International Journal of Biochemistry Research & Review
15(2): 1-6, 2016; Article no.IJBCRR.30768
ISSN: 2231-086X, NLM ID: 101654445

SCIENCEDOMAIN *international*
www.sciencedomain.org

Effect of Enzyme Replacement Therapy on Disease Burden Biomarkers in Gaucher's Disease

Mohammed H. Hassan[1*], Ayat A. Sayed[2], Ahmed El-Abd Ahmed[3], Tahia H. Saleem[2], Khalid I. Elsayh[4] and Norhan B. B. Mohammed[1]

[1]Department of Medical Biochemistry and Molecular Biology, Qena Faculty of Medicine, South Valley University, P.O.Box No. 83523 Qena, Egypt.
[2]Department of Medical Biochemistry and Molecular Biology, Faculty of Medicine, Assiut University, P.O.Box 71515, Assiut, Egypt.
[3]Department of Pediatrics, Qena Faculty of Medicine, South Valley University, P.O.Box No. 83523, Qena, Egypt.
[4]Department of Pediatrics, Faculty of Medicine, Assiut University, P.O.Box 71515, Assiut, Egypt.

Authors' contributions

Authors MHH and AAS carried out the whole biochemical assays, wrote the first draft of the manuscript, managed the literature searches and performed the statistical analysis. Authors AEA, THS and KIE were responsible for study concept, design, patients selection and wrote the protocol. Author NBBM was responsible for data collections and blood sampling. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/IJBCRR/2016/30768
Editor(s):
(1) Hector M. Mora Montes, Department of Biology, University of Guanajuato, Mexico.
(2) Cheorl-Ho Kim, Molecular and Cellular Glycobiology Unit, Department of Biological Science, Sungkyunkwan University, South Korea.
Reviewers:
(1) Mehmet Çelik, Trakya University, Turkey.
(2) Ying Sun, Cincinnati Children's Hospital Medical Center, USA.
Complete Peer review History: http://www.sciencedomain.org/review-history/17623

Original Research Article

Received 29th November 2016
Accepted 18th January 2017
Published 26th January 2017

ABSTRACT

Aims: We evaluate the effect of enzyme replacement therapy "ERT" on the plasma levels of some biochemical markers of the disease burden, in pediatric patients with Gaucher's disease "GD", in the form of plasma chitotriosidase "ChT", total acid phosphatase activity, ferritin and globulin to evaluate the therapeutic monitoring efficacy of such biomarkers.
Methodology: A cross sectional case control study, carried out on 26 GD pediatric patients, divided into group A (13): On ERT and group B (13): Not receiving ERT, and in addition to 20 healthy age

**Corresponding author: E-mail: Mohammedhosnyhassaan@yahoo.com, mohammedhosnyhassaan@med.svu.edu.eg;*

and sex matched controls. ELISA assays of plasma ChT and ferritin, colorimetric assays of plasma total acid phosphatase activity and globulin, were done for all groups, while, plasma proteins electrophoresis was done for GD patients only.
Results: Significant higher plasma levels of ChT, total acid phosphatase activity, ferritin and globulin among GD not receiving ERT versus both GD on ERT and Control group. Positive correlation between plasma ferritin and total acid phosphatase activity (r = 0.465 and P-value <0.05).
Conclusions: Plasma ChT, total acid phosphatase activity; ferritin and globulin are collectively helpful in evaluation and follow up the effect of ERT.

Keywords: Disease burden markers; Gaucher's disease; ERT.

1. INTRODUCTION

Gaucher's disease "GD" is one of the few lysosomal storage diseases "LSDs" for which treatment has been developed. ERT is one of the most used today, but substrate synthesis inhibition is also available [1].

Gaucher cells secrete many molecules that could be useful as biological markers of disease burden and/ or response to enzyme replacement therapy "ERT" [2]. These molecules include chitotriosidase, acid phosphatase and ferritin [3,4].

