Assessment of Creatine Levels in Patients with Vitamin B6-Responsive Gyrate Atrophy Using Magnetic Resonance Spectroscopy after Vitamin B6 Administration

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Abstract

Background: Gyrate atrophy of the choroid and retina (GACR) is an autosomal recessive metabolic disorder caused by mutations in the ornithine aminotransferase (OAT) gene.
Objectives: The aim was to assess the impact of vitamin B6 administration on the creatine amino acid level in the central nervous system (CNS) in patients with GACR.
Patients and methods: This observational study included 10 patients with GACR aged 12 to 18 years old. All patients with B6-responsive GACR and evidence of creatine deficiency were included, regardless of outcome. Exclusion criteria involved non-responsive GACR, renal or hepatic impairment, or creatine supplementation. Patients were selected from Tanta and Sohag University hospitals between 2022 and 2023, and data were gathered through a retrospective review of medical records, including clinical details and investigations such as DBS amino acid profiles and creatine phosphokinase (CPK) assays before and after vitamin B6 administration at doses of 515 ± 62.58 mg/day (400–600 mg/day).
Results: Following vitamin B6 treatment, both ornithine and CPK levels significantly decreased (43.4%-53.9%, and 67.9%-70.1%, respectively). Additionally, citrulline and arginine levels significantly increased. When compared to pre-treatment values, the post-treatment creatine peak increased dramatically in the basal ganglia, frontal, and occipital regions of the cerebral cortex, respectively.
Conclusions: Vitamin B6 is a crucial component of the gyrate atrophy treatment regimen. It was effective in restoring the creatine level in the CNS and lowering ornithine levels. The MRS is a reliable tool for monitoring of patients with gyrate atrophy.

Keywords: Gyrate atrophy; Creatine deficiency; MRS; Ornithine.
DOI: 10.21608/SVUIJM.2025.340477.2036
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Received: 7 December ,2024.
Revised: 7 January,2025.
Accepted: 10 January,2025.
Published: 10 January, 2025
Cite this article as: Amira Mobarak, Ahmed Elsharkawy, Rofaida M. Magdy, Mohamed M. Hassan, Alaa Mohamed Reda.(2024). Assessment of Creatine Levels in Patients with Vitamin B6-Responsive Gyrate Atrophy Using Magnetic Resonance Spectroscopy after Vitamin B6 Administration**.** *SVU-International Journal of Medical Sciences*. Vol.7, Issue 2, pp: 1046-1058.

Introduction

Gyrate atrophy of choroid and retina (GACR) (MIM # 258870) is an autosomal recessive metabolic condition that occurs due to mutations in the ornithine aminotransferase (OAT) gene (MIM # 613349) **(Kim et al., 2013)**. This gene encodes the mitochondrial OAT–enzyme, which is crucial for the metabolism of ornithine, (**Fig.1**), (**Montioli et al., 2021)**.

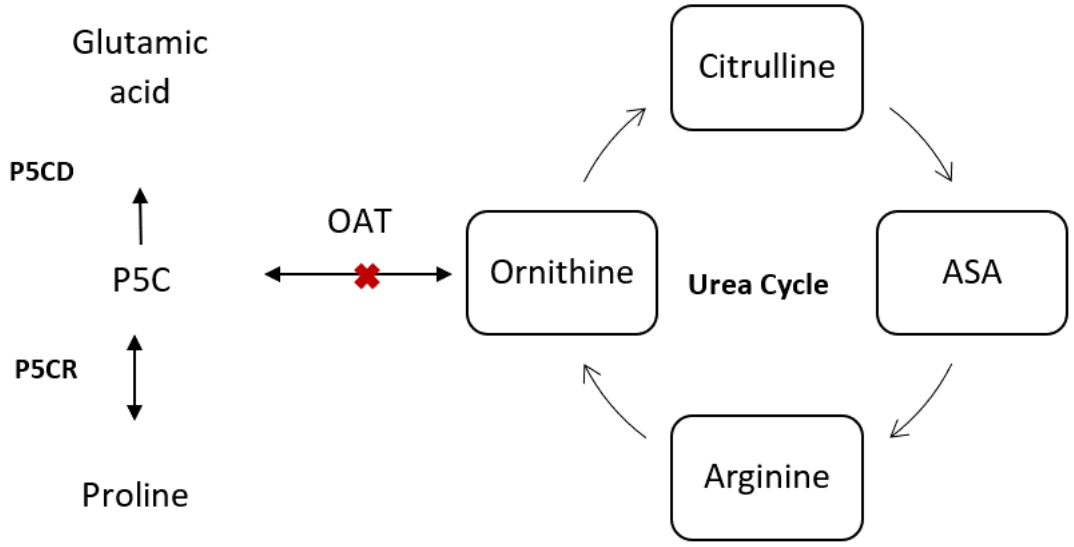


Fig.1. Ornithine amino acid metabolism

AGAT, Arginine: Glycine amidinotransferease; ASA, Argininosuccinic acid; GAA, Gaunidinoacetate OAT, Ornithine aminotransferase; P5C, Pyrroline-5-carboxylate; P5CD, P5C dehydrogenase; P5CR, P5C reductase. The *X* marks the enzyme defect in GACR.

