The Role of Vitamin D in the Assessment of Treatment Response in Children with Gaucher Disease

Faris K Khadir, Ph.D* Dena Mohammed Alsahaf, M.B.Ch.B, F.I.C.M.S **

ABSTRACT

Gaucher disease (GD) is a hereditary autosomal recessive disorder. Numerous biomarkers that have a role in the pathophysiology and etiology of GD are used for the diagnosis and prognosis of this disorder in pediatric populations. The study sought to examine the significance of vitamin D levels in assessing the treatment response of individuals with GD receiving enzyme replacement therapy (ERT) during follow-up evaluations. Case-control research was conducted including 70 children (both male and female) aged 1 to 13 years diagnosed with Gaucher disease, recruited from the Pediatric Department and the Unit of Rare Diseases. The vitamin D levels were assessed in samples from Gaucher patients classified as newly diagnosed GD patients that didn't receive treatment (n=7), those undergoing ERT for 3-6 months (n=20), 6-12 months (n=20), and patients received ERT for over one year (n=23), and these levels were compared with those of twenty gender- and age-matched control subjects. The investigation was conducted from December 2023 to May 2024. The vitamin D levels are evaluated using an enzyme-linked immunosorbent test (ELISA) kit. The data revealed that levels of vitamin D in patients were considerably lower ($p<0.05$) compared to age-matched controls. It was concluded that Vitamin D showed to have a diagnostic value in newly diagnosed GD patients that didn't receive treatment with a poor monitoring role as it elevated slowly with the treatment progress.

Keywords: Enzyme replacement therapy, Gaucher disease, imiglucerase, lysosomal storage disorder, Vitamin D.

INTRODUCTION

Gaucher disease (GD) is a disorder that observed predominantly in Ashkenazi Jewish community that classified genetically as an autosomal recessive disorder[1], and considered as the most prevalent lysosomal storage disorder resulting from glucocerebrosidase enzyme (sometimes referred to as acid β-glucosidase) deficiency[2], which lead to an accumulation of glucocerebroside in reticuloendothelial cells[3]. Glucocerebrosidase enzyme hydrolyses the β-glycosidic bond of glucosylceramide, yielding ceramide and glucose, with the former being degraded by lysosomal acid ceramidase into sphingosine and fatty acid. Lysosomes house several acid hydrolases that facilitate the breakdown of proteins, lipids, and carbohydrates internalized through endocytosis[4]. A significant array of genetically inherited deficiencies in lysosomal hydrolases is recognized, characterized by either the absence of a specific hydrolase or impaired activity, leading to the accumulation of undegraded substrates, a progressive enlargement and proliferation of lysosomes, and the emergence of a lysosomal storage disorder with its characteristic clinical features[5]. Enzyme replacement treatment (ERT) utilizing recombinant glucocerebrosidase Cerezyme® (imiglucerase for injection), an equivalent of the human enzyme ß-glucocerebrosidase, is the primary treatment for Gaucher disease, the first lipid storage disorder to be effectively treated. Prospective therapies may encompass oral enzyme replacement and/or gene therapy procedures[6-8].

Bone issues typically arise in children with Gaucher disease, diminishing their quality of life. Bone biomarkers have been utilized to evaluate disease status and to monitor therapeutic responses in various bone illnesses[9]. Previous studies examined Vitamin D as a bone biomarker potentially useful for monitoring the bone response to ERT[10].

This study aims to examine the significance of vitamin D levels in diagnosis of GD and as a monitoring tool for the treatment responses in patients with Gaucher's disease who received ERT (imiglucerase).

MATERIALS AND METHODS

Study Protocol: Case-control research was conducted including 70 children (both male and female) aged 1 to 13 years (mean ± SD age of 6.17 ± 3.16) diagnosed with Gaucher disease, recruited from the Paediatric Department at Al Karama Teaching Hospital and Central Child's Teaching Hospital in Baghdad, Iraq. Patients were divided into 4 subgroups; G1: newly diagnosed GD patients that didn't receive treatment (n=7), G2: patients with three months treatment with ERT (n=20), G3: patients with six months treatment with ERT (n=20) and G4: patients with one year treatment with ERT (n=23).

Research laboratories in Department of Physiology College of Medicine, University of Baghdad carried out the practical portion of the investigation in the period from December 2023 to May 2024. The outcomes of the patient cohorts were juxtaposed with twenty age- and gender-matched non-GD children, possessing a mean age of 6.3 ± 2.7 years, serving as a control group. Exclusion criteria encompassed individuals who suspected of having tuberculosis, those with chronic infections and inflammatory conditions such as chronic arthritis, along with other factors influencing enzyme activity, including asthma, haematological disorders (e.g., β-thalassemia), parasitic infections (e.g., malaria), fungal infections (e.g., Candida albicans), and other hereditary diseases (e.g., Niemann-Pick Disease).

