The role of alkaline phosphatase (alp) and γ-glutamyltransferase (ggt) enzymes activities in monitoring the effect of enzyme replacement therapy in children with gaucher disease

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| Article History | ABSTRACT |
|-----------------|----------|
| Received on: 10.08.2019 | To assess the role of γ-Glutamyltransferase (GGT) and alkaline phosphatase (ALP) in the diagnosis of Gaucher disease (GD) and monitoring the response of liver and bone to the treatment with enzyme replacement therapy (ERT). A case-control study was done on 67 children age range from 3-10 years who had Gaucher disease recruited from the Pediatric Department and Unit of rare disease at Al Imamain Al-Kathemeain medical city, Gastroenterology and Hepatology Teaching Hospital, Children Welfare Hospital Consultation Clinic and Central Child’s Teaching Hospital. The activity of Alkaline Phosphatase (ALP) and γ-Glutamyl Transferase (GGT) enzymes were measured in the samples of 67 Gaucher patients who were categorized as newly diagnosed untreated patients (n=9), patients receiving ERT for 3-5 months (n=18) 6-12 months (n=20) and patients receiving ERT for more than one year (n=20) and compared with twenty age-matched control subjects. The data indicated that the levels of both ALP and GGT in GD patients (216.82 ± 64.51, 35.94 ± 7.11; respectively) were significantly higher than those of controls (163.17 ± 49.34, 28.33 ± 3.36; respectively). Levels of ALP showed to return to levels comparable to those of controls after more than one year of treatment, while GGT levels become comparable to controls’ levels within 6-12 months. Negative significant (p<0.05) correlations were obtained between the levels of ALP and GGT activities with the duration of receiving ERT. Receiver Operating Characteristic (ROC) curve results revealed that GGT showed a higher area under the curve (AUC), sensitivity, and specificity than ALP activities in all studied groups and subgroups. The possibility of using ALP and GGT as supportive diagnostic markers and as powerful markers for the monitoring of the liver and bone improvement with the administration of ERT |

Keywords: Gaucher disease, Liver function test, γ-Glutamyltransferase (GGT), alkaline phosphatase (ALP) Enzyme replacement therapy, imiglucerase, β-glucocerebrosidase, Glucocerebroside

INTRODUCTION

Gaucher disease (GD) is considered an inherited autosomal recessive disease that commonly affects Ashkenazi Jewish population. It is caused by defective functioning in β-glucocerebrosidase enzyme that known to have a catabolic role in the degradation of glucocerebroside. So, the defect in such enzyme leads to glucocerebroside accumulation in the mono nuclear phagocyte system, especially histiocytes in bone marrow lymph nodes, and the spleen
in addition to Kupffer cells in the liver, alveolar macrophages, osteoclasts in bone neural microgria in CNS, and histiocytes in peritoneum, genitourinary tracts, and the gastrointestinal tracts (Schulze and Sandhoff, 2011; Chen and , 2008).

Several biological markers and tests were studied and reported as biomarkers that involve in the etiology and pathogenesis and can be used as a tool for diagnosis and prognosis of GD in children. This wide range of biomarkers gained their importance as a result of their role in assessing the complications in organs related to these biomarkers such as tests for liver functions, spleen indices and bone integrity (Stirnemann et al., 2017).

There are many lines of treatment for GD. Still, the most recent effective therapy is that with a recombinant glucocerebrosidase enzyme as an enzyme replacement therapy (ERT), which known as imiglucerase (Cerezyme®) that given intravenously to GD patients (Brady, 2006). Patients received this treatment showed a significant improvement in GD complications such as Hepatosplenomegaly, anemia, and thrombocytopenia within 6 months (Pastores et al., 2004; Arikan-Ayyildiz et al., 2011; Shemesh et al., 2015).

