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Assessment of the Role of Orexin A and Some Clinical Parameters in Children with Autism Spectrum Disorder

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ABSTRACT

Objective: Autism spectrum disorder [ASD] is characterized by impairments in emotional, social, and cognitive areas and is considered a neurodevelopmental disorder. These children often exhibit stereotypical and repetitive manners that may be inconsistent with their inner physiological cadence, leading to disturbances in the Wake-up and sleep cycle. This study aimed to investigate the serum Orexin A, 5-HT, glutamate, and oxidative stress status and compare the values with those of healthy controls. Methods: This study included 40 participants, 20 patients, and 20 controls. The samples of children with ASD were obtained from the AL-Shatra unit of Autism Care//Thi Qar. Children taking medication and those with neurological diseases were excluded from the study. Serum orexin A, 5-HT, glutamate, NO, and albumen levels were assessed in all participants. Results: The means of serum orexin A, 5-HT, glutamate, and NO levels of patients with ASD were significantly higher than those of the control group ($p < 0.05$). However, serum GSH and albumen levels were lower among the cases than the control group ($p < 0.05$). Conclusion: orexin A, 5-HT, and glutamate measurements taken together may even be utilized as markers for the development of sleep and eating disorders and insomnia. The rising levels of orexin A in uncontrolled autism spectrum disorder may be a cause of rising BMI. This assessment may be beneficial in developing an appropriate treatment plan for these children. This assessment may be instrumental in developing an appropriate treatment plan for these children and may form the basis for developing and directing targeted treatment in the future.

Keywords: ***Autism spectrum disorders, orexin A, 5- 5-hydroxytryptamine, glutamate, and BMI***

1. INTRODUCTION

Autism spectrum disorder[ASD] is characterized by impairments in emotional, social, and cognitive areas and is considered a neurodevelopmental disorder.It is diagnosed early in life, with 1 in 54 children diagnosed by the age of 8 years [1,2]. Symptoms of ASD appear during the first three years of life when the rapid formation and maturation of brain synapses occur [3]. Brain development processes such as synaptogenesis, arborization, migration, synaptic pruning, and plasticity aim to create a functional brain [4,5]. During growth (neurotransmitters and their receptors) play an important role.[3].Children with this disorder often suffer from a range of co-morbidities across multiple systems, with sleep disorders being specially mutual.[6]. There are some clinical features that children with [ASD] suffer from, including difficulty sleeping, light sleep, daytime sleepiness, and insomnia. All of these fall under the framework of what is called a sleep disorder[7]. Novel evidence suggests that sleep disturbances are correlating with a rise risk of cardiovascular diseases, like as obesity and insulin resistance [8]. The correlating between short sleep duration and obesity has been inspected in various studies, The main findings of these studies, mention that individuals with

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HSSD have a higher BMI compared to those with normal sleep duration [9]. During normal brain development, neuropeptides and neurotransmitters play a major role, and therefore any disturbance in them can cause impairment in brain development processes, which in turn can cause autism [10,11]. There are many types of neurotransmitters produced in the brain, each with different functions. Some of these neurotransmitters influence sleep, wakefulness, and appetite control. We will focus on some of these types. Serotonin (5- hydroxytryptamine) [5-HT] is a local hormone and neurotransmitter with numerous physiological functions in the body[12], including regulating sleep, emotions, and appetite [13]. Glutamate is a non-essential amino acid that plays an effective role in metabolic regulation. In addition, it stimulates approximately 70% synapses, making it the most important excitatory neurotransmitter in the central nervous [14,15]. If we want to mention the brain processes that are regulated by this transmitter, they are numerous. We will mention some of them, which include (motor function, mood, learning and memory, pain perception, in addition to controlling the sleep-wake cycle) [16–19].]. Orexin A, also known as hypocretin, is a lateral hypothalamic neuropeptide that has been linked to several physiological functions, including regulation of sleep, wakefulness, and appetite control [20,21]. In addition, orexin plays an substantial function in neuroprotection by prevent oxidative stress stuteuse and the inflammatory response via its "type I and type II" receptors [22]. Some studies have found that treatment with orexin A lower the secretion of some cytokines and also lower the production of reactive oxygen species [23]. The objective of this study is to evaluate the role of serum Orexin A, 5-HT,Glutamate and oxidant- antioxidant statues in patients with ASD. To assess sleep and appetite disorders, as well as anxiety, which are crucial for developing an appropriate treatment plan for these individuals.

