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Evaluation of the levels of some neurotransmitters in patients with thalassemia major

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ABSTRACT

This study was conducted to examine the variables related to the neurotransmitters and the effects of thalassemia disease due to the appearance of numbness and weakness in the extremities, especially the lower limbs. Around 60 blood samples were collected from patients with thalassemia major and 30 samples from healthy people and separated in a centrifuge to obtain the serum which was used to conduct biochemical tests. The results indicated a highly significant increase in the enzyme monoamine oxidase, serotonin N-acetyltransferase and galanin, which reached (1.160 ± 12.45) , (1.03 ± 12.40) and (37.8 ± 878.4) respectively compared to the healthy ones, where it reached (0.913 ± 7.45) and (1.52 ± 7.73) and (35.2 ± 653.0) . As for the value of glutamate carboxypeptidase 2 and dopamine, there was a significant decrease in the amount of (0.699 ± 2.470) and (1.09 ± 1.93) respectively compared to the healthy ones, which amounted to (1.18 ± 5.270) and (3.18 ± 7.24) . Neurological changes due to chronic iron accumulation, anemia, and iron chelation therapy.

1. Introduction

Thalassemia is also called Mediterranean anemia, (Aksu and Unal., 2021). It is a genetic disease resulting from a defect in the production of globin chains which is responsible of the formation of the hemoglobin molecule that leads to a reduction in the construction of one of the globin chains, formation of an abnormal hemoglobin molecule, and production of hemoglobin. Incomplete red blood cells do not have the ability to transport oxygen (Greer and Wintrobe, 2008; Runda and Rachmilewitz, 2005). Acute anemia, iron chelation therapy and chronic iron accumulation are the three main factors that are responsible for neurological changes in beta-thalassemia patients (Hesham *et al.*, 2022). Monoamine oxidase is a family of enzymes that convert biological and food amines, monoamine hormones, and neurotransmitters such as serotonin, dopamine, norepinephrine and epinephrine by removing the amine group and converting it to an aldehyde or ketone. (Bortolotto and Shih, 2011 ; Ramsay and Albrecht, 2018 ; Tipton, 2018). It also inhibits monoamine neurotransmitters due to this reason, it is involved in many psychiatric and neurological diseases that can be treated by MAOIs inhibitors that block the action of (MAOs), (Yeung *et al.*, 2019). There are two types of MAO: MAO-A and MAO-B. MAO-B is both found in neurons and outside the nervous system MAO-A is found in the liver and placenta and MAO-B is mostly found in platelets (Manzoor and Hoda, 2020), serotonin-N-acetyltransferase, also called arylalkylamine N-

acetyltransferase (AANAT) It is the responsible enzyme of producing melatonin day and night by converting serotonin, (Cazamea *et al.*, 2013). At night, melatonin increases, while its production decreases during day time (Kvetnoy *et al.*, 2022). Melatonin is synthesized by converting serotonin by AANAT enzyme to N-acetyl-serotonin (NAS) and then NAS is converted by ASMT enzyme to produce melatonin and these enzymes are under the control of endocrine glands that regulate the amount and time of melatonin (Amaral and Cipolla, 2018), Glutamate carboxypeptidase 2, also called N-acetyl-L-aspartyl-L-glutamate peptidase 1 (NAALADase 1) and prostate-specific membrane antigen (PSMA), is recognized as a target for therapeutic interventions in a variety of neurological disorders (Barink *et al.*, 2012). It is an enzyme found in membranes and also found outside the cell. It is a glycoprotein that catalyzes the breakdown of (NAAG) N-acetyl-aspartyl-glutamate to glutamate and (NAA) N-acetyl-aspartate. The GCPII enzyme is receiving increasing interest in the field of neurology, where increased expression of the enzyme is associated with impaired cognitive functions in humans. People with a gain-of-function mutation in the FOLH1 gene express higher levels of the enzyme, which leads to lower NAAG in the brain and lower IQ (Zink *et al.*, 2020). And NAAG is one of the most prevalent neurotransmitters in the central nervous system (Neale *et al.*, 2011). Galanin is a neuropeptide encoded by the GAL gene (Fonseca *et al.*, 2022) and is widely expressed in the brain, spinal cord and