OAT is a PLP-dependent transferase that catalyzes the metabolism of ornithine, which is an intermediate metabolite that links the urea cycle, proline metabolism, and creatine synthesis. Ornithine is a by-product of the rate-limiting step in the biosynthesis of creatine that is catalyzed by Arginine: Glycine amidinotransferease; hence it exerts a negative feedback inhibition on this biochemical reaction **(Singer et al., 2016).** Hence, deficiency in the OAT enzyme leads to high levels of ornithine in blood and other body fluids (**Pampalone et al., 2024)**.

High ornithine inhibits arginine: glycine amidinotransferase (AGAT), the rate-limiting enzyme in the creatine synthesis pathway, which causes secondary creatine deficiency in GACR (**Pintilie et al., 2021)**.

This deficiency can be detected by low creatine peaks in magnetic brain resonance spectroscopy studies **(Clark and Cecil, 2015; Ardon et al., 2016)**.

GACR patients mainly present with ocular symptoms like myopia, decreased visual acuity, retinal detachment, and cataract; however, other symptoms like muscle weakness and fatigue, CNS affection, and peripheral neuropathy were reported **(Balfoort et al., 2021)**.

PLP ts as a cofactor for the OAT enzyme and helps it exert its function (**Ginguay et al., 2017)**. Based on that, GACR patients are managed by vitamin B6 supplementation and an arginine-restricted diet. Depending on whether the level of ornithine decreases in response to vitamin B6 administration, GACR patients are stratified into B6-responsive or non-responsive. In contrast to non-responsive patients, who may carry more damaging mutations that impact a different region of the enzyme, pyridoxine-responsive individuals may have mutations in the binding site that significantly diminish coenzyme affinity **(Molaei Ramshe et al., 2024).**

We aimed to examine the impact of vitamin B6 administration on the amino acid profile, CNS creatine level, and CPK enzyme level as biochemical evidence of possible muscle tissue affection.

Patients and Methods

This observational study included 10 patients diagnosed with GACR through elevated ornithine in the DBS amino acid profile or genetic sequencing of the OAT gene showing biallelic mutations. The patients were gathered from Tanta and Sohag University hospitals in the duration from 2022 to 2023. The neuroimaging data interpretation was done at the Radiodiagnosis Department.

This study was approved by the research ethics committee, Faculty of Medicine, Tanta University with REB application number 36264PR216/6/23. The study was conducted per the declaration of Helsinki. Patients and/or caregivers provided written informed consent for participation in the research and publication.

The inclusion criteria were any pediatric patients diagnosed with vitamin B6-responsive at the age of 1-18 years with consistent vitamin B6 intake for at least one year and a brain MRS performed before starting B6 with evidence of decreased creatine level.

Exclusion criteria were patients with non-responsive gyrate atrophy to vitamin B6 treatment, patients with renal or hepatic impairment, or those receiving creatine supplementation **(Balfoort et al., 2021)**.

To avoid selection bias, we included all patients with B6-responsive GACR and evidence of creatine deficiency regardless of the outcome **(Guan et al., 2021)**. We also repeated the brain MRS within the current study. The following data were collected retrospectively through a review of the medical records:

Clinical data: Age at diagnosis, gender, ocular symptoms at presentation, neuropsychiatric symptoms, muscle symptoms, vitamin B6 dose, route of administration, and duration of treatment.

Investigations: The following investigations were reviewed at diagnosis and after at least 1 year of uninterrupted vitamin B6 treatment:

Dried blood spot (DBS) amino acid profile (to assess ornithine, citrulline, and arginine levels). The blood samples were collected in the early morning and put on DBS paper for examination after a four-hour fast. It was analyzed using liquid chromatography-tandem mass spectrometry (LC–MS).

CPK blood levels were collected in a heparinized tube. Human Creatine Phosphokinase (CPK) ELISA Kit was used. The detection range of this kit is 6.25U/L-200U/L. Standard Concentration Gradients (S6 to S1): 200,100,50,25,12.5,6.25U/L. Both Intra-assay CV (%) and Inter-assay CV (%) are less than 15%.

For the evaluation of chronic fatigue, we used the Fatigue Assessment Scale For behavioral symptoms; we used a self-reporting questionnaire and the Patient Health Questionnaire-9 (PHQ-9).

After the diagnosis was confirmed, all patients were placed on oral vitamin B6 supplements (pyridoxine hydrochloride) at doses of 515 ± 62.58 mg/day (400 – 600 mg/day). Only one patient chose IM injections due to gastritis. The dosing regimen was established based on pharmacological B6 doses documented in the literature. The patient's ornithine levels were closely monitored, and dose modifications were made accordingly.

