing, breathing difficulties, loss of test and anosmia, as well as positive rRT-PCR and IgM antibodies specific to SARS-CoV-2. All patients' post-acute rRT-PCR results were negative. We selected 60 family members or relatives from an exclusive locality as controls. We also selected control participants who were negative for rRT-PCR and did not have acute phase symptoms such sore throat, fatigue, dry cough, loss of appetite, fever, influenza-like symptoms, chills, or night sweats. In addition, the study did not include people who had neurodegenerative or neuroinflammatory disorders, multiple sclerosis, diabetes mellitus or other systemic autoimmune diseases, inflammatory bowel disease, psoriasis, liver disease, stroke, rheumatoid arthritis, scleroderma, or any other medical conditions. The study documented the administration of Sinopharm, Pfizer, and AstraZeneca vaccines. The BMI was calculated from formula of dividing the weight of the body (Km) by the height^2 (in M). In addition, the study excluded pregnant or lactating women. Previous to their involvement in the trial, both control(HC) and patient(LC) contributors, or their relevant parents or legal guardians, gave in print consent after obtaining thorough information. The study complied with ethical and privacy standards in Iraq and internationally, containing, the Belmont Report, the CIOMS Guidelines, the International Conference on Harmonization of Good Clinical Practice, and the World Medical Association's

Declaration of Helsinki. Institutional review boards at both the University of Kufa (1657/2023) and the Kerbala Health Directorate-Training and Human Development Centre (Document No.0030/2023) gave their approval to the study.

Assays

Blood was drawn at 9-12 a.m., after waking up and before breakfast. A 5-milliliter venous blood sample was placed in clean, empty tubes. Experiments that were hemolyzed were not used. Centrifuged at 4,000 rpm for 5 minutes, the coagulated blood samples were then collected 10 minutes later. Isolated serum was stored in three fresh Eppendorf tubes at -80 °C until thawed for analysis. To rule out inflammation, Spinreact® latex slide assays in Barcelona, Spain measured CRP levels in human serum. To measure the amounts of Galanin and GalR-1 in blood, ELISA kits from Nanjing Pars Biochem Co., Ltd. (Nanjing, China) were used. The GAL and GalR-1 assays had a sensitivity variety of 10 ng/L to 300 ng/L and a 10.0% intra-assay CV. We followed the company's recommendations step-by-step.

Statistical analysis

The Kolmogorov-Smirnov assessment examined the distributions of the findings in different groups. The distinction between nonparametric and normally distributed variable findings is made based on statistical distribution. Results were shown using the normal distribution variable's mean standard deviation. The Mann-Whitney U test compared control and suffering groups' measurements. The study found 25th–75th percentile medians and interquartile ranges for nonparametric variables. Rho from Spearman's correlations, we can estimate the extent of correlation between the parameters. ANOVA analysis was employed to examine variations in continuous variables across different groups, whereas contingency tables (χ2-test) were utilized to investigate relationships between nominal variables. Calculated variables include cut-off points, sensitivities, specificities, and Youdin's

statistic. We examined the variables' relationships using Pearson's product-moment correlation coefficients. Biomarkers' diagnostic efficacy was examined using ROC analysis. Statistics were calculated using SPSS 26. Two-tailed tests with 0.05 p-values were used. Statistics were organized with Excel 2019.

Results

Galanin system assay

The estimated serum level GAL in (pg/mL) for both the HC group and the LC patients' participation as showed below in figure1. The GAL levels of those classified as LC [63.211 (51.577-141.616)] pg/ml were significantly higher ($p<0.001$) than those of the HC group [47.350 (41.367-57.084)] pg/ml, according to the study's findings.