\( \gamma \)-Glutamyltransferase (GGT; EC 2.3.2.2) is a microsomal enzyme that distributes widely in mammalian tissues, especially in absorptive and secretory processes, as in the bile canaliculi and brush border of renal tubules. It also presents with a less extent in the lungs, pancreas, heart and seminal vesicles. GGT known to have a role in amino acid transport across the cell membrane, so it is a location in the cell membrane to cope with this function; it transfers amino acids in the form of \( \gamma \)-glutamyl amino acids. The activity of this enzyme in the bloodstream comes mainly from the Hepatobiliary system, so its activity affected by any type of liver disease, causing an elevation in GGT activity. In comparison with the closely related enzymes; ALP, aminotransferases and 5’ nucleotidase; GGT showed to be more sensitive in detecting cholangitis, obstructive jaundice, and cholecytitis since its activity rose earlier than the rest of liver enzymes with its superiority in persisting for a longer duration (Cabrera-Abreu and Green, 2002).

Alkaline phosphatase (ALP; EC 3.1.3.1) is considered as a group of enzymes that distributed in several organs in the human body and catalyzed phosphate ester hydrolysis in an alkaline medium (Sharma et al., 2014). Normally, circulating ALP derived mainly from the liver and bones, whereas lesser amounts were arisen from the placenta, intestines, leukocytes and kidneys (Saif et al., 2005). Due to its origin, elevated levels of ALP activities denote several diseases related to organs from which it derived, such as a hepatobiliary disorder, which considered as the most common cause of ALP activities increment. Slight ALP elevation to levels less than three times the normal level is not considered as a specific and sufficient tool for diagnosis (Wiwanitkit, 2001; Nargis et al., 2014).

Activities of the ALP enzyme is an age-related level that makes it difficult to interpret in childhood. There is also a significant incidence of raising in the activities of a bone isoenzyme of ALP in the serum of subjects as a result of vitamin D deficiency. ALP activities showed to elevate in the serum of children frequently and invariably as a result of the bone isoenzyme. The interpretation of serum GGT and its elevation in children is easier than that of ALP given that it is not derived from bone and the main cause of its alteration is the administration of some drugs, so, as fewer children taking drugs than adults and if they are, this is likely to be known the change in GGT activities will be easy to predict. Finally, GGT may help in the differentiation between the situations where plasma ALP is increased as a result of bone or liver problems. It was also documented that the normal GGT levels are age dependent, and the advice is often requested to interpret the elevated levels, which are, in fact, normal for age (Cabrera-Abreu and Green, 2002).

This study was aimed to determine the activity of GGT and ALP enzymes in Gaucher patients receiving ERT in an attempt to evaluate the assumed improvement in hepatobiliary functions in the subjected children and to assess the possibility of using these two enzymes as a biomarker for the diagnosis and prognosis in these patients. Furthermore, the current study aimed to determine the possibility of using GGT alone or in combination with ALP as more sensitive markers for follow-up and monitoring of treatment.

MATERIALS AND METHODS

Sixty-seven children (35 females and 32 males) suffering from Gaucher disease were subjected to a case-control study their age ranged from three to ten years (mean ± SD; 5.72 ± 1.56) who had Gaucher disease collected from the frequently visitors for the Pediatric Department and Unit of rare diseases at Al Imamain Al-Kathameaain medical city, Gastroenterology and Hepatology Teaching Hospital, Children Welfare Hospital Consultation Clinic and Central Child’s Teaching Hospital.

The activity of Alkaline Phosphatase (ALP) and \( \gamma \)-Glutamyl Transferase (GGT) enzymes were assessed
in the samples of GD patients. Patients categorized according to the duration of ERT intake into four subgroups; the first group comprises nine patients who do not receive treatment yet while the rest of the subgroups comprise 18, 20 and 20 patients received treatment for 3-5 months, 6-12 months and more than one year; respectively. Levels of studied markers in GD patients were compared with age and sex-matched twenty control subjects (9 males & 11 females) with age ranged from three to ten years (mean ± SD; 5.95 ± 1.88). The exclusion criteria for healthy control subjects include those with chronic infections and those who suspected to have tuberculosis (TB) in addition to patients suffering from chronic inflammatory conditions such as chronic arthritis and other factors that affect enzyme activity such as:

1. Fungal infection e.g., Candida albicans
2. Parasitic infection e.g., Malaria
3. Blood disorder e.g., β-thalassemia
4. Asthma
5. Other inherited disease e.g., Niemann-Pick Disease

The practical part of the study was conducted at research laboratories in the Department of therapeutic and clinical pharmacy, Baghdad College of Medical Sciences, Baghdad-Iraq from March to July 2019. The activities of these enzymes were determined by colorimetric methods according to manufacturer information supplied by Biomaghreb, Tunisia for ALP and Abcam, USA for GGT.

