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RESEARCH ARTICLE

The Oxidative status in Children with Autism receiving Melatonin

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ABSTRACT:

Objectives: To determine the changes in the levels of Malondialdehyde (MDA) and antioxidant enzymes such as superoxide dismutase (SOD) and catalase (CAT) in autistic children receiving melatonin supplementation to evaluate its antioxidant role in autism. **Methods:** A follow-up study was carried out in the Department of therapeutics and clinical pharmacy, Baghdad College of Medical Sciences, Baghdad - Iraq. The study was performed on 55 autistic children who had recruited from several private institutions specialized in autistic children care, Baghdad, Iraq between June 2018 and November 2018. The levels of melatonin, MDA, SOD and CAT were measured in the serum of 55 patients before and after receiving melatonin supplementation for three months. **Results:** The results revealed statistically significant differences in the levels of melatonin, MDA, SOD and CAT between patients before and after receiving melatonin supplement. Furthermore, melatonin levels showed significant positive correlations with both SOD and CAT in addition to a significant positive correlation between SOD and CAT while MDA levels showed significant negative correlations with melatonin, SOD and CAT in autistic patients before and after receiving the supplement. **Conclusions:** Melatonin levels, CAT and SOD activity showed to be improved significantly by melatonin supplementation with a concomitant reduction in the levels of MDA as an indicator of a decrease in an oxidative stress in autistic children.

KEYWORDS: Autism, Melatonin, Malondialdehyde (MDA), Superoxide dismutase (SOD), catalase (CAT), oxidative stress, Reactive oxygen species, lipid peroxidation.

INTRODUCTION:

Autism is a neurodevelopmental disorder with a manifestations appear clearly within the first 36 months of the child's life that is also related closely to many neural growth changes and that occur either pre- or postnatally[1].

Patients suffering from autism specially children showed a wide variety of symptoms ranged from repetitive patterns of behaviour to the more serious manifestation that include the impairment of children's ability to interact normally with the surrounding people as a consequence of language abnormalities beside the poor eye contact that lead to failure in social communication [2].

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Furthermore, children with autism also showed an avoidance behavior that might be caused by abnormalities in sensory input threshold[3]. Autism incidence showed a sex related pattern in which that male formed the majority of autistic patients with a ratio ranged from 5.6:1 to 2:1 against female autistic patients [4]. The difficulties in early diagnosis of autism arise from the fact that the appearance of obvious clinical signs starts after the age of three years, whereas previous researches the age of revealed that the signs actually appear earlier and may be manifested at the age of 6–12 months[5].

Many effectors including neurohormones, neurotransmitters in addition to hormones and immunological mediators may participate effectively in the manifestation of clinical signs beside their crucial role in the severity and pathogenesis of autism. Melatonin considered as one of the neurohormones that play an essential role in the regulation and maintenance of circadian sleep-wake rhythm which is synthesized mainly in the pineal gland from tryptophan[6,7]

Moreover, melatonin regulates many physiological functions with pleiotropic effects on the immune system since it serves as an immunomodulatory compound. It is still unclear how it controls the activity of the immune system[8]. Previous literatures revealed that melatonin has an immune-stimulant effect, while other studies described its anti-inflammatory properties. Carrillo-Vico et al. review prove the concept of the immune-buffer activity of melatonin that behave as a stimulant under basal or immunosuppressive conditions or may serves as an anti-inflammatory mediator in the presence of exacerbated immune responses, such as acute inflammation[6,8].

Recent researches conducted on autistic children obviate that melatonin levels were abnormally decreased as consequence of an assumed reduction in melatonin secretion. Recently, Melatonin acquires great interest in autistic children because of its possible involvement in neurodevelopment in addition to its role in controlling sleep-wake rhythm in these patients[9].