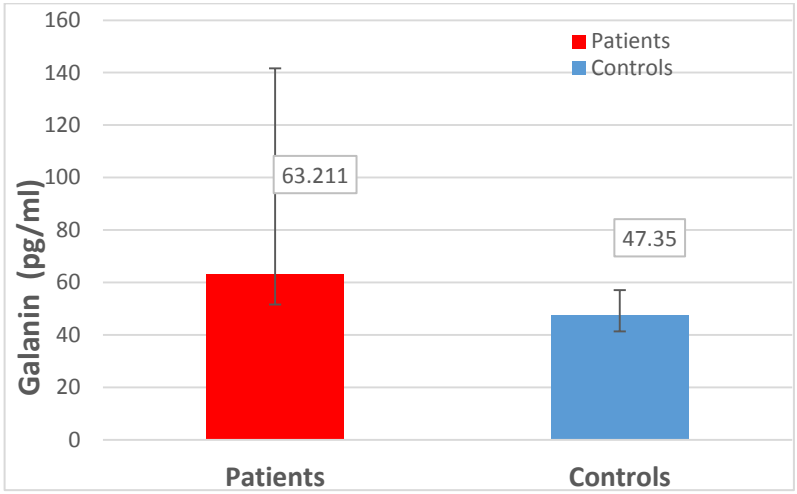

Figure1. Serum Galanin in patients and control groups.

The serum GalR-1 results for the HC and LC groups as showed below in figure 2. The results of the investigation revealed a statistically significant difference in GalR-1 levels between individuals classified as LC [1.965 (1.661-2.547)] pg/ml and the HC group [1.770 (1.487-1.878)] pg/ml ($p = 0.007$).

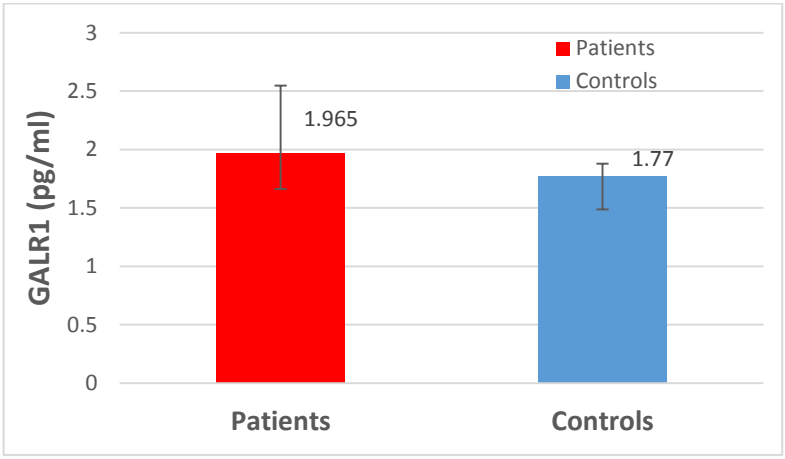

Figure 2 Serum Galanin Receptor-1 in patients and control groups.

The results of an experiment measuring the Gal/GalR-1 ratio using serum samples from individuals with LC and a control group of healthy individuals are shown in Figure 3. The levels of Gal/GalR-1 in those with LC diagnoses [32.957 (26.627-60.056)] were significantly higher (p=0.019) than in the HC group [26.741 (22.650-35.396)], according to the study's findings.

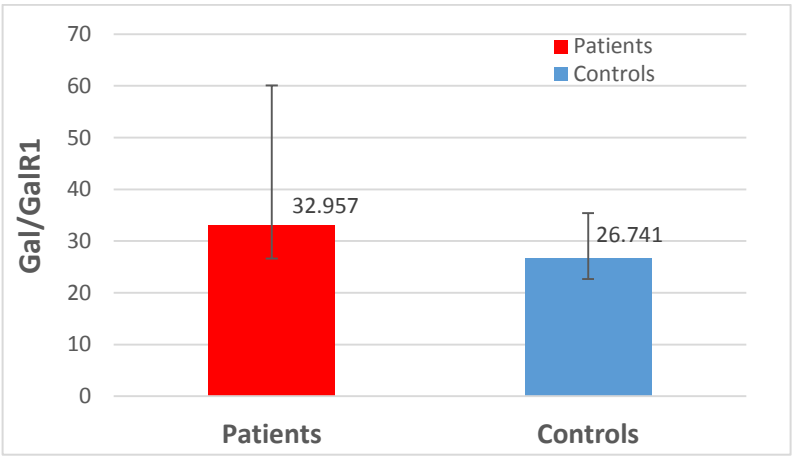

Figure 3 Serum Gal/GalR-1 in patients and control groups.