The local Scientific and Review Board of the Baghdad College of Medical Sciences, Baghdad, Iraq, approved this study. Additionally, the parents or the legal guardians of all subjected children signed the informed written consents of participation in accordance with the Helsinki principles

**Statistical analysis**

The results of the current study were firstly stored in a Microsoft Excel format and expressed in the form of mean ± SD. All the comparisons that performed were accomplished statistically by using an independent t-test to compare two independent groups (patients and controls). The relationships between the different biomarkers studied in the present study were performed by using the Pearson correlation test to assess the significance and the strength of the correlation between these biomarkers. All statistical analyses used in this study were carried out by using the IBM SPSS Statistics for Windows, Version 20.0 (Armonk, NY: IBM Corp). The normality of distribution was checked using Shapiro- Wilk and Kolmogorov-Smirnov tests.

Receiver operating characteristic (ROC) analyses were accomplished as a comprehensive way for the determination of the importance, sensitivity and specificity of the studied biomarkers. In this analysis, area under the curve that is denoted as AUC which considered statistically as a powerful tool for comparison between the studied biomarkers given that the value of AUC which become closer to one, indicates that this parameter would be considered as an excellent biomarkers for the diagnosis and prediction whereas the biomarkers that showed a curve lies closer to diagonal (AUC=0.5) considered as non-significant diagnostic marker. AUC value that is closer to one is always accompanied by satisfactory values of sensitivity and specificity (hajian, 2013).

**RESULTS AND DISCUSSION**

Results illustrated in Table 1 revealed that the activities of Alkaline Phosphatase (ALP) and Gamma Glutamyl Transferase enzymes in Gaucher children (216.82 ± 64.51, 35.94 ± 7.11; respectively) were significantly higher than those of controls (163.17 ± 49.34, 28.33 ± 3.36; respectively) whereas the age of both patients and controls was non-significantly different between each other.

The activities of ALP showed the highest values in newly diagnosed patients who didn’t receive treatments yet and decreased gradually with the increase in the duration of receiving treatment. ALP activities in newly diagnosed patients were non-significantly higher than those in children receiving ERT for 3-5 months and 6-12 months, while they were significantly higher than those of controls (163.17 ± 49.34, 28.33 ± 3.36; respectively) whereas the age of both patients and controls was non-significantly differ between each other.

The activities of ALP showed the highest values in newly diagnosed patients who didn’t receive treatments yet and decreased gradually with the increase in the duration of receiving treatment. ALP activities in newly diagnosed patients were non-significantly higher than those in children receiving ERT for 3-5 months and 6-12 months, while they were significantly higher than the levels of ALP in patients receiving treatment for more than one year and control subjectsTable 2. Patients receiving treatment for 3-5 months showed a non-significant difference in ALP activities with patients receiving ERT for 6-12 months but significantly higher than ALP activities in patients receiving treatment for more than one year and controls. Additionally, Gaucher patients receiving treatment for 6-12 months showed to be non-significantly differ in the activities of ALP with patients receiving the same treatment for more than one year but still significantly higher than controls’ ALP activities. On the other hand, patients receiving treatment for more than one year showed to be the only patients’ subgroup that non-significantly.

Gamma Glutamyl Transferase enzyme activities showed a pattern nearly similar to that of Alkaline phosphatase, as demonstrated in a Table 3 with some differences in the data obtained in which the
Table 1: The comparison of the studied parameters between GD patients and control group by independent t-test

| Parameter        | Control      | Patients     | P-value |
|------------------|--------------|--------------|---------|
|                  | N=20         | N=67         |         |
| ALP (U/L)        | 163.17 ± 49.34 | 216.82 ± 64.51 | 0.001*  |
| GGT (U/L)        | 28.33 ± 3.36  | 35.94 ± 7.11  | <0.001* |
| Age (year)       | 5.95 ± 1.88   | 5.72 ± 1.56   | 0.576   |

activities in patients receiving the treatment for 6-12 months were non-significantly differ from those of controls. These patients also showed significantly lower levels of GGT activities than those of newly diagnosed patients and those receiving ERT for 3-5 months.