Oxidative stress can be defined as an imbalance between the metabolic reactions producing free radical and antioxidant defences (enzymatic and non-enzymatic) which responsible for the protection against free radicals [10]. This imbalance may be either due to overproduction of free radicals such as superoxide ($O_2^{\bullet}$) and resulting hydrogen peroxide (H_2O_2) or a reduction in the antioxidant capacity. An increase in oxygen supply (hyperoxia) may cause an elevation in free radicals leading to damage which is considered as side effects that is manifested in series of functional changes of many biological reactions. Highly reactive nature of free radicals cause an oxidation and alteration in the normal function of many molecules such as ion pumps, receptors and enzymes and react with nucleic acid causing gene mutations that in turn cause an alteration in proteins structure and production leading to cancer[11,12]. Endogenous protictive maechanism against free radicals include some antioxidant enzymes such as Superoxide Dismutase (SOD) and Catalase (CAT) that account for the first line defence against the injurious effects of $O_2^{\bullet}$ and resulting H_2O_2 by catalyzing the conversion of $O_2^{\bullet}$to H_2O_2 and oxygen[13]. The reaction also can proceed spontaneously, but SOD can increase the rate of intracellular dismutation by thousand times[14]. H_2O_2 produced by SOD are reduced by glutathione peroxidase (GPx) to water or destroyed d by CAT to free oxygen and water[13].

The formation of free radical-mediated tissue damage can compromise several cell elements, causing changes in proteins or nucleic acid damage in a reaction called lipid peroxidation that changes the lipid leading to the formation of malondialdehyde (MDA) that considered as one of the most popular product of this process. So, MDA levels considered as an indicator for the extent of lipid peroxidation and also a marker for oxidative stress [15,16].

Several previous studies were considered melatonin and its metabolites as antioxidant since they have several effects including ROS scavenging effect and radical-associated reactants, stimulation of SOD, GPx and CAT expression in addition to its assumed role in reducing the expression of prooxidants[17].

The purpose of this study was to determine the changes in the level of MDA as an indicator of oxidative damage and antioxidant enzymes such as SOD and CAT which involved in the defense mechanism against free radicals produced in autistic children receiving melatonin supplementation to evaluate its antioxidant role in autism.

MATERIALS AND METHODS:

An experimental study was done for 55 male autistic children who had recruited from several private institutions specialized in autistic children care, Baghdad, Iraq between June 2018 and November 2018. Their ages ranged between 3 and 12 years (mean± SD 6.73±2.71 years) and received 1mg melatonin as a daily supplementation for three months.

The patients met the diagnostic criteria of autism according to the Diagnostic and statistical manual of mental disorders (5th ed.)[18]. All participants had normal results for urine analysis and with the inclusion criteria that include that the patients had no associated neurological diseases (such as cerebral palsy, tuberous sclerosis), no associated metabolic disorders (eg. Phenylketonuria) that may affect the level of serotonin and melatonin in autistic children and all participants were not receiving any other medications.

The local Scientific and Review Board of the Baghdad College of Medical Sciences, Baghdad, Iraq, approved this study. In addition, an informed written consent of participation in the study was signed by the parents or the legal guardians of the investigated subjects according to the Helsinki principles.

Sample collection:

About five milliliters of blood samples were collected from overnight fasting autistic children in plain tubes at 9 a.m. Serum was obtained and kept at −20°C until analysis time for measurement of serum melatonin, MDA CAT and SOD.

BIOCHEMICAL ASSAYS:

Melatonin measurement:

The ELISA assay kit was a product of Cusabio, China. This assay employs the quantitative sandwich enzyme immunoassay technique. Antibody specific for MT has been pre-coated onto a microplate. Standards and samples were pipetted into the wells and any MT present was bound by the immobilized antibody. After removing any unbound substances, a biotin-conjugated antibody specific for MT was added to the wells. After washing, avidin conjugated Horseradish Peroxidase (HRP) was added to the wells. Followed by a wash to remove any unbound avidin-enzyme reagent, a substrate solution was added to the wells and color develops in proportion to the amount of MT bound in the initial step. The color development was stopped and the intensity of the color was measured spectrophotometrically at 450 nm. . The concentration of melatonin in the serum was then determined by comparing the samples optical density to the standard curve.