Table1 presents the results, which show a statistically significant positive association between Gal/GalR-1 (ρ=0.641, $p<0.01$) and between GAL and GalR-1 (ρ=0.212, $p<0.05$). The analysis showed a negative association with Gal/GalR-1 (ρ=-0.489, $p<0.01$) and a statistically significant relationship between GalR-1 and GAL (ρ=0.212, $p<0.05$).

Table 1: Correlations between among biomarkers and cations levels characteristics in patients.

	Galanin	GALR1	Gal/GalR1
Galanin	1.000	0.212*	0.641**
GALR1	0.212*	1.000	-0.489**
Gal/GalR1	0.641**	-0.489**	1.000

* Correlation is significant at the 0.05 level (2-tailed).
** Correlation is significant at the 0.01 level (2-tailed).

ROC study of Galanin system biomarker

In the context of Long-COVID, the analytical specificity and sensitivity of GAL, GalR-1, and the Gal/GalR-1 ratio were assessed using the Receiver Operating Characteristic (ROC) analysis. Figure 4 displays the receiver operating characteristic (ROC) curves used for analysis. The ROC research demonstrates that the coordinates and concentration cut-off values reported in table 2 achieve optimal levels of sensitivity and specificity when appropriately adjusted. The results of GAL and GALR-1 have the highest levels of sensitivity, precisely 71.7% and 60.0% respectively, in distinguishing persons with LC from those who are HC as presented in table 2 and demonstrate in figure 4. While the statistical relevance of Gal/GalR-1 as a diagnostic marker is not established ($p > 0.05$), the information present in this table can be deduced as: With a sensitivity and specificity of 71.7% and 73.3%, respectively, those whose GAL levels are higher than the cutoff point of 53.302 pg/ml may be positive for LC. Patients with LC have higher than threshold levels of GALR-1 (pg/ml) with a sensitivity and specificity of 60.0% over the defined cutoff of 1.815 pg/ml. When the concentration of Gal/GalR-1 surpasses the predefined threshold of 30.740 pg/ml, it signifies that individuals with LC have a specificity of 57.6% and a sensitivity of 58.3%.

Table 2 AUC study of receiver operating characteristics of Galanin, GALR1 and Gal/GalR1 in LC. group. CI: Confidence interval.

Variable(s)	Cut-Off	Sensitivity	Specificity	AUC(SE)	p-value
Galanin Pg/ml	53.302	71.7	73.3	0.790(0.049)	<0.001
GALR1 (Pg/ml)	1.815	60.0	60.0	0.674(0.055)	0.005
Gal/ GalR1	30.740	58.3	57.6	0.652(0.058)	0.713