Patients are divided into pyridoxine-responsive and non-responsive groups depending on their biochemical responses to vitamin B6 supplementation. Ornithine level is anticipated to drop in the responsive group.

Radiological assessment

After reviewing the pre-treatment brain MRI and MRS results, patients who met the inclusion criteria received requests for

post-treatment brain MRI and MRS. All patients had their metal pins removed and entered the machine headfirst while supine. Two neuroradiologists with 10 years of neuroradiology expertise simultaneously examined imaging data at the Radiodiagnosis Department, Tanta University, Egypt.

MRI and MRS were done using a standard 16-channels head coil at 1.5 T (GE Signa Explorer, closed magnet, United States). Axial and sagittal T1-weighted images (TR: 560, TE: 15 msec) and axial and coronal T2-weighted images (TR: 4530, TE: 100 msec) were obtained. Single-voxel proton MRS was performed by using the point-resolved spectroscopy sequence (TR: 1500, TE: 31 ms). Voxels (2 x 2 x 2 cm) were placed, including the frontal and parieto-occipital subcortical white and cortical gray matter. After automatic shimming and gradient tuning, water suppression with a water-selective excitation pulse was interactively optimized on the display console. Analysis of the spectra was performed with the manufacturer-supplied spectroscopy software package of the magnetic resonance system on workstation advantage window 4.7, GE Medical Systems. The spectra were referenced to creatine (3.02 ppm).

The MRI protocol was as follows: the field of view was 220-240 mm, the matrix was 256 x 256, and the slice thickness was 4 mm. Axial T1WI (TR/TE = 300-600/10-30 m/sec), axial FLAIR pictures (TR/TE/Inversion time (TI) = 6000/140/1400), axial T2WI images (TR/TE = 700-2000/80-100 m/sec). Axial 3D diffusion tensor imaging was obtained using a single-shot spin echo EPI (TR/TE = 7400/60 ms, 56 slices), and MRS protocol was acquired with sequential short (35 milliseconds) and long (144 milliseconds) echo acquisitions using a repetition time of 2000 milliseconds. Shimming was performed, multi-voxels MR spectroscopy localization was done, and the patient was positioned within both frontal and occipital regions and both basal ganglia.

Post-processing was performed using MRI workstation software (ADW 4.7 Vantage, GE Medical Systems), and quantitative metabolite ratios to creatine signal were determined for each spectrum. Low creatine was detected by long echo spectrum, where lower height indicated a drop in creatine level, and higher NAA /creatine and choline/creatine ratios were recorded at both long and short echo times.

Statistical analysis

Data were collected in Microsoft Excel for Windows. Microsoft Excel for Windows and JASP 0.14.1.0 were used for graphs and statistical analysis. Kolmogorov–Smirnov test was used for normality of data. Data are presented as mean ± standard deviation (SD), percentage, and range. One-sample *t*-test was used to compare the creatine peaks and MRS metabolite ratios, as well as amino acids and CPK levels pre- and post-treatment with a significance value of $P < 0.05$.

Results

This observational study included 10 individuals who were identified as having GACR either by molecular testing identifying biallelic mutations in the OAT gene and/or by high ornithine levels. Vitamin B6-responsive patients who took the medication consistently for at least a year and had ~~b~~rain MRS imaging that demonstrated decreased creatine levels were included in this study. Vitamin B6 responsiveness was defined as a decrease in ornithine of at least 25% or more after treatment. The mean age of diagnosis was 15.2 ± 1.93 years (12-18), with 60% of the patients being females with an average duration of treatment of 2.9 ±1.32 years (1.5-5).

The most prevalent presenting ocular sign was decreased visual acuity in 80% of patients, while ocular pain was the

least prevalent (10%). CPK elevation was noted in all our patients with chronic fatigue being the only muscle symptom found in 60%. Behavioral symptoms such as irritability, emotional lability, and mild depression were present in 40% of the patients. After twelve months of vitamin B6 supplementation, both the muscle and psychiatric symptoms improved (**Table.1**).

Table 1. Demographic data and clinical symptoms

Variables	
Age at diagnosis (years) mean ± SD (range)	15.2 ± 1.93 years (12 -18)
Gender N (%) ▪ Males ▪ Females	 4/10(40%) 6/10(60%)
Protein-restricted diet N (%)	10/10 (100%)
B6 administration route: N (%) ▪ Oral ▪ IM injection	 9/10 (90%) 1/10 (10%)
Muscle symptoms: N (%) ▪ Chronic easy fatigability and elevated CPK ▪ Asymptomatic elevated CPK	 6/10 (60%) 4/10 (40%)
Presenting ocular symptoms: N (%)	
▪ Decreased VA	8/10 (80%)
▪ Myopia	4/10 (40%)
▪ Retinal detachment	2/10 (20%)
▪ Nyctalopia	3/10 (30%)
▪ Ocular pain	1/10(10%)
Psychiatric symptoms: N (%) ▪ Irritability ▪ Mild depression ▪ Emotional liability	4/10 (40%) 4/10 (40%) 2/10 (20%) 4/10 (40%)
Genetic variants in 4/10 patients	▪ c.240C > G (homozygous) ▪ c.240C > G (homozygous) ▪ c.710G > A (homozygous) ▪ c.240C > G, c.473A>C (double heterozygous)

