Journal of Medicinal and Chemical Sciences 6 (2023) 1344-1351

Journal of Medicinal and Chemical Sciences

Journal homepage: http://www.jmchemsci.com/

Original Article

Association of Serum Serotonin Levels, and Hyperglycemia with Ageusia and Anosmia in Iraqi COVID-19-Infected Patients

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ARTICLE INFO

Article history

Receive: 2022-08-26
Received in revised: 2022-09-09
Accepted: 2022-11-14
Manuscript ID: JMCS-2209-1765
Checked for Plagiarism: **Yes**
Language Editor:
Dr. Fatimah Ramezani
Editor who approved publication:
Dr. Majid Darroudi

DOI:10.26655/JMCHEMSCI.2023.6.14

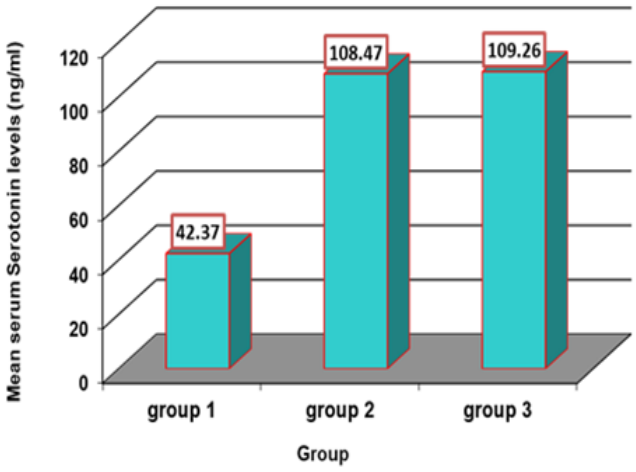
KEYWORDS

Serum serotonin levels
hyperglycemia
anosmia
ageusia
Iraq

ABSTRACT

Background: Serotonin plays a significant neuro-modulatory role in olfactory neurons and taste receptor cells.
Aim: This study was conducted to examine and comparison between serum levels of serotonin in active Iraqi COVID-19 patients (with or without anosmia and ageusia) and healthy individuals. Also, the correlation between serotonin serum levels and hyperglycemia in each of the study groups was evaluated and compare it with control group.
Methods: The participants were divided into three groups: 30 active COVID-19-infected patients who had anosmia and ageusia (Group 1), 30 patients who were infected and had no anosmia and ageusia (Group 2), and 30 healthy subjects who served as controls (Group 3).
Results: All the samples were thawed and used to evaluate the levels of serum serotonin. Likewise, a colorimetric approach was employed to measure the levels of serum FBG and the (SPSS, version 25) was used to analyze the data, it was observed that the mean amounts of serum serotonin were significantly (p < 0.0001) lower in group 1 compared with groups 2 and 3, while mean, the average serum levels of glucose were significantly higher in group 1 (p < 0.0001), as compared with groups 2 and 3.
Conclusion: Serotonin levels was decreased in patients who had COVID-19 infections along with anosmia and ageusia, which helps with the prediction of anosmia and ageusia associated with the other diseases. In addition, there is an inverse relation between blood glucose level and serotonin.

GRAPHICAL ABSTRACT



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Introduction

Anosmia in common coronavirus infections has already been documented [1]. Hwang documented an instance of anosmia in 2006 that persisted for two years after the onset of SARS [2]. The preponderance of the evidence points to sustentacular cells as the SARS-CoV-2 main targets in the OE. As demonstrated by the presence of cell debris in the lumen of the nose in numerous experiments, the OE bulk appears to be destroyed through desquamation after infection. A portion of the olfactory sensory neurons (OSN) population will be eliminated by this desquamation, but it also may cause the OSN dendritic layer, which is necessary for olfactory transduction, to disappear [3]. Viral infection and inflammatory reactions may alter the makeup of saliva, how taste is transmitted and how taste buds continually regenerate [4].