2. MATERIALS AND METHODS

This study included 40 participants in this study 20 patients and 20 controls. The Stephen Thompson equation was used to get the sample size. the samples of children with ASD they were obtained AL-Shatra unit of Autism Care//Thi Qar. From 2024 to 2025. The age range of children with "ASD and controls" between [7-12] years . "The medical history of these children was taken, and their daily routine and the most important disorders they suffer from were identified, such as (sleep disorders, eating disorders, appetite disorders, walking on tiptoes, and facial expressions)." Approximately five milliliters of blood was collected and allowed to clot at room temperature in empty disposable tubes,then centrifuged at 3000 xg for 10 minutes . The serum samples were maintained and separated at [-20] degrees until use or used immediately to analyses biochemical parameters". This serum it was used to determine some [neurotransmission and biochemical] parameters in this study. Serum glutamate, orexin A and 5-HT were estimated by enzyme linked immunoassay method by ELISA Reader. Using kits supplied by MyBioSource. Serum Albumin was analyzed by colourimetric method by spectrophotometer, using kit supplied by (Biolabo ,france). Serum NO was measured depending on the method of Dervisevic et al.[24] . Serum GSH was measured depending on the method of Ellman[25]. SPSS version 23 was used for statistical analysis. Results were expressed using mean ± standard deviation (mean±SD) . A t-test was performed to compare parameters across the study group at a significance level ($P \leq 0.05$) to determine statistical

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significance. Pearson correlation coefficients (r) will be used to describe relationships among various parameters within each group.

3. RESULTS AND DISCUSSION

Table 1 shows the clinical characteristics of the study groups . The current study included 40 cases, divided into 20 children with autism spectrum disorder and 20 apparently Health,the mean ages of the two groups were similar".

Table 1: Distribution of age and sex in groups of study

Groups	N	Age(years) Mean±SD	Sex(M/F)
Controls	20	8.39±1.78	8/12
Patients	20	8.95±1.95	8/12
p-value		0.071	

N: Number of subjects, M: male, F: Female, SD: standard deviation

Table2:shows the distribution of clinical features and symptoms in children with autism spectrum disorder, the results of the current study showed that 80% with eating disorders, 80 % with appetite, 85% with sleeping disorders, 70% with abnormal communication, 70 % with facial expressions and 35% have an eye contact .

Table 2: Distribution of clinical features and symptom in children with ASD

Clinical features and symptom	Children with ASD number (%)
Eating disorders	16(80%)
Appetite	16(80%)
Sleeping disorders	17(85%)
Abnormal communication or play	14(70%)
toe-walking	13(65%)
Facial expressions	14(70%)
Eye contact	7(35%)

Table3: shows the BMI levels in the study groups. The current study focused on accurately calculating BMI levels, given their importance, as a child's body changes in composition from infancy to adulthood. It was calculated BMI based on growth charts specific to the child's age

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and sex. It was found the significant difference in percentile of BMI between patients and controls. The current study showed that children with autism spectrum disorder have Overweight.

Table3:Levels of BMI in the study groups

Groups	N	Percentile of BMI
Controls	20	65%
Patients	20	87%
p-value		0.007

Number of subjects, BMI: body mass index

Table4: shows the levels of orexin A, 5-HT and glutamate in patients and controls . In this table, its observed that there was a significant increase in the levels of serum orexin A, 5-HT and glutamate in the patient group compared to the controls group .

Table 4: Serum levels of orexin A,5-HT and glutamate in patients and controls group

Groups	N	Orexin A (Mean±SD) ng/mL	5-HT (Mean ±SD) ng/mL	Glutamate (Mean±SD) µmol/L
Controls	20	0.72± 0.05	198.24±33.76	6.31±1.07
Patients	20	2.18± 0.42	352.21±45.67	20.48±3.25
p-value		0.001	0.000	0.000

N: Number of subjects, 5-HT : 5- hydroxytryptamine (Serotonin)

Table5: shows the levels of NO, GSH and albumen in patients and controls. In this table, its observed that there was a significant increase in the levels of serum NO in the patient group compared to the controls group . While it is observed that there was a significant decrease in the levels of serum GSH and albumen in the patient group compared to the controls group .

Table5:Serum levels of NO, GSH and Albumen in patients and controls group .

Groups	N	NO Mean±SD (µmol/mL)	GSH Mean ±SD (µmol/L)	Albumen Mean±SD (mg/dL)
Controls	20	3.56±0.71	409.73±86.51	4.53±0.71
Patients	20	6.12±1.19	398.95±88.45	4.12±0.33
p-value		0.004	0.002	0.007

N: Number of subjects, NO: nitric oxide : GSH: glutathione SD: standard deviation

Pearson's correlation explains the link between orexin A and other parameters in this research. Table 6 reveals a statistically significant positive correlation among orexin A, 5-HT glutamate and NO. While a statistically significant negative correlation was seen between orexin A,GSH and albumen .