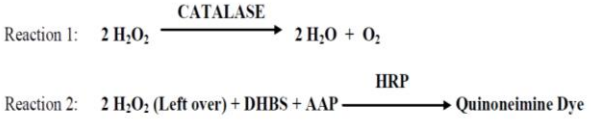
The quantitative measurement of Malondialdehyde levels (MDA):

This measurement performed by Competitive-ELISA technique using kit provided by AMSBIO, UK. The microtiter plate provided in this kit has been pre-coated with MDA. During the reaction, MDA in the sample or standard competes with a fixed amount of MDA on the solid phase supporter for sites on the Biotinylated Detection Antibody specific to MDA. Excess conjugate and unbound sample or standard are washed from the plate, and HRP-Streptavidin (SABC) is added to each microplate well and incubated. Then a TMB substrate solution is added to each well. The enzyme-substrate reaction is terminated by the addition of a sulfuric acid solution and the color change is measured spectrophotometrically at a wavelength of 450 nm. The concentration of MDA in the samples was determined by comparing the O.D. of the samples to the standard curve in the corresponding microtitere plate. The concentration of MDA in each serum sample was expressed in ng/ml for comparison among all studied groups.

Catalase (CAT) Activity Assay:

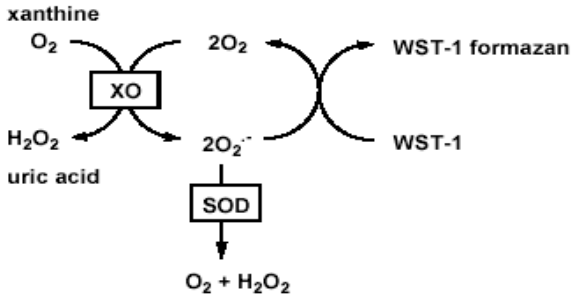
The kit provided by CELL BIOLABS, USA for the assay of Catalase Activity involves two reactions. The first reaction is the catalase induced decomposition of hydrogen peroxide H_2O_2 into water and oxygen. The rate of disintegration of hydrogen peroxide into water and oxygen is proportional to the concentration of catalase (See Reaction 1). A catalase-containing sample can be incubated in a known amount of hydrogen peroxide. The reaction proceeds for exactly one minute, at which time the catalase is quenched with sodium azide.

The remaining hydrogen peroxide in the reaction mixture facilitates the coupling reaction of DHBS and AAP in conjunction with an HRP catalyst (See Reaction 2). The quinoneimine dye coupling product is measured at 520nm, which correlates to the amount of hydrogen peroxide remaining in the reaction mixture.



Assay of Superoxide Dismutase (SOD) Activity:

The sensitive SOD assay kit provided by Mybiosource, USA utilizes WST-1 that produces a water-soluble formazan dye upon reduction with superoxide anion. The rate of the reduction with a superoxide anion is linearly related to the xanthine oxidase (XO) activity, and is inhibited by SOD (below). Therefore, the inhibition activity of SOD can be determined by a colorimetric method.



Statistical analysis:

Results were expressed as mean ± standard deviation (SD) and all statistical comparisons were made by means of independent t-test and the correlation was done between all parameters using Pearson correlation test [19]. Values were considered to be significant when **P<0.01, *P<0.05. All statistical analysis was carried out using Statistical Package for the Social Sciences (SPSS) software 20.

RESULT:

The melatonin and MDA levels in addition to CAT and SOD activities were measured in patients with varying levels of autism severity before and after receiving a melatonin supplementation for three months and the results obtained in table 1 revealed a significant increase ($p<0.05$) in the level of serum melatonin with a concomitant increase in the activity of CAT and SOD enzymes. On the other hand the level of MDA showed a significant reduction after three months of melatonin supplementation.