Studies on various biomarkers of Gaucher disease will predict the clinical severity and assess the response in patients receiving ERT. A collection of plasma biomarkers in the form of plasma ChT, total acid phosphatase activity,ferritin and globulin were studied to test and confirm if their plasma levels will be affected by ERT in GD patients or not.

2. MATERIALS AND METHODS

2.1 Study Population

The present study is a cross sectional case control study, was carried out on 26 pediatric patients divided into two groups; Group A: It includes 13 GD patients receiving the ERT 4 years or more with imiglucerase (Cerezyme, 60 U/kg/2weeks by intravenous infusion over 1–2 hrs) and Group B includes 13 GD patients not receiving the ERT, in addition to 20 apparently healthy children, age and sex matched selected as control group (group C). They were recruited from the pediatric outpatient clinics and inpatients Pediatric departments of Assiut and Qena University hospitals, which are of the major tertiary referral pediatric hospitals in Upper Egypt after approval of the university hospital ethical committee. Prior to initiation of the study; every subject and his/her parents were informed about the aim of the study and gave a written consent.The study was carried out during the period from January 2015 to May 2016.

2.2 Laboratory Workup

Complete blood counts "CBC", albumin, globulin, alkaline phosphatase "ALP", bilirubin (total and direct), liver enzymes (ALT and AST) were taken already from the file of the patients. Complete blood counts "CBC" were measured using (Cell Dyn 1800-Abbott diagnostics, Germany). Liver function tests including albumin, globulin, A/G ratio, alkaline phosphatase "ALP", bilirubin (total & direct) and liver enzymes (ALT & AST) were estimated using [Cobas C311 (Roche diagnostics, Germany)].

Three cc of venous blood was drawn from the included children on EDTA tubes, centrifuged at 3500 rpm for 15 min and the separated plasma from each tube was stored into aliquots using 1 ml cryotubes at- 20℃ until the biochemical analysis for GD burden biomarkers.

Using commercially available enzyme-linked immune-sorbent assay (ELISA) assay kits(using ELISAmultiskan EX microplate photomter, thermo scientific, STAT FAX-2100, USA) according to manufacturer protocol for measurements of plasma ChT (supplied by Elabscience Biotechnology Co.,Beijing, Catalog No: E-EL-H5620) and plasma Ferritin (supplied by BIOCHECK, INC, 323 Vintage Park Dr., Foster City, CA 94404- Catalog Number: BC-1025).

Using commercially available colorimetric assay kit (using T60 UV visible spectrophotometer. PG INSTRUMENTS LIMITED, Alma park wibtoft, Leicester shreshire, England. LE17SBE. Serial No. 20-1650-01-0010) according to manufacturer

protocol for measurement of plasma total acid phosphatase activity (supplied by Bio-Diagnostic Co. Cairo, Egypt, Catalog No: AC 10 10).

Plasma protein electrophoresis was performed using Sebia capillary electrophoresis, Sebia, Inc. according to manufacturer's guidelines. Plasma proteins were separated into 6 components; albumin, alpha1, 2, beta1, 2 and gamma globulin. The separation was carried out on silica capillaries according to their electrophoresis mobility and elctroosmotic flow at high voltage in alkaline media.

2.3 Statistical Analysis

Data were analyzed using SPSS (statistical program for social science version 12): Quantitative variables were described as mean ±SD. Qualitative variables were described as number and percentage. Unpaired t-test was used for comparison between quantitative variables, in parametric data. Correlation co-efficient test was used to rank variables positively or inversely. P value >0.05 insignificant, P<0.05 significant, P<0.001 highly significant.

3. RESULTS

Regarding the demographic and clinical data of the studied groups; Group A (mean age 6.20 years ±5.88 SD) (6 males, 7 females); group B (mean age 6.04 years ±4.99SD) (8 males, 6 females); Group C (mean age 8.07 ±5.50SD) (12 males, 8 females), 20 (77%) have GD type 1 and 6 (23%) have GD type 3.