Tryptophan is converted into the monoamine neurotransmitter serotonin (5-hydroxytryptamine). Most serotonin in the body is found in the gastrointestinal tract, where it affects bowel movements via regulating smooth muscle contraction. In addition, it functions in the brain as a neurotransmitter and as a modulator of cardiovascular vasoconstriction. Thus, any abnormalities in the serotonergic system may lead to various clinical diseases, including those that affect the heart's function, regulate mood and memory, and cause bowel motility difficulties [5]. L-tryptophan, a precursor, is converted into serotonin in enterochromaffin cells (EC cells). The transformation of L-tryptophan to 5-hydroxy-L-tryptophan (5-HTP), which is mediated by tryptophan hydroxylase (Tph), is the first determinant in the serotonin creation. The subsequent decarboxylation of 5-HTP by L-amino acid decarboxylase caused the second step's creation of serotonin. For this process, cofactors like magnesium, vitamin B3, and vitamin B6 are needed.

The serotonin system is modulated by the Tph enzymes in two separate ways. Tph1 is mostly prevalent in the EC, while Tph2 occurs in the central and enteric nervous systems [6]. The blood-brain barrier does not allow serotonin to pass through. The EC cells express mechanical and chemically sensitive ion channels, ligand-gated ion channels, G protein-related receptors, and release serotonin in a controlled way in response to various mechanical and chemical stimuli [7]. In the bloodstream, surrounding tissue, and intestinal lumen, serotonin generated by EC cells have several roles [8]. Serotonin is transported via the serotonin reuptake transporter (SERT) into the neighboring epithelial cells, where it is converted into 5-hydroxy indole acetic acid (5-HIAA). Likewise, serotonin mediates many roles in the intestine by acting on many receptors in smooth muscle, enterocytes, immune cells, and intestinal neurons. Human islets include the genes for the essential TPH and aromatic-L-amino-acid-decarboxylase, which are necessary for the production of serotonin by pancreatic β-cells [9].

The study was conducted to determine the correlation between serum levels of serotonin in Iraqi COVID-19 patients (with or without anosmia and ageusia) in the active state and healthy individuals. Furthermore, the correlation between serum levels of serotonin and hyperglycemia in both study groups was examined.

Materials and Methods

Study groups

The Alatta Hospital for Communicable Illnesses, in Iraq, Baghdad, Ministry of Health, and a private outpatient clinic, was the sites of this case-control study. Recruitment for the study started in November 2021 and finished in January 2022. Ninety people were classified into three groups based on the collected cases: group 1 included thirty people with COVID-19 who also had anosmia and ageusia, group 2 included thirty subjects with COVID-19 without anosmia and ageusia, and group 3 included thirty subjects who were healthy as control.

Exclusion criteria and ethical clearance

The age range for groups 1 and 2 was 25 to 55 years old, while the age range for group 3 was 22 to 55 years old. In the study, people with heart disease, diabetes, hyperglycemia, hypertension, autoimmune disease, cancer, and patients taking

alcoholics, obesity, or antipsychotics were excluded. All the subjects offered their informed consent and the research were carried out in line with the declaration of Helsinki approved by the Ethics Committee of the University of Baghdad's College of Pharmacy, Iraq [10].

Blood collection

Five milliliters of venous blood were obtained from each participant in groups 1-3. The blood samples were placed into autoclaved gel tubes and allowed to clot for 15 minutes at ambient temperature. The serum was then obtained by centrifuging the blood for 15 minutes at 3000 rpm. After that, the serum was frozen at -20 °C until needed for further analysis.

Biochemical analysis

All the samples were thawed. Human ELISA Kit (BT LAB; Bioassay Technology Laboratory) was used to evaluate the levels of serum serotonin. Likewise, a colorimetric approach (Glucose-MR/Enzymatic colorimetric) was employed to measure the levels of serum FBG (Linear Chemical Company in Spain).

Statistical analysis

The statistical package for social sciences (SPSS, version 25), was utilized for data analysis and the descriptive statistics were employed for their presentation. The statistical differences among the three groups were carried out to describe the dissimilarities between the groups using one-way ANOVA. For the correlation between two quantitative variables, it was determined that the value of Pearson correlation coefficient (r) was either positive (direct correlation) or negative (inverse correlation), when $p<0.05$, statistical significance was considered.