The results illustrated in table 2 reveled that there were significant correlations among the studied parameters that obviously demonstrated from the significantly positive correlations between melatonin and both CAT and SOD activities in addition to the positive significant correlation between SOD and CAT activities in autistic children before and after receiving the melatonin supplementation. On the other hand, MDA level showed significant negative correlation with the level of serum melatonin in addition to those with the activities of SOD and CAT enzymes in both pre- and post-supplementation period.

Table 1: Levels of melatonin MDA, CAT and SOD in autistic children befor and after receiving melatonin supplement.

Parameters	Before treatment	After treatment	P- value
Melatonin	24.23±7.19	28.24±6.51	0.002**
MDA	5.46±2.67	3.08±1.46	<0.001**
CAT	60.31±9.28	69.52±9.76	<0.001**
SOD	144.23±28.96	159.38±27.79	0.004**

**P<0.01, *P<0.05

Table 2: the Correlations among all studied groups in autistic children before and after receiving melatonin supplement.

		Before Treatment			After Treatment		
		MDA	**CAT**	**SOD**	**MDA**	**CAT**	**SOD**
Melatonin	**r**	-0.353**	0.326*	0.363**	-0.303*	0.367**	0.277*
	p	0.006	0.011	0.004	0.019	0.004	0.032
MDA	**r**		-0.314*	-0.413**		-0.260*	-0.383**
	p		0.015	0.001		0.045	0.003
CAT	**r**			0.443**			0.286*
	p			0.000			0.027

**P<0.01, *P<0.05

DISCUSSION:

Many previous studies revealed that autistic children showed a high oxidative stress level as a consequence of low defense mechanisms against free radicals which also reported that they participate in the pathogenesis of autism[20,21]. In the current study, the goal was to examine the possible effect of melatonin administration that used widely to correct sleep problems in affected patients on the oxidative stress through its assumed anti-oxidant activity[22,23].

The results obtained in this study confirm the concept of anti-oxidant role of melatonin in the children suffer from autism that revealed by the significant elevation in the serum melatonin level after an administration of daily 1 mg supplement for three months that accompanied by elevation in the anti-oxidant defense mechanism enzymes activities that have a crucial impact on the oxidative status that represented by the reduction in the Malondialdehyde (MDA) level. Several explanations were reported previously for the role of melatonin in reducing oxidative stress by either direct or indirect manner that contribute to the scavenging activity of melatonin and/or its effect on other anti-oxidant endogenous enzymes such as CAT and SOD[17].

In children subjected to this study, there were an elevation in the level of MDA with a concomitant decrease in the levels of CAT and SOD enzymes before receiving the supplement that my explain the high oxidative stress which is also accompanied by low melatonin level which is in agreement with many previous researches [6,9,24]. So, the possible explanation is that the defect in melatonin synthesis in autistic children may participate in reducing the activity of CAT and SOD that lead to increase the MDA levels.

After melatonin administration, Melatonin level in serum become improved significantly which is in compatible with many studies that illustrate the enhancement of sleep pattern in autistic children as an indicator of improved melatonin level in the sera of autistic patients after receiving the supplementation as reviewed by Sanchez-Barcelo and his colleagues [23]. The significant increase in melatonin level thought to have a role in CAT and SOD enzymes expression as stated by various studies which may contribute to its indirect anti-oxidant activity beside its direct scavenging anti-oxidant effect that lead to reduce MDA levels[25-28].

Furthermore, the significant correlation obtained between melatonin level and oxidative stress indicators before and after receiving the supplementation prove the possible contribution of melatonin in the prevention of oxidative damage that may participate in the pathogenesis of autism in addition to its neuro-modulatory effect that originate from melatonin capability to cross blood brain barrier[6].

Moreover, the results obtained elucidate that there were positive significant correlation among melatonin, CAT and SOD in autistic patients before and after receiving melatonin while significant negative correlations were obtained between MDA on one hand and melatonin level, CAT and SOD activity on the other hand. These findings may indicate that the gradual increment in the serum melatonin may lead to increase the expression of defense mechanism enzymes that result in a reduction in the MDA level as a marker for oxidative status which is consistent with many previous reviews[25].