Research Ethical Committee in the College of Medicine - University of Baghdad approved the present study. Furthermore, informed written

* Department of Physiology, Faculty of Medicine, University of Baghdad, Baghdad, Iraq.

** Al-Mustafa University, Department of Radiology, Baghdad, Iraq.
Email: dina-rt@almustafauniversity.edu.iq

Table 1. Demographic characteristics of all studied groups

	control	GD patients (total)	G1	G2	G3	G4
N	20	70	7	20	20	23
Male (n, %)	9 (45%)	35(50 %)	4 (57.14%)	9 (44%)	10 (50%)	12 (52.17%)
Female (n, %)	11(55%)	35(50 %)	3 (42.85%)	11 (55%)	10 (50%)	11 (47.82%)
OR		1.22	1.63	1	1.22	1.33
95% CI		0.45- 3.32	0.29-9.26	0.29- 3.48	0.35- 4.24	0.4- 4.44
P-value		0.69	0.58	1	0.75	0.64
Age	6.3±2.7	6.17±3.16	4.97±2.94	5.88± 3.76	5.96± 2.87	6.97± 2.76
P-value with control		0.84	0.19	0.57	0.71	0.62
Weight	20.87± 8.87	19.32± 7.76	15.66± 6.33	18.82± 10.41	19.64± 7.51	20.61± 5.31
P-value Vs. control		0.45	0.14	0.43	0.57	0.84

OR: Odd Ratio, CI: confidence interval.

Table 2. Total Vitamin D levels in all studied groups

Group	Levels of Vitamin D (ng/ml) (Mean ± SD)
Control (n= 20)	37.12 ± 6.74 A
Whole GD patient	23.73 ± 7.43 BC
G1: Newly diagnosed (n = 7)	18.94 ±6.24 B
G2: 3-5 months treatment (n = 20)	23.38 ± 5.91 BC
G3: 6-12 months treatment (n = 20)	23.73± 6.74 BC
G4: >1 year treatment (n = 23)	25.49 ± 5.51 C

Values denoted with different letter are differ significantly ($p<0.05$)

consent for participation in the study was obtained from the parents or legal guardians of the subjects in accordance with the Helsinki standards.

Sample collection and preparation: Blood samples (5 ml) were collected from fasting patients and controls at 9-11 AM and the sample put into serum separating tube (SST) to obtained the serum that used to evaluate the total vitamin D level in all subjects by enzyme-linked immunosorbent assay (ELISA) technique according to manufacturer instruction.

Statistical analysis: The study's data were saved in a Microsoft Excel spreadsheet and analysed using SPSS software version 20 and Microsoft Excel 2010. Numeric variables were presented as mean ± SD, and all statistical comparisons were conducted using independent t-tests and ANOVA, with $P < 0.05$ being statistically significant. The correlation among all numerical factors was conducted utilizing the Pearson correlation test, and all statistical analyses were performed utilizing the SPSS software[11]. Odds ratio was also calculated to elucidate the difference in the gender distribution among all studied groups in comparison with controls[12]. Receiver Operating Characteristic (ROC) analysis was conducted to thoroughly evaluate the accuracy of the examined markers. The area under the curve (AUC) serves as an effective metric for comparing various biomarkers. An AUC value approaching 1 signifies an exceptional diagnostic and predictive marker, while a curve on the diagonal (AUC = 0.5) lacks diagnostic relevance[13]. An AUC near 1 is consistently associated with favorable specificity and sensitivity values of biomarker[14].

RESULTS

Demographic characteristics of all studied groups were illustrated in table 1 which showed that the incidence of Gaucher disease in male children was non-significantly ($p>0.05$) lower than that of female children in all studied groups. The age and sex in male children were non-significantly differ from that of female in all studied groups showed.

Results illustrated in table 2 showed that Children with Gaucher disease showed a significantly lower levels ($p< 0.05$) of Vitamin D (23.73 ± 7.43) in comparison with control (37.12 ± 6.74).
Vitamin D levels as illustrated in Table (2) were reduced significantly ($p<0.05$) in all patients' groups in comparison with control. In contrary, non-significant differences ($p>0.05$) were obtained between G1, G2 and G3 patients. Moreover, vitamin D level in patients received more than one year treatment (G1) showed a significant increase in a comparison with that obtained in newly diagnosed GD patients that didn't receive treatment.