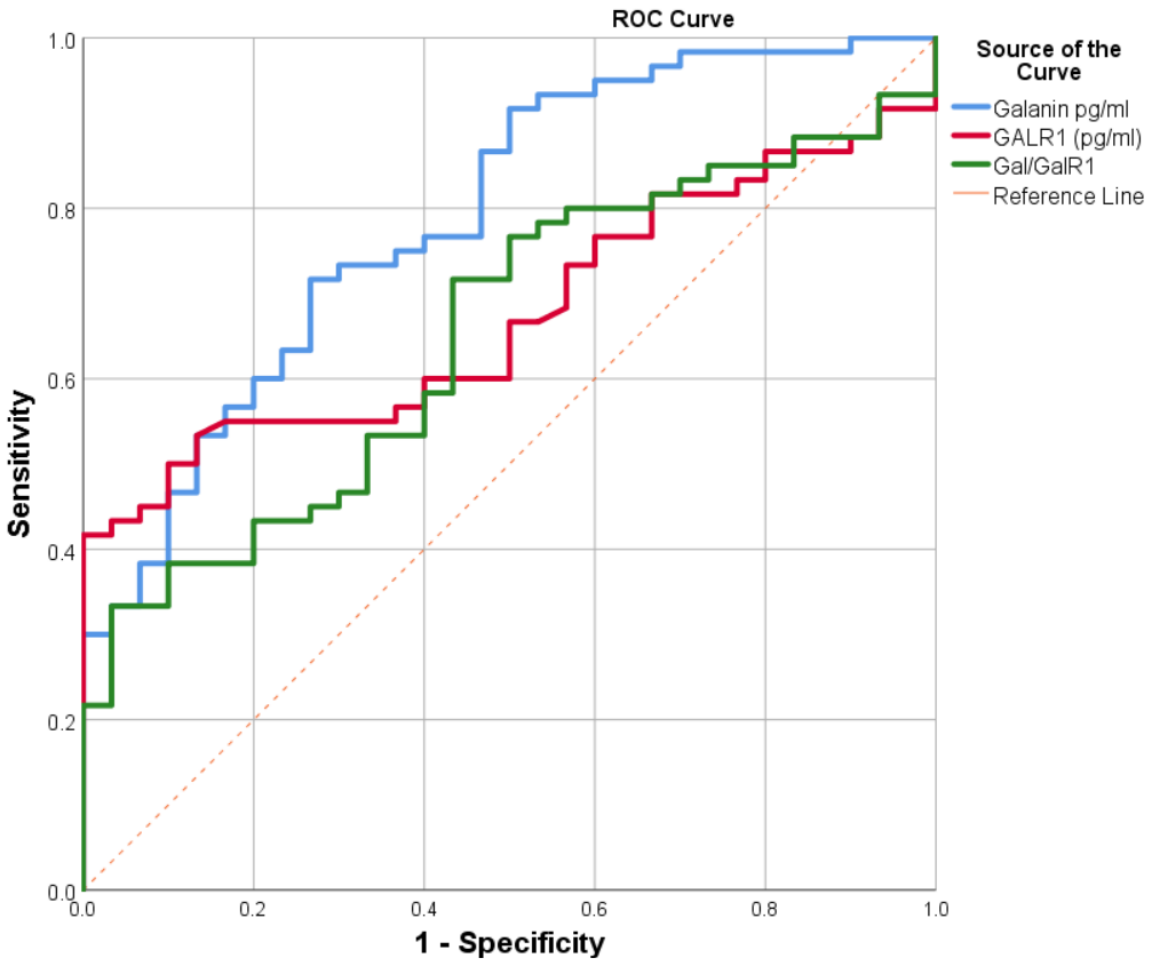

Figure 4. Receiver operating characteristic curves of GAL, GALR-1 and Gal/GalR1 in LC group.

Discussion

Current study revealed that the three biomarkers associated with LC, namely Galanin, Galanin Receptor-1, and Galanin/Galanin Receptor-1 ratio, account for a significant portion of the variability in finding individuals with LC. The serum GAL (pg/ml) values for individuals with LC and HC individuals as displays in figure 1. LC patients exhibited significantly high levels of Gal compared to the HC. It has been noted that influenza causes an increase in GAL expression in the lung tract. This increase may have an effect on inflammation and local immune responses [32, 33]. GAL is associated with numerous inflammatory illnesses, while other regulatory peptides alter the responses of the immune system [34]. The Galanin system is associated with age-related chronic diseases [35]. Examine the Gal/GalRs ratio system as a potential target for pain, diabetes, cancer depression, epilepsy, and neurodegenerative diseases [36]. GAL exerts an influence on smooth muscle, hence impacting the functioning of the gastrointestinal and respiratory systems [37]. The distribution of GAL in guinea pigs suggests that it may have an impact on the functioning of the airways, blood vessels, and secretory processes