Additionally, melatonin thought to have a protective role against free radical formation by reducing the expression of pro-oxidants and its metabolites N(1) -acetyl-N(2) -formyl-5-methoxykynuramine (AFMK) and N(1)-acetyl-5-methoxykynuramine (AMK) have free radical scavenging ability. So, melatonin and its metabolites can serve as an efficient team of scavengers that deactivate a wide range of reactive oxygen species, under different conditions. So, the results of current study support the previous findings that melatonin exert a continuous anti-oxidant protection in cascade manner to scavenge reactive oxygen species [29,30].

CONCLUSION:

The levels of melatonin and MDA in addition to CAT and SOD activity showed to be affected significantly by melatonin supplementation that lead finally to reduce the reactive oxygen species effect among autistic children. This study also revealed that there were significant correlations between studied parameters that can be used in future work as a powerful tool for emerging a new treatment regime that reduce the pathogenesis of autism and improve patients' mental health in addition to reduce the sleep disorder and other miscellaneous manifestations.

CONFLICT OF INTEREST:

The authors have no conflicts of interest regarding this investigation**.**

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AUTHORS CONTRIBUTIONS:

Mohammed B. Mohammed was responsible for the collection of samples from patients before and after the administration. Yasir SJ. Alrubaye was responsible for the biochemical work in addition to his role in the follow-up of patients during the period of drug administration.

REFERENCES:

1. Abdulamir HA, Abdul-Rasheed OF, Abdulghani EA. Serotonin and serotonin transporter levels in autistic children. Saudi Med J 2018;39:487-94.
2. Abdulamir HA, Al-Awadi SJA, Abdul-Rasheed OF. The correlation of single nucleotide polymorphism in oxytocin and oxytocin receptor genes and the serum oxytocin level in the Iraqi children with autism. Int J Pharm Bio Sci 2016; 7: (B) 668 - 75
3. Yadav S. Autism Spectrum Disorder- A Review. Int. J. Nur. Edu. and Research. 2016; 4(2): 223-226.
4. Hasan AA. A Descriptive Study among Children suffering from Autism in Baghdad, Iraq. Research J. Pharm. and Tech. 2017; 10(6): 1919-1923.
5. Dhariyal RS, Kimothi V, Singh S. A Review on Autism. Res. J. Pharmacology and Pharmacodynamics.2019; 11(2):76-80.
6. Abdulamir HA, Abdul-Rasheed OF, Abdulghani EA. Low oxytocin and melatonin levels and their possible role in the diagnosis and prognosis in Iraqi autistic children. Saudi Med J 2016; 37: 29-36.
7. Son CM, Min BI, Moon HW. The Effects of Combined Exercise on Blood Lipids and Melatonin in Developmental Disorder Students. Research J. Pharm. and Tech 2018; 11(12): 5543-5647.
8. Carrillo-Vico A, Lardone PJ, Alvarez-Sanchez N, Rodriguez-Rodriguez A, Guerrero JM. Melatonin: buffering the immune system. Int J Mol Sci 2013; 14: 8638-83.
9. Tordjman S, Anderson GM, Cohen D, Kermarrec S, Carlier M, Touitou Y, et al. Presence of autism, hyperserotonemia, and severe expressive language impairment in Williams-Beuren syndrome. Mol Autism 2013; 4: 29.
10. Saha D, Tamrakar A. Xenobiotics, Oxidative Stress, Free Radicals Vs. Antioxidants: Dance Of Death to Heaven's Life. Asian J. Res. Pharm. Sci. 2011; 1(2)36-38.
11. Vaishali M. Antioxidants in Health and Diseases. Research J. Pharm. and Tech. 2014; 7(4)489-493.
12. Patlevič P, Vašková J, Švorc P, Vaško L, Švorc P. Reactive oxygen species and antioxidant defense in human gastrointestinal diseases. Integr Med Res 2016;5:250-8.
13. Vadivelan R, Dhanabal SP, Raja Rajeswari, Shanish A, Elango K, Suresh B. Oxidative Stress in Diabetes- A Key Therapeutic Agent. Research J. Pharmacology and Pharmacodynamics. 2010; 2(3): 221-227.
14. Sheng Y, Abreu IA, Cabelli DE, et al. Superoxide Dismutases and Superoxide Reductases. Chemical Reviews 2014;114:3854-918.
15. Suresh PK, Sah AK, Daharwal SJ. Role of free radicals in ocular diseases: An overview. Research J. Pharm. and Tech. 2014;7(11)1330-1344.
16. Chakraborty P, Kumar S, Dutta D, Gupta V. Role of Antioxidants in Common Health Diseases. Research J. Pharm. and Tech. 2009;2(2) 238-244.
17. Mahurkar N, Hasan SMSU, Malpani AA, Chetan M. Influence of Single and Multiple Dose Regimen of Melatonin, Rabeprazole and their Combination in Gastroprotection in Pylorus Ligated Albino Rats. Research J. Pharm. and Tech. 2013; 6(5)562-568.
18. American Psychiatric Association. Diagnostic and Statistical Manual of Mental Disorders. 5th ed. Washington (DC): American Psychiatric Publishing; 2013.
19. Norman G. Likert scales, levels of measurement and the "laws" of statistics. Adv Health Sci Educ Theory Pract 2010; 15: 625-32.
20. Hernández DQ, Cruz AL, González SB, Vila DT, Robert YC, Forment GR, Tamargo barbeito TO. Variation of the REDOX Status in Patients with Primary Autism after Antioxidant Therapy. J Neurol Transl Neurosci 2017:5: 1085.
21. Altun H, Şahin N, Kurutaş EB, Karaaslan U, Sevgen FH, Fındıklı E. Assessment of malondialdehyde levels, superoxide dismutase, and catalase activity in children with autism spectrum disorders, Psychiatry and Clinical Psychopharmacology 2018; DOI: 10.1080/24750573.2018.1470360
22. Cortesi F, Giannotti F, Ivanenko A, Johnson K. Sleep in children with autistic spectrum disorder. Sleep Med 2010;11:659-64.
23. Sánchez-Barceló EJ, Mediavilla MD, Reiter RJ. Clinical uses of melatonin in pediatrics. Int J Pediatr 2011;2011:892624.
24. Ruggeri B, Sarkans U, Schumann G, Persico AM. Biomarkers in autism spectrum disorder: the old and the new. Psychopharmacology (Berl) 2014; 231: 1201-16.
25. Rodriguez C, Mayo JC, Sainz RM, Antolin I, Herrera F, Martin V, Reiter RJ. Regulation of antioxidant enzymes: a significant role for melatonin. J. Pineal. Res 2004; 36: 1-9.
26. Reiter RJ Tan D.X, Osuna C, Gitto E. Actions of melatonin in the reduction of oxidative stress. A review. J. Biomed. Sci 2000;7: 444-58.
27. Tan DX., Chen L, D, Poeggeler B, Manchester LC, Reiter RJ. Melatonin: a potent, endogenous hydroxyl radical scavenger. Endocrine J 1993; 1:57-60.
28. Galano A, Tan DX, Reiter RJ. On the free radical scavenging activities of melatonin's metabolites, AFMK and AMK. J. Pineal. Res 2013; 54: 245-57.
29. Büyükavci M, Ozdemir O, Buck S, Stout M, Ravindranath Y, Savaşan S. Melatonin cytotoxicity in human leukemia cells: relation with its pro-oxidant effect. Fundam Clin Pharmacol 2006 Feb;20:73-9.
30. Galano A, Tan DX, Reiter RJ. On the free radicals scavenging activities of melatonin's metabolites, AFMK and AMK. JPineal Res 2013; 54:245-57