Results and Discussion

There was no significant (p = 0.551) difference in mean age between infected patients in either group (1 and 2) and healthy control group 3 (Table 1 and Figure 1). The average age of groups 1, 2, and 3 were 38.12±2.92 (the age range between 25 and 55), 38.0±2.44 (the age range between 25 and 55), and 38.55±2.99 (the age range between 22 and 55). There were significantly ($p<0.0161$) different body mass index (BMI) values between the COVID-19 patients (groups 1 and 2) and the healthy control group 3, as depicted in Table 1. The mean BMI values for groups 1, 2, and 3 patients were 23.46±0.18, 23.36±0.17, and 22.70±0.23.

As indicated in Table 2 and Figure 2, there was reduced amount of mean serum serotonin in group 1 compared with groups 2 and 3. There was a significant difference ($p<0.0001$) between groups 1 and 2, as well as the healthy control group 3. Nevertheless, no significant difference exists between groups 2 and 3 (control). According to Table 2 and Figure 2, group 1 had higher mean serum glucose levels than groups 2 and 3 (control), and these differences were statistically significant ($p<0.0001$). In contrast, group 2 and healthy control group 3 did not differ significantly from one another.

Table 1: Demographic characteristics of patients with COVID-19 infection and healthy control groups

P-value	Group 3 (n=30)	Group 2 (n=30)	Group 1 (n=30)	Demographics
Age (years)				
0.551	38.55±2.99[a]	38.0±2.44[a]	38.12±2.92[a]	Mean ± SD
	22-55	25-55	25-55	Range
BMI (kg/m2)				
<0.0161[**]	22.70±0.23[b]	23.36±0.17[a]	23.46±0.18[a]	Mean ± SD
	20-25	22-25	22-25	Range

Group 1: Active COVID-19-infected patients with anosmia and ageusia, Group 2: Active COVID-19-infected patients without anosmia and ageusia, Group 3: Healthy control subjects, [**]: Highly significant difference; Superscripts (a and b) among different groups indicate the significance level. Similar letters indicate no significant difference, whereas different letters indicate significant difference. Letter "a" is assigned the highest value, followed by letter "b", and then letter "c". Means having different letters in the same column differ significantly; [**]: P < 0.01.

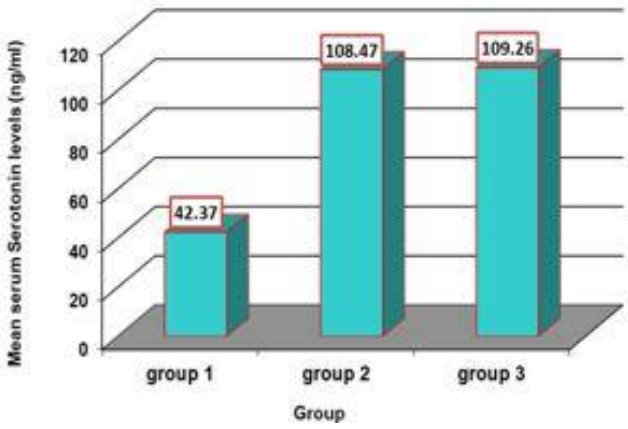

Figure 1: Mean Serum levels of serotonin for the studied groups, Group 1: Active COVID-19-infected patients with anosmia and ageusia, Group 2: Active COVID-19-infected patients without anosmia and ageusia, and Group 3: Healthy control subjects

Table 2: Serum levels of the studied parameters among the different groups

Variable	Group 1 (n = 30)	Group 2 (n = 30)	Group 3 (n = 30)	*p*-value
Serotonin				
Mean ±SD	42.37±2.07[b]	108.47±6.23[a]	109.26±5.98[a]	<0.0001**
Range	26.11-2.89	74.62-00.30	75.72-200	
Glucose				
Mean ± SD	13.59±0.34[a]	5.44±0.11[b]	5.22±0.08[b]	<0.0001**
Range	10.50-6.60	4.50-6.50	4.50-6.50	