Significant higher plasma levels of chitotriosidase, ferritin and lower platelet count among group B in comparison with group A and group C (P-value was <0.05 and <0.001 respectively for each) (Table 1 and Table 2).

Significant higher plasma levels of total acid phosphatase activity and lower hemoglobin levels among group B when compared with group A and group C (P-value was <0.001 and <0.001respectively for each) (Table 1 and Table 2).

Significant higher plasma levels of globulin among group A and group B in comparison with group C with polyclonal hypergammaglobulinemia in 46% of group A and in 61.5 % of group B (Table 1).

There was positive correlation between plasma ferritin level and plasma total acid phosphatase activity (r = 0.465 and P-value was <0.05) (Fig. 1).

Table 1. Comparison between the studied groups (group A, group B and group C) regarding complete blood count and liver function tests

Variables	Group A (n =13)	Group B (n= 13)	Group C (n= 20)	P-value		
				P_1	P_2	P_3
Complete blood count parameters:						
Hemoglobin (g/dl, mean ± SD)	12.30±1.30	9.13±2.22	12.92±0.85	.000**	.070	.000**
White blood cell count (x 10^9/L, mean ± SD)	7.32±2.37	5.80±2.61	6.94±1.63	.134	.554	.088
Platelet count (x 10^9/L, mean ± SD)	220.85±104.25	109.00±58.35	264.20±61.40	.003*	.095	.000**
Liver function tests:						
Total bilirubin (mg/dl, mean ± SD)	8.62±4.30	12.33±4.70	10.15±2.98	.051	.185	.079
Direct bilirubin (mg/dl, mean ± SD)	1.96±1.13	2.43±0.89	2.80±0.93	.072	.061	.699
Aspartate transaminase (AST) (U\l, mean ± SD)	35.85±14.62	47.41±33.12	30.93±5.52	.264	.114	.011*
Alanine transaminase (ALT) (U\l, mean ± SD)	18.615±9.55	20.58±10.06	21.17±6.68	.322	.128	.068
Alkaline phosphatase (ALP) (U\l, mean ± SD)	188.15±47.97	160.67±78.11	194.90±59.87	.296	.312	.082
Albumin (g/dl, mean ± SD)	3.64±0.73	3.68±0.57	3.88± 0.30	.866	.122	.141
Globulin (g/dl, mean ± SD).	3.23±0.63	4.23±0.88	2.53±0.33	.006*	.000**	.000**
Polyclonal hypergammaglobulinemia (NO., %).	6 (46%)	8 (61.5%)	----	----	----	----
A/G ratio(mean ± SD)	1.24±0.28	0.91±0.27	1.55±0.19	.008*	.000**	.000**

P_1: A versus B - P_2: A versus C - P_3: B versus C.
*P value >0.05 insignificant, * P<0.05 significant, **P<0.001 highly significant*

Table 2. Comparison between the studied groups regarding plasma chitotriosidase level, ferritin level and total acid phosphatase activity

Variables	Group A (n =13)	Group B (n= 13)	Group C (n= 20)	P-value		
				P_1	P_2	P_3
Plasma chitotriosidase (pg/ml, mean ± SD))	1366.46±444.83	2134.27±965.43	624.55±375.24	0.038*	0.000**	0.000**
Plasma ferritin (ng/ml, mean ± SD))	190.42 ± 144.93	476.69 ± 336.84	69.89 ± 59.93	0.017*	0.001*	0.000**
Plasma total acid phosphatase activity (U/L, mean ± SD))	13.53± 1.73	26.99 ± 12.58	11.59 ± 4.17	0.000**	0.067	0.000**

P_1: A versus B - P_2: A versus C - P_3: B versus C.
*P value >0.05 insignificant, * P<0.05 significant, **P<0.001 highly significant*