intestine of humans as well as other mammals. Galanin signaling occurs through three protein-bound receptors (Mitsukawa *et al.*, 2008). G is also involved in inhibiting and modulating action potentials in neurons and plays an important role in many functions, including pain sensation (Xu *et al.*, 2008), awakening, cognition, mood regulation and nutrition (Barson *et al.*, 2010) and blood pressure regulation (Mechenthaler, 2008) and is associated with many diseases including Alzheimer's, epilepsy, depression, as well as eating disorders (Lundström *et al.*, Berger *et al.*, 2005.). Dopamine is a chemical that sends messages within the midbrain into the substantia nigra. It is a substance similar to the hormone dopamine that is regulated into a group of catecholamine amines (Sherwood *et al.*, 2005) and is broken down by the enzyme Catechol-O-methyltransferase, which is found in extracellular fluid as well as in Nerve endings are also destroyed by endogenous enzymes (MAO) monoamine oxidase (López *et al.*, 2015). The nerve impulse is transmitted between nerve cells in the brain, which number about 100 billion cells through a chemical carrier, and the chemical transmission is through the brain By moving from one cell to another after being secreted into one brain cell (Schultz, 2008). Dopamine transmits information related to feelings in cases of pleasure or recovery, in the case of feeling happy to eat delicious foods or when obtaining something that brings happiness, even if in a simple way, through dopamine. In addition to activating memory, dopamine production in brain cells under normal conditions and at constant rates (Sherwood *et al.*, 2005) dopamine transmits chemical signals between neurons that control movement and regulate feelings and mood (Solomon *et al.*, 2005).

2. Materials and Methods

This study was conducted in Al-Karama Teaching Hospital in Baghdad Governorate from December to March 2022. The study included 60 samples of

patients with beta-thalassemia major and 30 healthy samples whose ages ranged from (35 - 5) years. All the information about each person was recorded according to the research form, which included information such as name, gender, age and height for each patient, and the variables were measured using the following kits:

MAO-B (Amine Oxidase [Flavin-containing B) ELISA Kit

AANAT (Serotonin N-acetyl transferase) ELISA Kit

PSMA/FOLH1 (Glutamate carboxy peptidase2) ELISA Kit

GAL (Galanin peptides) ELISA Kit

DA (Dopamine) ELISA Kit

These kits are from the Chinese company (Fine Test), where the method of interaction between antibodies and antigens was adopted. The principles of kits were based on sandwich enzyme-linked immune-sorbent assay technology. The capture antibody was pre-coated onto 96- well plates, and the biotin conjugated antibody was used as a detection antibodies. The standards, test samples and biotin conjugated detection antibody were added to the wells subsequently, and washed with wash buffer. HRP-Streptavidin was added and unbound conjugates were washed away with wash buffer. TMB substrates were used to visualize HRP enzymatic reaction. TMB was catalyzed by HRP to produce a blue color product that changed into yellow after adding acidic stop solution. The density of yellow is proportional to the target amount of sample captured in the plate. Read the O.D. absorbance at 450nm in a micro plate reader, and then the concentration of target can be calculated.

3. Results and Discussion

Table (1) shows the Mean $\pm$ SD of enzymes (MAO-B, Serotonin-N-acetyl transferase and Glutamate carboxy peptidase) in patients and healthy subjects, the number of groups, and the p-Value.