Following the commencement of vitamin B6 treatment for one year, both the ornithine and the CPK levels significantly decreased by (43.4% - 53.9%), and (67.9% - 70.1%), respectively; $p < 0.001$. Additionally, citrulline and arginine levels significantly increased; $p < 0.001$. (**Table.2** and **Fig.2**). No significant difference was found in proline levels before (158.8 ± 10.4 µmol/l) and after treatment (158 ± 9.2 µmol/l); ($p = 0.59$).

Table 2.Amino-acids profile pre- and post-vitamin B6 supplementation

Parameters	mean ± standard deviation (minimum-maximum)	P-value
Ornithine Level: µmol/l		
▪ **Pretreatment**	529.8 ± 26.61 (498-580)	< 0.001*
▪ **Post-treatment**	280 ± 8.43(268 -290)	

Citrulline level: µmol/l		
▪ **Pretreatment**	23.5 ± 2.54 (20-28)	< 0.001*
▪ **Post-treatment**	33.2 ± 2.29 (30-37)	
Arginine level: µmol/l		
▪ **Pretreatment**	20.8 ± 1.81 (18-24)	< 0.00*
▪ **Post-treatment**	44.4 ± 1.34 (43- 47)	
Creatine Phosphokinase: IU/l		
▪ **Pretreatment**	190.1 ± 4.9 (185-199)	< 0.001*
▪ **Post-treatment**	54.2 ± 5.73 (45-62)	

*significance; Student t-test.