[38]. Galanin analogs have the potential to reduce the occurrence of respiratory collapse following a seizure [39]. Galanin exerts an influence on pain threshold, anxiety, and behavior sadness [26]. GAL inhibits the release of acetylcholine, so the use of a receptor antagonist may enhance cognitive function [40]. The results of our investigation indicate a potential link between higher amounts of GAL and the persistence or progression of LC symptoms, suggesting that Galanin may play a role in the underlying development of the disease. Patients with Long-COVID exhibit increased GalR-1 levels. Galanin and Galanin-like peptides have been shown to activate GalR-1, which is a receptor for Galanin[41]. Stimulating GalR-1 suppresses cell growth [42]. GalR-1 stimulates definite GALRs subtypes in order to safeguard neurons of excitotoxic harm [43]. The GalR-1 gene is found to be increased in nerve tissues [44]. The regulatory effect of cloned GAL1 receptors on cAMP is characterized by its potency profile [45]. GALRs facilitate distinct signal pathways, and the activation of GalR-1 has been found to have inhibitory effects on cell proliferation. This indicates that GALRs could potentially serve as therapeutic targets and diagnostic markers for specific types of cancers [46]. The levels of GalR-1 may influence the signaling and neuroplasticity of the locus coeruleus (LC) [47]. Elevated GalR-1 levels have been found to be associated with persistent symptoms of COVID. Several factors can increase GalR-1 levels: LC or other health problems may enhance the functionality of GalR-1. The advancement or difficulty of a disease may have enhanced the transmission of Galanin signals. Changes in GalR-1 can impact the immune response, pain perception, and inflammatory processes. The increase of GalR-1 may be explained by the methodology, setting, and settings employed by the researchers. Further research is required to investigate the impacts of differences in receptor expression. The Gal/GalR-1 ratios of LC patients and HC as displays in figure 3. Healthy controls had significantly lower Gal/GalR-1 ratios compared to Long-COVID patients. Reliable outcomes: The Galanin/Galanin-receptors system holds potential in identifying treatment targets for a range of ailments, such as epilepsy, diabetes, mental illnesses, pain, cancer and neurodegenerative diseases [36]. Gal/GalR-1 function as tumor suppressors [48]. Reduced Gal/GalR-1 amounts may possibly play a role in the progression of gastric cancer [29]. Numerous studies have connected the expression of Gal/GalR-1 to the differentiation of tumors [49]. A potential disturbance in the Galaninergic system, which influences inflammation of illness, immunological responses, and pain regulation, was suggested by the severe symptoms linked to the found rise of GAL levels relative to GalR-1 levels. One way to measure the concentration of GAL in relation to its receptor is by looking at the Gal/GalR-1 ratios. This ratio is shown to be more in severe LC patients. GAL was found to be positively associated with both GalR-1 and Gal/GalR-1, respectively. The GalR-1 gene was linked to the Gal/GalR-1 pathway. The findings demonstrated a hydrophobic interaction between GAL and GalR-1. This receptor