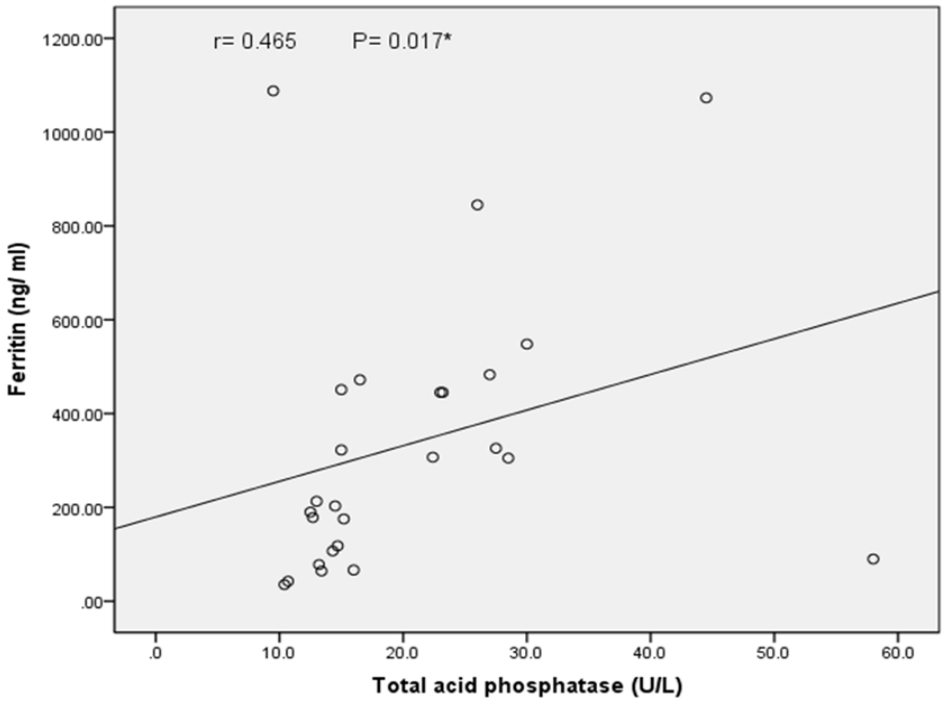

Fig. 1. Correlation between plasma ferritin and total acid phosphatase activity among GD patients showing positive correlation

4. DISCUSSION

Regarding the effect of ERT on some laboratory parameters related to pediatric patients with GD, the present study showed significant higher plasma levels of ChT, ferritin and lower platelet count among group B in comparison with group A and group C, significant higher total acid phosphatase activity and lower hemoglobin levels among group B in comparison with group A and group C, while, total leukocytic count (TLC) showed non-significant differences among group B in comparison with group A and group C.

Although the comparison regarding the previous laboratory parameters wasn't in the same group of GD patients pre and post-ERT because the study was a cross sectional, but still significant differences between group A and B were present. In accordance with these findings, Souza et al. [5] found that GD patients had significantly lower levels of hemoglobin and platelet count at diagnosis when compared with two years following the ERT with non-significant changes for TLC after treatment. Also, a study done by Sumaracet al. [6] on patients with GD, reported higher plasma levels of ChT, total acid phosphatase and ferritin before ERT with

significant initial decrease in their plasma levels following ERT. Another study done by Stein et al. [7] on patients with a diagnosis of GD, demonstrated an impressive elevation of plasma ferritin in GD with significant decrease in ferritin level after the ERT.

According to the human body physiology, when the macrophages, present in the spleen, digest the red blood corpuscles, the hemoglobin will be disappeared with subsequent rise in the lysosomal ferritin granules number and activity of acid phosphatase [8]. In the present study, there was a positive correlation between plasma ferritin level and total acid phosphatase activity with non- significant correlation neither between plasma ferritin levels and plasma ChT levels nor between total acid phosphatase activity and plasma ChT levels. On the contrary, a study done by Cabrera-Salazar et al. [9] found significant positive correlation between plasma total acid phosphatase activity and plasma ChT activity among GD patients. Also another study done by Sumaracet al. [6] reported a significant positive correlation between plasma total acid phosphatase activity and plasma ChT activity and non-significant correlations between ferritin and any of the other two biomarkers among GD patients.