Levels of vitamin D that illustrated in Table 3 indicates that the area under the curve (AUC) for vitamin D levels is notably high at 0.91, with accuracy metrics of specificity at 83% and sensitivity at 92% when comparing Gaucher patients to healthy control children. It was also demonstrated that the levels of vitamin D exhibited high AUC values (1) with high sensitivity and specificity (100% and 100%) in newly diagnosed GD patients that didn't receive treatment patients (G1) comparing with healthy control children.

As shown in table 4, levels of vitamin D showed AUC value of 0.726 with poor sensitivity (54.7%) and specificity (64.4%) in G1 group in comparison with that of G2 group.

Results obtained in table 5 revealed that the levels of vitamin D demonstrated a low AUC (0.531) with week specificity and sensitivity (69.7 % and 39.8%, respectively) in patients received 3-6 months treatment (G2) in comparison with patients received 6-12 months treatment (G3).

Moreover, Results obtained in table 6 revealed that the ROC curve results of vitamin D showed a low AUC (0.565) with week specificity

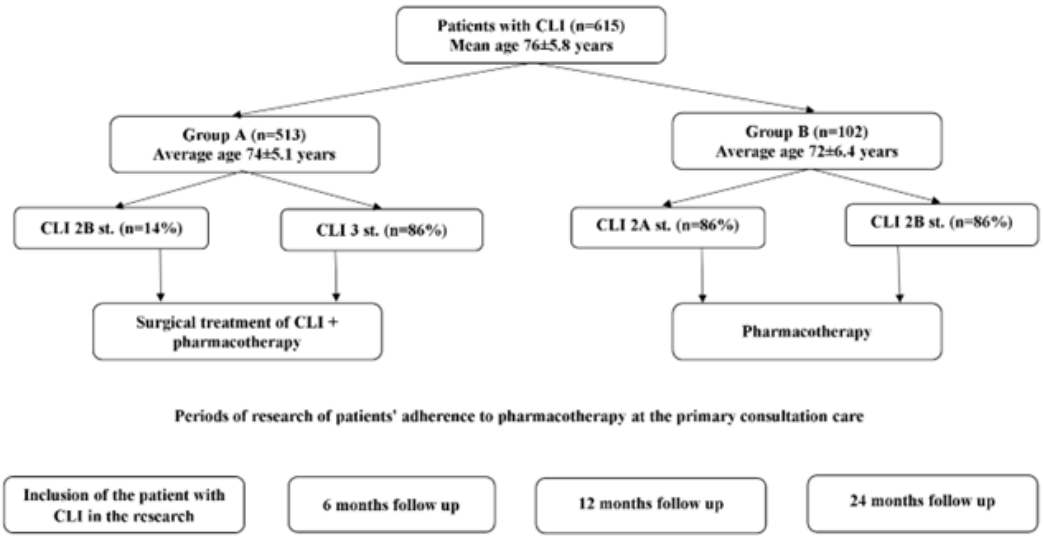

Figure 1. The design-program of the research

Table 3. Results of ROC curve analysis for vitamin D in whole GD children and newly diagnosed GD children (G1) in comparison with controls

Group	Specificity (%)	Sensitivity (%)	AUC	Cut-off value
Whole GD patient	83	92	0.91	29.89
G1: Newly diagnosed (n = 7)	100	100	1.00	26.31

Table 4. Results of ROC curve analysis for vitamin D levels in newly diagnosed GD children (G1) in comparison with children received ERT for 3-6 months (G2)

Group	Sensitivity (%)	Specificity (%)	AUC	Cut-off value
G1 Vs. G2	64.4	54.7	0.726	20.96

Table 5. Results of ROC curve analysis for vitamin D levels in children received ERT for 3-6 months treatment (G2) in comparison with children received ERT for 6-12 months (G3).

Group	Sensitivity (%)	Specificity (%)	AUC	Cut-off value
G2 Vs. G3	39.8	69.7	0.531	27.24

Table 6. Results of ROC curve analysis for vitamin D levels in children received ERT for 6-12 months (G3) in comparison with children received ERT for more than one year (G4).

Group	Sensitivity (%)	Specificity	AUC	Cut-off value
G3 Vs. G4	58.9	59.3	0.565	24.22

and sensitivity (59.3 % and 58.9%, respectively) in patients received 6-12 months treatment (G3) in comparison with patients received more than one year treatment (G4).

DISCUSSION

Results obtained in this study as demonstrated in table 2 revealed that the level of vitamin D was significantly lower than that of control in consistent with previous studies[10,15]. Additionally, the levels of vitamin D still significantly lower than that of control even after received more than one year treatment as shown in table 2. Vitamin D level showed a slight increment with treatment but the levels still significantly lower than that of control. Non-significant differences were observed among the patient subgroups as demonstrated in table 2. The possible explanation of these results is that the ERT effect was slower on bone complications than that on the liver and other complications which is in agreement with the previous literature[16,17]. The only exception is the significant increase in the level of vitamin D in children on treatment for more than one year in comparison with that of newly diagnosed GD patients that didn't receive treatment which indicate that there was a slow improvement in bone turnover after long period of treatment.