*Group 1: Active COVID-19-infected patients with anosmia and ageusia, Group 2: Active COVID-19-infected patients without anosmia and ageusia, Group 3: Healthy control subjects, **: Highly significant difference, and Superscripts (a and b) among different groups indicate the level of significance. Similar letters indicate no significant difference, whereas different letters demonstrate a significant difference. Letter "a" is assigned the highest value, followed by letter "b", and then letter "c". Means having different letters in the same column differ significantly, **: P <0.01.*

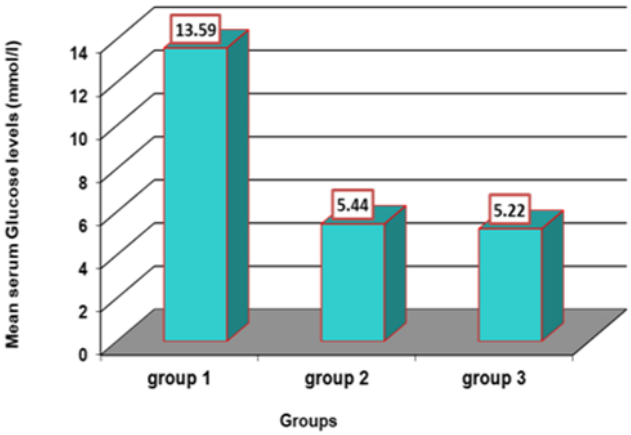

Figure 2: Mean serum levels of glucose for studied groups; Group 1: Active COVID-19-infected patients with anosmia and ageusia, Group 2: Active COVID-19-infected patients without anosmia and ageusia, and Group 3: Healthy control subjects

Correlation between serotonin and FBG in the experimental groups

Table 3 provides the correlation analysis among the biochemical indicators in each group of participants in the current study (n=30). Serum serotonin levels and serum glucose levels showed a statistically significant negative association in group 1 (r = -0.87, $p < 0.0001$), an insignificant negative weak relationship in group 2 (r = -0.03, $p = 0.848$), and an insignificant positive weak correlation in group 3 (r = 0.02, $p = 0.263$).

Table 3: Correlation coefficient between the studied parameters and FBG in various groups

Parameter	Correlation coefficient (r)	*P*-value
Serotonin and Glucose in Group 1	-0.87	<0.0001**
Serotonin and Glucose in Group 2	-0.03	0.848
Serotonin and Glucose in Group 3	0.02	0.263

*Group 1: Active COVID-19-infected patients with anosmia and ageusia, Group 2: Active COVID-19-infected patients without anosmia and ageusia, Group 3: Healthy control subjects, r: Correlation coefficient based on Pearson's correlation test, and **: Highly significant correlation at p < 0.01.*

Correlation between serotonin and FBG in COVID-19 infected group

As presented in Table 4, a correlation analysis was conducted among biochemical markers in participants in the current study who had contracted COVID-19 (groups 1 and 2). The infected groups 1 and 2 showed a statistically significant negative correlation between the amounts of serotonin and glucose in their serums (r=-0.75, $p<0.0001$).

COVID-19 patients (no history of ear, nose, or throat illness) need to be actively examined for the related symptoms at presentation time, as reports of taste and smell abnormalities are subjective [11]. According to Mao *et al.*'s findings, the most common complaints in individuals with peripheral nervous system symptoms associated with COVID-19 were hypogeusia (5.6%) and hyposmia (5.1%) [12], the more recent studies may have evaluated these individuals explicitly for the olfactory symptoms as they gained the widespread recognition, leading to a larger prevalence of olfactory symptoms. In addition, few researchers used the approved equipment to accurately evaluate "loss of odor" [13].