Table 6: Correlation between orexin A and other parameters

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Orexin A with	r	p-value	Result
5-HT	0.51	0.002	Significant positive correlation
Glutamate	0.47	0.006	Significant Positive correlation
NO	0.45	0.011	Significant positive correlation
GSH	- 0.43	0.023	Significant negative correlation
Albumen	- 0.32	0.037	Significant negative correlation

a. The most important core symptoms of [ASD] are disturbances in stereotyped and social communication , activities and repetitive demeanor . These symptoms can affect children's daily lives to diverse point, inclusive their sleep manner. Social disorders can lead to many difficulties, including understanding social cues and routines for these children, including daily habits related to sleep, in addition to the non-verbal signals that parents expect [26].These children many times exhibit stereotypical and rrepetitive manners that may be inconsistent with their inner physiological cadence, leading to disturbances in the cycle of Wake-up and sleep cycle[27]."The mechanism of the orexin signaling pathway in sleep disturbances in children with autism spectrum disorder is a complex process that includes many aspects, the most important of which are (regulation of the neurotransmitter system, interactions that occur between brain regions, as well as modulation of the response to stress) [28]. Orexin foster the action of arousal-promoting neurotransmitters in the brain, while dampen sleep-promoting neurotransmitters, such as 5-HT, affecting the sleep-wake cycle [28]. In addition, orexin plays an substantial role in neuroprotection by prevent oxidative stress stuteuse and the inflammatory response via its (type I and type II) receptors[22]. Therefore, the current study was designed to shed light on the levels of orexin and some neurotransmitters, in addition to oxidant-antioxidant system indicators. As well as the effect of anxiety, excitement and agitation on the levels of neurotransmitters. It was found a significant in rise in the levels of Orexin A in children with ASD. Children with autism spectrum disorder may have difficulty regulating their emotions, which can lead to increased anxiety and agitation at night, making it difficult for them to calm down and sleep [29]. Studies have found that one of the symptoms of insomnia linked with autism and attention deficit hyperactivity disorder is increased activity of the orexin system [30] . Therefore, it can be concluded that increased orexin levels are a result of insomnia and persistent anxiety in these children, and vice versa. Also,Direct relation among orexin and the domain arousal/regulatory system (sleep-wakefulness and arousal) is previously determined[31]. Many studies have found that one of the basic components of the arousal system is orexin, and it is also considered a major factor in its stability[32,33]. Monoamine neurons in the brainstem are strongly stimulated by orexin neurons[34].This may perform the sensible commentary for the potential function of orexin in neurodevelopmental disorders. The current study also found a significant rise in the level of 5-hydroxytryp. The rise serum serotonin (5-hydroxytryptamine) levels are considered a 1st biomarker being discovered in autism research.In spontaneous physical activity, energy expenditure is enhanced across multiple sites by orexin.One of the important targets shown by recent studies for energy

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expenditure and orexin stimulation of spontaneous physical activity is the dorsal nucleus of the raphe.The dorsal nucleus raphe neurons in the brainstem are a major site of sserotonin/5-hydroxytryptophan producing in the central nervous system[35]. They are essential for regulating behavior, mood, and appetite. In the neurons of the dorsal raphe nucleus, the primary transmitter is serotonin. So appear a probable nominee for the stimulating effects of orexin on SPA and NEAT in the DRN [36]. Glutamate is one of the most excitatory neurotransmitters in the brain, leting irritation of about all synapses [37]. Glutamate is converted to glutamine after entering astrocytes, then transported to neurons to produce gamma-aminobutyric acid and glutamate. Any change affecting one of these transmitters will affect the other [38]. The balance between the neurotransmitter GABA and the neurotransmitter glutamine is crucial for the efficient functioning of the brain[39,40]. Variation in these systems may contribute to the pathophysiology of autism, assuming neural network organization and information processing . This condition has been linked with lower social competence, repetitive sentimental manner , anxiety disturbance, and oxidative stress[40–42]. High levels of synaptic glutamate lead to raise levels of calcium ions within cells, occasion a transient activation cascade [43]. High levels of synaptic glutamate lead to raise levels of calcium ions within cells, occasion a transient activation cascade.Specifically, high levels of intracellular calcium cause toxic effects through alteration of the mitochondrial membrane, disrupting cellular energy production and activating calcium-dependent enzymes such as calpains and neuronal nitric oxide synthase, with subsequent raise in producing of nitric oxid [43,44]. Such may lead to an raise in reactive oxygen species which, together with raise protease and lipase activity, are involved in oxidative stress and cell death by apoptosis [45].

4. CONCLUSION

Based on the results, it was concluded that chiled with autim spectrum disorder have a high levels of orexin A, 5-HT, glutamate and BMI. At the same time, they have disturbed levels of markers of oxidative stress. orexin A, 5-HT, glutamate measurements taken together may even be utilized as markers for development of sleep and eating disorders and insomnia. the rise levels of orexin A in uncontrolled autism spectrum disorder may be a cause of rise BMI . A positive correlation was shown between orexin A and A, 5-HT, glutamate and NO. This assessment may be very helpful in promote an suitable treatment plan for these children, and may form the basis for promote and directing targeted treatment in the future .

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