Table 1: The enzymes level in Patients and healthy subjects

Parameters	people	number	Means $\pm$ SD	P- Value
MAO-B ng/ml	Patients	60	12.45 $\pm$ 1.160	0.0007
	healthy	30	7.45 $\pm$ 0.913	
Serotonin-N-acetyl transferase ng/ml	Patients	60	12.40 $\pm$ 1.03	0.0006
	healthy	30	7.73 $\pm$ 1.52	
Glutamate carboxy peptidase II ng/ml	Patients	60	2.470 $\pm$ 0.699	0.0007
	healthy	30	5.270 $\pm$ 1.18	

There was a significant increase in the level of monoamine oxidase (B) as its concentration in patients reached 12.45 $\pm$ 1.160 ng/ml compared to the control groups where its concentration reached 7.45 $\pm$ 0.913 ng/ml. Iron accumulation in patients with thalassemia leads to an increase in the concentration of monoamine oxidase B, and an increase in its level in the blood (Youdim *et al.*, 1990). There was also a significant increase in the level of serotonin N-acetyltransferase enzyme in patients, where its

concentration reached 12.40 $\pm$ 1.03 ng/ml compared to the control, where its concentration reached 7.73 $\pm$ 1.52 ng/ml. Numbness and weakness in the lower extremities in thalassemia patients may be due to increased activity of the enzyme Serotonin N-acetyltransferase, which leads to the conversion of the neurotransmitter serotonin to melatonin resulting in a decrease in serotonin and nerve impairment (Sawaya *et al.*, 2006). It was also observed that there was a significant decrease in the enzyme glutamate

carboxypeptidase 2, where its concentration in patients was 2.470 ± 0.669 ng/mL compared to the control groups, where its concentration reached 5.270 ± 1.18 ng/mL. The level of folate in the blood (Castald *et al.*, 1983), which in turn affects the decrease in the level of glutamate carboxypeptidase 2 (Sherwood and Melton, 2013). Furthermore, the results of table (2) show the Mean $\pm$ SD of enzymes (Galanin, and Dopamine) in patients and healthy subjects, the number of groups, and the p-Value.

Table 2: The neurotransmitters (Galanin and Dopamine) level in Patients and healthy subjects

Variable		Number	Means $\pm$ SD	P- Value
Galanin pg/ml	Patients	60	878.4 ± 37.8	0.0005
	healthy	30	653.0 ± 35.2	
Dopamine ng/ml	Patients	60	1.93 ± 1.09	0.0004
	healthy	30	7.24 ± 3.18	

The results of Table 2 indicated that there was a significant difference in the level of galanin compared to the healthy group, where its concentration in the patients was 878.4 ± 37.8 pg/ml and 653.0 ± 35.3 pg/ml in the healthy group. The increase in the concentration of calnin causes the blocking of nerve conduction, so symptoms of numbness and nerve weakness occur (Sawaya *etal.* 2006). Finally, a significant decrease in dopamine was observed as its concentration was 1.93 ± 1.09 ng/ml compared to the healthy control groups where the concentration was 7.24 ± 3.18 ng/ml. A low level of dopamine occurs due to disorders in the liver or kidneys due to the presence of disease, which leads to a low level of dopamine in the blood (Sturgill *etal.*,

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2011). Or the low level of dopamine occurred due to the patients who use the drug Desferal, which causes a drop in dopamine levels (Jiang *et al.*, 2006).

In this study, the correlation coefficient was also found between the studied variables, where r values were found to determine the strength of the correlation between the variables for healthy patients, as shown in Tables 3 and 4.

Table 3: The correlation coefficient of the variables for patients group

	DA	PSMA	MAO-B	AANAT
PSMA	-0.071			
MAO-B	0.189	-0.013		
AANAT	0.043	-0.362	0.127	
GLA	0.017	-0.033	0.102	0.036

Table 4: The correlation coefficient of the variables for healthy group

	DA	PSMA	MAO-B	AANAT
PSMA	-0.132			
MAO-B	0.022	0.151		
AANAT	0.054	-0.284	-0.296	
GLA	-0.092	-0.262	0.126	-0.160

Conclusions

The accumulation of iron led to an increase in the enzyme monoamine oxidase, and a decrease in the concentration of folic acid may be the reason that leads to a decrease in the concentration of glutamate carboxypeptidase 2 and the appearance of numbness and weakness in the lower extremities, also an increase in the level of serotonin N-acetyl transferase and an increase in galanin may be the causes Finally, desferal drug may be the cause of elevated dopamine levels.

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