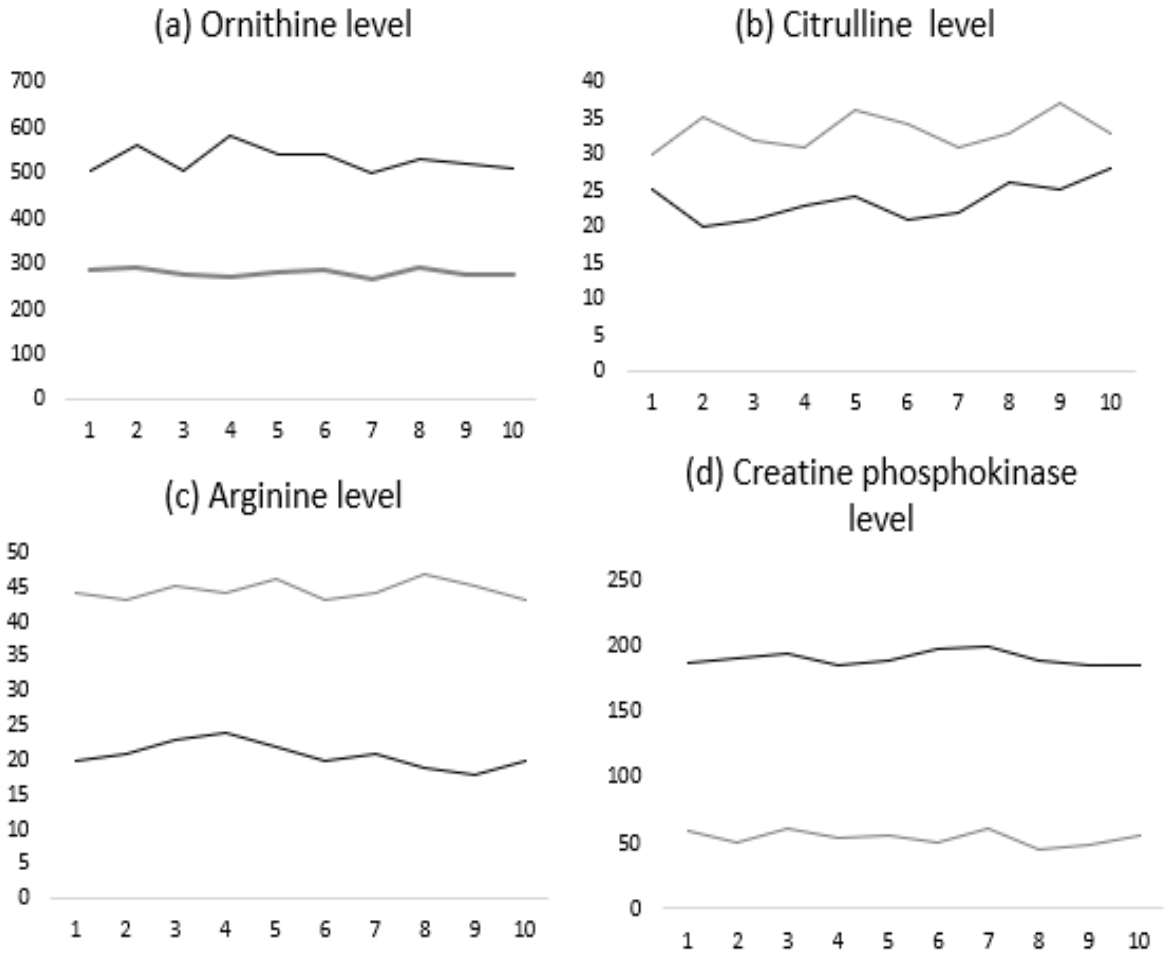


Fig.2. Laboratory test for amino-acids levels

When compared to pre-treatment values, the creatine peak post-treatment significantly increased in the basal ganglia, frontal, and occipital regions of the cerebral cortex ($p = 0.014$, $p < 0.001$, and $p < 0.001$, respectively). Furthermore, the ratio of brain metabolites in the basal ganglia, frontal, and occipital areas (NAA/Creatine, Choline/Creatine) significantly improved after therapy ($p < 0.001$) (**Tables.3** and **4**, **Figs .3** and **4**).

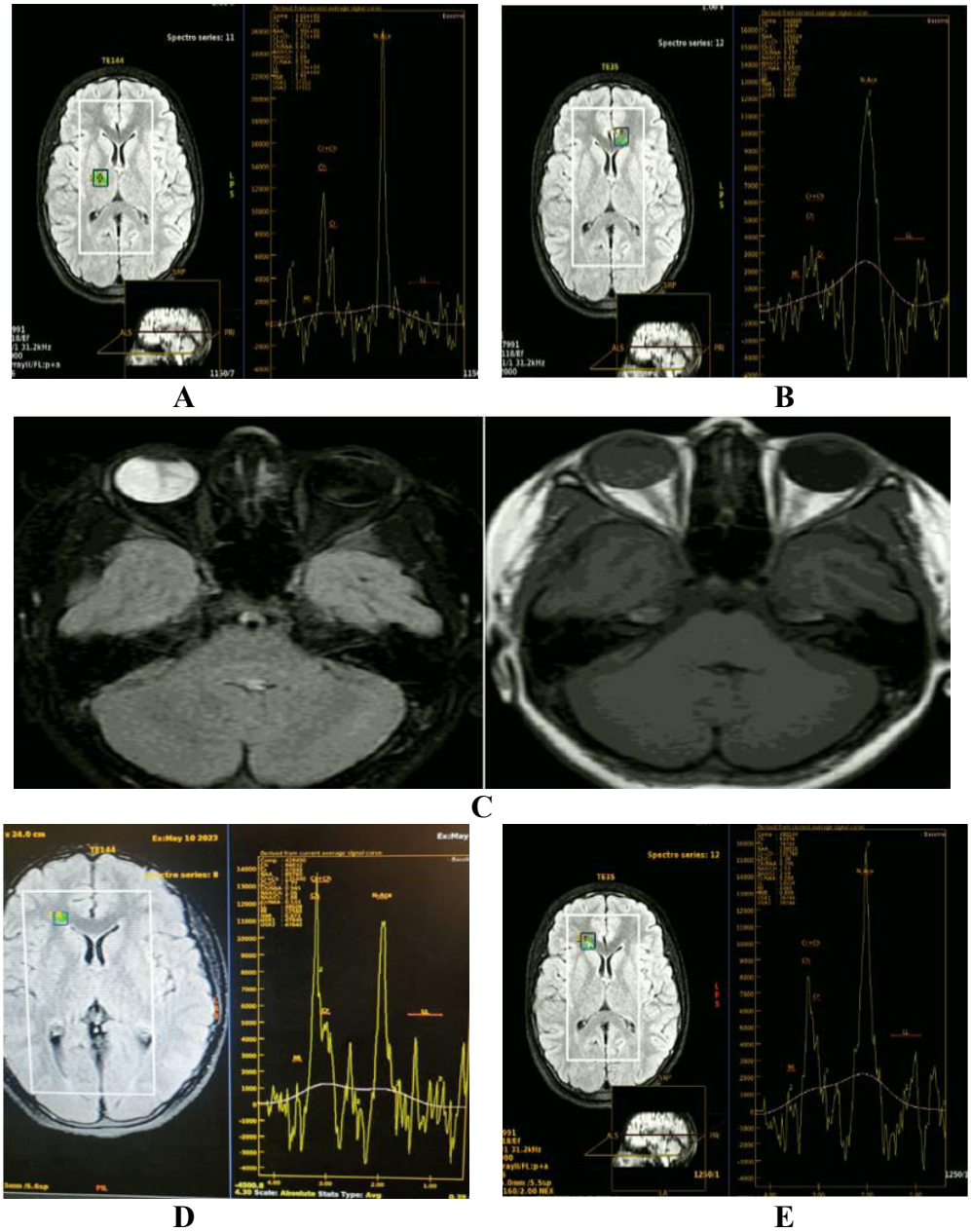
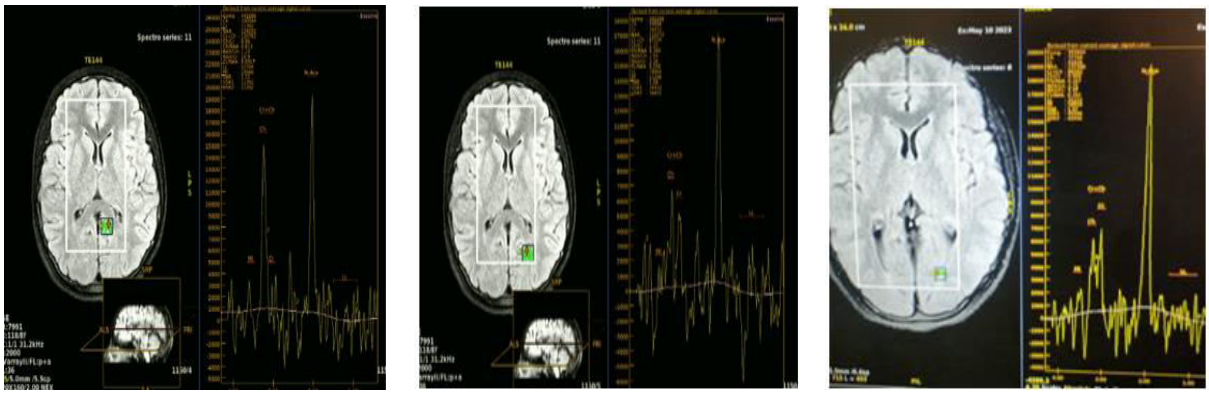