ROC curve results also confirm the above results and revealed that the level of vitamin D in whole patients and newly diagnosed GD patients that didn't receive treatment can be considered as an excellent marker for diagnosis of the disease but poorly used as biomarker for the monitoring the treatment with ERT due to slow response of these parameter to the treatment as revealed in tables (3-6).

CONCLUSION

Total Vitamin D levels showed a significant reduction in Gaucher patients comparing with controls as an indication of a bone complications which is not reversed rapidly. That is why the levels of vitamin D didn't increase significantly among the patients received ERT in this study. So, vitamin D can be considered as diagnostic biomarker for GD but poorly used as monitoring biomarkers especially during the first

year of received ERT.Authorship Contribution: All authors share equal effort contribution towards (1) substantial contributions to conception and design, acquisition, analysis and interpretation of data; (2) drafting the article and revising it critically for important intellectual content; and (3) final approval of the manuscript version to be published. Yes.

Potential Conflicts of Interest: None

Competing Interest: None

Acceptance Date: 30 June 2025

REFERENCES

1. Alcalay RN, Dinur T, Quinn T, et al. Comparison of Parkinson risk in Ashkenazi Jewish patients with Gaucher disease and GBA heterozygotes. JAMA neurol 2014;71(6):752-7.
2. Kanneganti M, Kamba A, Mizoguchi E. Role of chitotriosidase (chitinase 1) under normal and disease conditions. J Epithel Biol Pharmacol 2012;5:1-9
3. Deegan PB, Cox TM. Imiglucerase in the treatment of Gaucher disease: a history and perspective. Drug Des Devel Ther 2012;6:81-106.
4. Ben Bdira F, Artola M, Overkleeft HS, et al. Distinguishing the differences in β-glycosylceramidase folds, dynamics, and actions informs therapeutic uses. J Lipid Res 2018;59(12):2262-76.
5. Schulze H, Sandhoff K. Lysosomal lipid storage diseases. Cold Spring Harb Perspect Biol 2011;3(6):a004804.
6. Abdulamir HA, Aldafaay AA, Al-Shammari AH. The Role of Liver Function Tests in Monitoring the effect of Enzyme Replacement Therapy in Children with Gaucher Disease. Res J Pharm Technol 2022;15(8):3490-6.
7. Awadh AI. The role of lipid profile assessment in monitoring the effect of imiglucerase in children with gaucher disease. Res J Pharm Technol 2023;16(2):573-80.
8. Chen M, Wang J. Gaucher disease: review of the literature. Arch Pathol Lab Med 2008;132(5):851-3.
9. Giuffrida G, Cingari MR, Parrinello N, et al. Bone turnover markers in patients with type 1 Gaucher disease. Hematol Rep 2012;4(4):e21.
10. Mikosch P, Reed M, Stettner H, et al. Patients with Gaucher disease living in England show a high prevalence of vitamin D insufficiency with correlation to osteodensitometry. Mol Genet Metab 2009;96(3):113-20.
11. Aldafaay AA, Amir HAA, Abdulhussain HA, et al. The use of Urinary α-amylase level in a diagnosis of Chronic renal failure. Res J Pharm Technol 2021;14(3):1597-600.
12. Abdulhusseın HA, Alwasıtı EA, Khıro NK, et al. The potential impact of vascular endothelial growth factor rs699947 polymorphisms on breast tumors susceptibility in a sample of Iraqi females. Acta Pharm Sci 2024;62(2):268-77.
13. Manna MJ, Baqir LS, Abdulamir HA. The assessment of the antimicrobial effect of gemfibrozil alone or in combination with ceftriaxone or gentamycin on several types of bacteria. Acta Pharm Sci 2024;62(3):565-74
14. Abdulhussein HA, Alwasiti EA, Khiro NK. The role of VEGF levels in the differentiation between malignant and benign breast tumor. J Res Pharm 2024; 28(3): 603-11.
15. Stirnemann J, Belmatoug N, Camou F, et al. A Review of Gaucher Disease Pathophysiology, Clinical Presentation and Treatments. Int J Mol Sci 2017;18(2):441
16. Smid BE, Ferraz MJ, Verhoek M, et al. Biochemical response to substrate reduction therapy versus enzyme replacement therapy in Gaucher disease type 1 patients. Orphanet J Rare Dis 2016;11:28.
17. Linari S, Castaman G. Clinical manifestations and management of Gaucher disease. Clin Cases Miner Bone Metab 2015;12(2):157-64.