The average amounts of serum serotonin were the lowest in group 1 compared with groups 2 and 3 (Figure 1 and Table 2). With a range of serotonin receptor types present in the olfactory bulb, serotonin is mostly engaged in modifying

the sensory gain. The role of serotonin in odor detection and differentiation is somewhat complex. This is because the mitral cells can be in excitatory, inhibitory, or mixed mode (inhibition followed by excitability) [14, 15]. The overall action of serotonin on mitral cells is usually bidirectional (excitatory and inhibitory). The obvious suppressive effects may enhance the perception of some odors over the others by enhancing the ability to distinguish between different odors or blocking sensory information that may compete for attention [16]. It can block the spontaneous firing of sensory cortical neurons (preserving sensory-driven firing), which may lead to an enhanced signal-to-noise ratio. Thus, serotonin plays a vital function in olfaction [17]. Although tryptophan depletion and serotonin deficiency are the main causes of inability to smell in COVID-19 infected patients. In more severe cases where the patient experiences long-term olfactory dysfunction, the nerve damage and dysfunctional olfactory bulbs (and related structures) should be taken into account [18]. Although intestinal infections are quite prevalent in COVID-19, a small proportion of patients likely avoid them, protecting them from tryptophan depletion and potentially preventing anosmia and ageusia. The fact that females typically have lower brain serotonin levels than males can help to explain why women are more likely to develop anosmia and ageusia [19].

Table 4: The correlation coefficient between serotonin and FBG in subjects infected with COVID-19

Parameter	Correlation Coefficient (r)	*P*-value
Serotonin and Glucose (Groups 1 and 2)	-0.75	<0.0001**

*Group 1: Active COVID-19-infected patients with anosmia and ageusia, Group 2: Active COVID-19-infected patients without anosmia and ageusia, FBG: Fasting blood glucose, r: correlation coefficient based on Pearson's correlation test, and **: Highly significant correlation at p < 0.01.*

A recent study [20] found that serotonin levels were lower in patients with coronavirus, researchers have hypothesized that a decrease in serotonin can be caused by altered enterochromaffin cell function or increased degradation of serotonin by enzymes of the MAO family, where MAO-A converts serotonin to 5-HIAA [20]. In cases of SARS and COVID-19 infection, hyperglycemia and diabetes are recognized as autonomous indicators of mortality and morbidity [21]. It has been shown that one or more existing medical abnormalities of insulin sensitivity (such as obesity or high BMI) increase the susceptibility to stress hyperglycemia in serious illnesses and may ultimately lead to diabetes [22].

Notably, hyperglycemia raises the pneumonia prevalence in COVID-19 patients [23] and is linked to the higher morbidity and mortality in the ICU patients [24]. The IR is characterized by increased lipolysis, decreased muscle glucose absorption, and increased hepatic glucose synthesis. The liver, adipose tissue, and muscles are unresponsive to insulin in this syndrome. IR increased the risk of cardiovascular disease, type 2 diabetes, and hypertension (greater risk for COVID-19-related severe illness) [25].

Serotonin and glucose concentrations have a significant negative correlation in group 1 of this study. There is also a significant negative correlation in groups 1 and 2, while group 2 and group 3 have insignificant negative weak correlations and insignificant positive weak correlations, respectively. Numerous *in vitro* and expression experiments suggest that exterior serotonin may further control the hepatic synthesis of glucose and lipids [26]. This intriguing link provides the substantial support for the idea that COVID-19 causes a serotonin deficiency condition and ageusia results from a serotonin deficit in coronavirus patients [27].

Conclusion

Serotonin levels decreased in patients who had COVID-19 infections along with anosmia and ageusia, which may help with the prediction of anosmia and ageusia associated with the other diseases. In patients who had COVID-19 infections, serotonin played a role in the prediction of anosmia and ageusia. The need for estimating these factors in blood glucose monitoring is demonstrated by the inverse relationship between blood glucose level and serotonin.

Funding

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Authors' contributions

All authors contributed to data analysis, drafting, and revising of the paper and agreed to be responsible for all the aspects of this work.

Conflict of Interest

The author declared that they have no conflict of interest.

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HOW TO CITE THIS ARTICLE

Sameerah Jasim Shanyoor, Zahraa Mohammed Ali Naji, Ghanyia Jasim Shanyoor. Association of Serum Serotonin Levels, and Hyperglycemia with Ageusia and Anosmia in Iraqi COVID-19-Infected Patients. *J. Med. Chem. Sci.*, 2023, 6(6) 1344-1351

https://doi.org/10.26655/JMCHEMSCI.2023.6.14

URL: http://www.jmchemsci.com/article_160673.html