Fig.3 (case 1): A) A 13-year-old patient underwent ophthalmology evaluation after presenting with worsening visual acuity (particularly so on the right side). Results showed right-side retinal detachment and signs of gyrate atrophy. Genetic testing identified a VUS in the OAT gene: c.240C > G (homozygous); brain MRI and MRS were performed before therapy; follow-up was done one year after intramuscular vitamin B-6 delivery. Shows long TE144 of MR spectroscopy, region of interest (ROI) is in right basal ganglia, decreased level of creatine peak, normal level of NAA nd choline peaks, creatine peak = 37312, elevated NAA/creatine ratio ranging from 2.6 to 4 and choline/creatine ratio ranging from 1.15 to 8.1. Reduced creatine peaks in different voxels, (consistent with creatine deficiency): [In both frontal lobes' voxels ranging from 6560 to 12416, in both basal ganglia voxels ranging from 37312 to 52736, and in both occipital lobes' voxels ranging from 11392 to 2098], B) Shows short TE35 of MR spectroscopy of the same patient ROI is in the left frontal region, decreased level of creatine peak, normal level of N-acetyl aspartate and choline peaks, mild elevated peak of lipids/lactate, C) FLAIR (Fluid attenuation inversion recovery sequence) and T1WI (T1 weighted image) of MRI of the same patient show hyperintense

signal intensity in the vitreous of right eye globe with inverted V-shaped low signal intensity linear area, consistent with associated retinal detachment with hemorrhage, D) Shows MRS spectroscopy findings of the same patient after treatment, long TE144 of MR spectroscopy, ROI is in the right frontal region, improved level of creatine peak, normal level of N-acetyl aspartate and choline peaks, creatine peak = 47640, NAA/Creatine ratio ranging from 0.6 to 1.9 and choline/creatine ratio ranging from 0.8 to 1.7, improvement noted of creatine peaks in different voxels, especially frontal regions: [In both frontal lobes voxels ranging from 80790 to 14672, in both basal ganglia voxels ranging from 49812 to 66880 and in both occipital lobes voxels ranging from 41936 to 59432] and E) Shows the short TE35 of MR spectroscopy, ROI is in the right frontal region, mildly improved level of creatine peak, and normal level of N-acetyl aspartate and choline peaks.



A B C

Fig. 4. (case 2): A patient aged 15 years old, presented with decreased visual acuity, ocular pain, mild muscle pain, and easy fatigability. Shows long TE144 of MR spectroscopy, region of interest (ROI) is in the left occipital lobe, decreased level of creatine peak, normal level of N-acetyl aspartate and choline peaks, creatine peak= 13472.7, elevated NAA/Creatine ratio=3.84 and choline/creatine ratio=7.55. Reduced Creatine peaks in occipital lobes voxels, (consistent with creatine deficiency), ranging from 14562 to 170497, B) Shows short TE35 of MR spectroscopy, ROI is in the left occipital region, decreased level of creatine peak, normal level of N-acetyl aspartate and choline peaks, no significant elevation of lipids/lactate peaks and C) Shows MRS spectroscopy findings of the same patient after treatment, long TE144 of MR spectroscopy, ROI is in the left occipital region, mild improvement of the level of creatine peak, normal level of N-acetyl aspartate and choline peaks, creatine peak = 46569.6, NAA/Creatine ratio ranging from 0.61 to 0.79 and choline/creatine ratio ranging from 0.75 to 1.41, improvement noted of Creatine peaks in different voxels in both occipital regions ranging from 40492 to 52645.