acts differently from other G protein-coupled receptors that depend on interactions between their peptide ligands and water either close to or far from the plasma membrane. Additionally, it was discovered that the agonist/GalR-1 combination rapidly enters cells following binding [50]. The GalR-1 receptor exclusively triggers the Gi pathway, whereas GalR-2 triggers Go, Gq/G11, and Gi. This suggests that the way GalR-1 and GalR-2 are distributed in tissues and how they transmit signals may be the cause of the many functional effects of GAL that are mediated by these receptors. Additionally, they adjust the various physiological roles of GAL [51]. This study demonstrated the presence of interactions within the Galanin system. Coordinated reactions were identified between GalR-1 and the Gal/GalR-1 ratio, indicating positive connections. On the other hand, there were detected negative correlations, suggesting complex regulatory interactions. The GAL, GalR-1, and Gal/GalR-1 were assessed for their analytical sensitivity and specificity in detecting LC using receiver operating characteristic (ROC) curves, as depicted in figure 3. Galanin and GalR-1 exhibit the highest levels of sensitivity (71.7% and 60.0%, respectively) in accurately identifying Long-COVID patients (LC) compared to healthy controls (HC), as depicted in table 1 and figure 4. Given that GAL and GalR-1 do not offer an accurate diagnosis, the data in the table can be interpreted as follows: Galanin levels above 53.302 pg/ml have a 71.7% sensitivity and 73.3% specificity, suggesting the possibility of LC. A sensitivity and specificity of 60.0% each indicate the existence of LC in patients if GalR-1 levels are higher than the cutoff of 1.815 pg/ml. Gal/GalR-1 levels above 30.74 pg/ml have a 58.3% sensitivity and 57.6% specificity, indicating the presence of LC in patients. These conclusions are consistent with the findings of the research studies. The Galaninergic system can also impact conditions such as alcoholism, chronic pain, and inflammatory diseases of the intestine and skin [52]. Galanin plays a function in the development of experimental autoimmune encephalomyelitis [53]. The regulation of sleep and wakefulness is influenced by neuropeptides such as Galanin [54]. Elevated Galanin levels were associated with moderate OSAS [55]. Galanin, released by neurons in the retrotrapezoid nucleus, may act as a counterweight to glutamatergic inputs in respiratory centers, reducing excessive, promoting neuroplasticity and hyperventilation by slowing ventilation [56]. GalR-1 regulates Galanergic signaling by functioning as a dimer in extracellular space and translocating intracellularly upon activation by GAL [57].The Gal/GalR-1 peptide receptor regulates the sensitivity of nerve terminals in cases of long-term tissue damage and pain through an inhibitory autocrine mechanism at the presynaptic level [58]. There was no observed correlation between GAL binding and proliferation [59]. Long-term inflammation and damage to brain cells can lead to neurological and cognitive impairments because the SARS-CoV-2 virus is able to cross the protective barrier of blood vessels in the brain [60]. The ROC analysis demonstrated that the GAL and GalR-1 biomarkers

had the highest sensitivities, indicating their potential to differentiate LC patients from HCs. Nevertheless, the ratio of Gal/GalR-1 did not exhibit substantial diagnostic capability, underscoring the necessity for suitable indicators.

In conclusion,

This is the first study to report that patients with COVID, particularly those with persistent symptoms like anosmia, have elevated serum GAL and GALR-1 levels. GAL and GALR-1, vascular biomarkers associated with Long COVID, may aid in the management of the disease in patients with Long COVID. We require confirmation of our findings in a larger population. These findings permit additional research into the role of GAL and GALR-1 in infectious respiratory diseases.

Limitation

The study's limited sample size and need for a minimum of two months of hospitalization may restrict the applicability of the findings and establish a bias in the selection process. Moreover, the use of a cross-sectional design makes it difficult to demonstrate causal links, and the dependence on ELISA assays for biomarker quantification may introduce variability. The study did not adequately consider exclusion criteria and potential confounders. Additionally, the neural network model is sophisticated and requires confirmation in other groups of individuals.

Statement of ethics

The study with human subjects was reviewed and approved by the Institutional Ethics Committee (1657/2023) of the University of Kufa. patients or participants provided written learned approval to participate in this study.

Rights of humans and animals

The research followed ethical norms, including the World Medical Association Declaration of Helsinki and Iraqi and international privacy laws. Our Institutional Review Board (IRB) follows the International Guidelines for the Protection of Human Research Subjects, which are based on the ICH-GCP, the Declaration of Helsinki, the Belmont Report, and the CIOMS Guidelines.

CONSENT FOR PUBLICATION

Prior to participation in this study, every participant provided written informed consent.

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