The actual mechanistic relationship between GD and the associated immunoglobulinopathies is still unknown [10]. The findings of the present study revealed a significant higher plasma levels of globulin among group A and group B in comparison with group C, with statistically significant lower albumin/ globulin ratio (A/G ratio) in group A and group B in comparison with group C. Plasma protein electrophoresis revealed polyclonal hypergammaglobulinemia in 46% of group A and in 61.5% of group B. In accordance with these findings, Arıkan-Ayyıldız et al. [11] found that hyperimmunoglobulinemia was present in 66% and 60% before the administration of ERT, and after ERT intake, respectively among GD pediatric patients involved in their study.

5. CONCLUSIONS

The present study proves that plasma ChT, total acid phosphatase activity, ferritin and globulin are together helpful prognostic biomarkers in monitoring patient response, with GD, to the effect of ERT.

ACKNOWLEDGEMENTS

We would like to acknowledge the team work of the Metabolic and Genetic Disorders Unit- Faculty of Medicine- Assiut University, where the laboratory work of this study was done.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Jmoudiak M, Futerman AH. Gaucher disease: Pathological mechanisms and modern management. Br J Haematol. 2005;129:178-188.
2. Aerts JM, Hollak CE, van Breemen M, Maas M, et al. Identification and use of biomarkers in Gaucher disease and other lysosomal storage diseases. Acta Paediatr Suppl. 2005;94:43-46.
3. Thomas AS, Mehta A, Hughes DA. Gaucher disease: Haematological presentations and complications. Br J Haematol. 2014;165:427–440.
4. van Dussen L, Hendriks EJ, Groener JE, Boot RG, et al. Value of plasma chitotriosidase to assess non-neuronopathic Gaucher disease severity and progression in the era of enzyme replacement therapy. J Inherit Metab Dis. 2014;37:991-1001.
5. Souza AMA, Muniz TP, Brito RM. Study of enzyme replacement therapy for Gaucher Disease: Comparative analysis of clinical and laboratory parameters at diagnosis and after two, five and ten years of treatment. Rev Bras Hematol Hemoter. 2014;36:345–350.
6. Sumarac Z, Suvajdžić N, Ignjatović S, Majkić-Singh N, et al. Biomarkers in Serbian patients with Gaucher disease. Clinbiochem. 2011;44:950–954.
7. Stein P, Yu H, Jain D, Mistry PK. Hyperferritinemia and Iron Overload in Type 1 Gaucher Disease. Am J Hematol. 2010;85:472-476.
8. Spor S. Electron microscopic study on acid phosphatase of macrophages during the breakdown of erythrocytes in the spleen of the rat. Blut. 1970;21(4):244-250.
9. Cabrera-Salazar MA, O'Rourke E, Henderson N, Wessel H, et al. Correlation

10. of surrogate markers of Gaucher disease: Implications for long-term follow up of enzyme replacement therapy. Clin Chim Acta. 2004;344:101-107.
11. de Fost M, Out TA, de Wilde FA, Tjin E, et al. Immunoglobulin and free light chain abnormalities in Gaucher disease type I: data from an adult cohort of 63 patients and review of the literature. Ann Hematol. 2008;87:439–449.
12. Arıkan-Ayyıldız Z, Yuce A, Uslu-Kızılkan N, Demir H, et al. Immunoglobulin abnormalities and effects of enzyme replacement therapy in children with Gaucher disease. Pediatr Blood Cancer. 2011;56(4):664–666.

Peer-review history:
The peer review history for this paper can be accessed here:
http://sciencedomain.org/review-history/17623