Table 3.Brain Magnetic resonance spectroscopy findings

Metabolite (Mean ± SD)	**Before treatment**	**After treatment***	**P-value**
Creatine peak			
▪ **Frontal region**	9081.8 ± 2288.48	38063.5 ± 30177.02	0.014*
▪ **Basal Ganglia**	41602.5 ± 5368.97	54251.5 ± 5634.273	< 0.001*
▪ **Occipital region**	13472.7 ± 3587.04	46569.6 ± 6076.82	< 0.001*
NAA/creatine ratio			
▪ **Frontal region**	3.13 ± 0.51	1.26 ± 0.28	< 0.001*
▪ **Basal Ganglia**	3.33 ± 0.6	0.76 ± 1.33	< 0.001*
▪ **Occipital region**	3.84 ± 0.18	0.7 ± 0.09	< 0.001*

Choline/creatine ratio			
▪ **Frontal region**	5.995 ± 2.13	1.264 ± 0.42	< 0.001*
▪ **Basal Ganglia**	7.59 ± 0.49	0.98 ± 0.12	< 0.001*
▪ **Occipital region**	7.55 ± 0.5	1.08 ± 0.33	< 0.001*

*significance; student t-test.

Variation in Vitamin B6 treatment duration did not affect the creatine and ornithine levels, ($p > 0.05$) (**Table.4**).

Table 4.Effect of vitamin B6 duration of treatment on ornithine and creatine levels

Duration of treatment (Mean ± SD)	**2.5 years or less**	**More than 2.5 years**	**P-value**
Number of patients	5	5	
Age at diagnosis (years)	15 ± 2.4	15.4 ± 1.5	
Ornithine level µmol/l (pretreatment)	524.2 ±16.5	535.4 ± 31.5	0.59
Ornithine level µmol/l (post-treatment)	280.6 ± 71.3	279.4 ± 87.8	0.8
Pretreatment creatine peaks in MRS:			
• Frontal lobe	8988.6 ± 2633	9185 ± 2196.3	0.88
• Basal ganglia	39904.8 ± 4572	43300 ± 6062	0.42
• Occipital lobe	13517± 4198.69	13428.4 ± 3363.9	0.97
Post-treatment creatine peaks in MRS:			
• Frontal lobe	38880 ± 32709	37247 ± 31263.7	0.5
• Basal ganglia	56812.6 ± 6992.8	51690.4 ± 2475.5	0.9
• Occipital lobe	47889.2 ± 7930.9	45250 ± 3979.2	0.7

Student t-test, SD= standard deviation.

Discussion

Vitamin B6 is a cofactor and a chaperone for the OAT enzyme **(Guan et al., 2021)**.PLP) concentration is crucial for stabilizing the overall structure of the OAT enzyme and influencing its turnover as it encourages the availability of folded OAT enzyme, inhibits apoenzyme breakdown, and boosts the enzyme's thermal stability at physiological temperatures (**Clayton, 2006; Montioli et al., 2017)**.

In light of the above, vitamin B6 has been a crucial component of GACR management. Supplying vitamin B6 in pharmacological doses can help boost the reaction in favor of pyrroline 5 carboxylate formation, thus lowering ornithine levels to < 200 µmol, which is the main goal of GACR treatment **(Balfoort et al., 2021)**.

In our study, the ornithine level decreased on average by 47% ± 0.02% while taking 400–600 mg/day of vitamin B6 and adhering to a reduced protein diet since the patients could not handle an arginine-restricted diet. According to earlier studies, ornithine levels decreased by 19% to 68% in patients receiving vitamin B6 as monotherapy **(Balfoort et al., 2021)**.

Weleber and Kennaway (1981) reported that 3/7 patients responded to oral B6 supplementation with over 50% reduction of serum ornithine. McCulloch et al. **McCulloch et al. (1978)** showed that among approximately 70 Finnish GA cases reported to date, none have been responsive to vitamin B6.

Various factors, including the type of mutation, can influence the response to vitamin B6 (**Clayton, 2006)**. Mutations that result in lower affinity and affect binding to PLP can cause the vitamin B6-responsive type. Only 4/10 of the patients were able to undergo molecular testing.

The creatine synthesis pathway is catalyzed by the rate-limiting enzyme AGAT that transfers the amidino group from arginine to the amino group of glycine yielding guanidinoacetic acid (GAA) that is converted to creatine by guanidinoacetate methyltransferase (GAMT) and ornithine as a secondary product **Mulik and Mercimek-Andrews (2023)**. High levels of ornithine negatively regulate AGAT, slowing down the reaction.

The secondary creatine deficiency noted in GACR patients can be explained by the negative feedback high ornithine levels exert on the AGAT enzyme (**Valayannopoulos et al., 2009; Balfoort et al., 2021)**. This concept served as a rationale for using creatine supplements to treat GACR (**Michel et al., 2015)**.

It has been postulated that normal creatine levels can be restored by reducing ornithine levels by ameliorating negative feedback. This can be achieved by implementing an arginine-restricted diet (precursor of ornithine in diet) and supplementing vitamin B6, with or without creatine supplements. **Balfoort et al.,(2021)** found that controlling ornithine levels in this manner, it is possible to normalize creatine levels, which is of potential benefit in various contexts. MRS can be used to detect creatine deficiency in the CNS by measuring creatine peak together with other metabolite ratios, i.e., NAA/creatine and choline/creatine. Indicators of creatine deficiency, such as low creatine peak, high NAA/creatine, and choline/creatine ratios, were present in the pre-treatment MRS results in our cohort; however, the post-treatment MRS results demonstrated a considerable improvement. This can be explained by the negative feedback of high ornithine on the AGAT enzyme, which was relieved upon ornithine level normalization following vitamin B6 administration.

Forty percent of our patients experienced mild depression symptoms, irritability, and emotional lability. **(Valayannopoulos et al., 2009)** showed that patients reported improvement in their symptoms within one year of B6 supplementation. Neuropsychiatric symptoms, such as neurocognitive impairment, failure in school, visuospatial dyspraxia, aggressive behavior, and seizures, that have been observed in GACR may be attributed to secondary creatine deficiency.

At the time of diagnosis, 60% of the participants reported mild muscle clinical symptoms and all of them had an elevated CPK. However, within a year of receiving vitamin B6, the CPK normalized, and the muscle symptoms improved. Although muscle symptoms are usually minor or subclinical, manifestations such as persistent exhaustion and type II muscle fiber atrophy were present in gyrate atrophy (**Fleury et al., 2007)**.
Heinänen et al. (1999), **Sipilä et al. (1981) and Valtonen et al. (1999)**, and suggest that the symptoms mentioned above could be caused by a deficiency of creatine in the muscles and CNS and that the improvement occurred after the reduction in ornithine following B6 supplementation removed the negative feedback inhibition it exerted on the AGAT enzyme.

We noted that there was no statistically significant effect of the duration of Vitamin B6 treatment on the creatine level or the ornithine level. This observation could have been influenced by the variability in baseline creatine and ornithine levels.

The retina is a high-energy-consuming tissue that contains a

substantial amount of creatine, which appears to be crucial to ATP homeostasis and synthesis coupled with phosphocreatine. Therefore, in addition to increased intraocular ornithine levels, proline shortage in the choroid and retina, and catabolic products of ornithine, secondary creatine deficiency may be another contributing factor to the retinal and choroidal pathogenesis in GACR (**Acosta et al., 2005)**.

The levels of citrulline and arginine were found to be low before treatment, but there was a significant increase after the treatment. In the case of GACR, arginine becomes an essential amino acid, and since OAT-catalyzes the reversible conversion of ornithine to pyrroline-5-carboxylate, the net flux of the OAT catalyzed reaction may favor either ornithine (and arginine) degradation or synthesis (**Zubarioglu et al., 2016)**. We presume that if ornithine is pathologically elevated, the reaction will not favor ornithine synthesis. Hence, it functions to catabolize excess ornithine and arginine, leading to low levels of arginine as well as its derivative, citrulline. After treatment, the ornithine level was normalized/lowered, and the balance in the net flux of the reaction was restored.

In infancy, low levels of citrulline and arginine can be explained by shifting the whole body's OAT reaction in the direction of ornithine production. Therefore, an OAT deficiency could result in an increase in proline and a relative shortage of ornithine and its derivatives arginine and citrulline, leading to impairment of the urea cycle, which causes hyperammonemia (**Ginguay et al., 2017; Kaczmarczyk et al., 2022)**.

Limitations of this study included the small sample size that may produce insignificant results and lack of a control group. Further study with a larger sample size and comparator group was needed.

Conclusion

GACR may be associated with secondary creatine deficiency due to high levels of ornithine, which contribute to both systemic and ocular symptoms. Vitamin B6 administration in B6-responsive patients can lead to improvement in the CNS creatine level, normalization of the CPK level, and improvement of behavioral and muscle complaints.

Abbreviations: MRI: Magnetic resonance imaging, MRS: Magnetic resonance spectroscopy, CNS: central nervous system, GACR: gyrate atrophy of the choroid and retina, CPK: creatine phosphokinase, OAT: ornithine aminotransferase, PLP: pyridoxal phosphate, AGAT: arginine: glycine amidinotransferease

Acknowledgments: Nil

Financial support and sponsorship: Nil

Conflict of Interest: Nil

Author Contributions: All authors contributed to the study's conception and design. Material preparation, data collection, and analysis were performed by Amira Mobarak, Alaa Mohamed Reda, Rofaida M. Magdy, Mohamed M. Hassan, and Ahmed Elsharkawy. Amira Mobarak wrote the first draft of the manuscript. All authors read and approved the final manuscript.

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