The two studied parameters showed to be significantly and positively correlated with each other and both of them showed a significant negative correlation with age of patients and the duration of receiving the treatment as postulated in a Table 4.

Results of ROC revealed that the activities of GGT showed a higher area under the curve (AUC), sensitivity and specificity (0.857, 74.6% and 85%; respectively) than those of ALP (0.747, 68.7% and 65% respectively) as clarified in a Table 5. Moreover, Table 6 revealed that the activities of ALP showed relatively moderate AUC, sensitivity and specificity in newly diagnosed patients and those receiving ERT for 3-5 months in comparison with control subjects whereas all other ROC curve results for ALP activities among studied groups showed a low AUC, sensitivity and specificity.

On the other hand, GGT activities showed high AUC with excellent sensitivity and specificity in newly diagnosed patients when compared with other subgroups subjected to the present study, including a control group. Additionally, patients receiving treatment for 3-5 months showed also high AUC values with excellent sensitivity and specificity in comparison with patients receiving the treatment for longer periods.

GD defined as a metabolic disorder and a sphingolipidosis (a sub-type of lysosomal storage disease), which considered as a rare disorder that is inherited as an autosomal recessive metabolic disorder that results in in from deficiency glucocerebrosidase enzyme which is responsible for the degradation of glucocerebroside. It is chronic and progressive in its clinical presentation that owned to the involvement of several organs such as liver, spleen and bones (Rizk et al., 2015) that emphasize the importance of discovering and assess biomarkers to evaluate the progression of GD, so ALP and GGT that have known value in the assessment of hepatobiliary function were the targets of the present study due to their levels that assumed to elevate in GD patients as a result of liver and bone involvement. The previous assumptions confirmed by the results demonstrated in the Table 1 in which a significant increase in the levels of ALP and GGT were obtained in comparison with healthy children.

In the current study, the level of ALP activities might be elevated as a result of liver and bones involvement and reduced with a continuous treatment with enzyme replacement therapy (ERT) which is consistent with previous literature which stated that the treatment with ERT causes an improvement in liver and spleen sizes and also improves their functions within the first six months of treatment while the bone response to the ERT was much slower and needed more than one year (Linari and Castaman, 2015; Smid et al., 2016).

These findings were in agreement with the results obtained in the present study that demonstrates a significant reduction in ALP activities in patients receiving treatment for more than one year to levels nearly comparable to those of controls. However, the levels of ALP in patients receiving the treatment for less than one year still significantly higher than those of healthy controls and non-significantly differ from that of newly diagnosed patients which is possibly due to that the source of this enzyme is the bone in addition to liver, so, with treatment, the improvement possibly was in liver with a reduction in its isoenzyme while the bone isoenzyme still elevated due to the late response of bone to ERT which collectively lead to a non-significant decrease in the ALP level within the first year of treatment.

This suggested explanation is proved by results demonstrated in Table 2 in which the levels of ALP in all studied subgroups were listed in which significant increases in the level of ALP in newly diagnosed patients who didn’t receive treatment yet, patients receiving treatment for 3-6 months and patients receiving treatment for 6-12 months com-
Table 2: Comparison between the levels of ALP (U/L) in all studied groups.

|                  | Mean±SD     | 3-5 months | 6-12 months | > 1-year treatment | Control |
|------------------|-------------|------------|-------------|--------------------|---------|
| Newly diagnosed  | 254.68±68.84| 0.338      | 0.181       | 0.01               | <0.001  |
| 3-5 months       | 230.58±56.14| 0.502      | 0.024       | <0.001             |         |
| 6-12 months      | 216.62±69.06| 0.152      | 0.008       |                    |         |
| > 1-year treatment| 187.6±55.79  |           |             |                    | 0.151   |

Table 3: Comparison between the levels of GGT (U/L) in all studied groups.

|                  | Mean±SD     | 3-5 months | 6-12 months | > 1-year treatment | Control |
|------------------|-------------|------------|-------------|--------------------|---------|
| Newly diagnosed  | 45.24±6.77  | <0.001     | <0.001      | <0.001             |         |
| 3-5 months       | 40.97±4.9   | <0.001     | <0.001      | <0.001             |         |
| 6-12 months      | 32.14±3.65  | 0.927      | 0.052       |                    |         |
| > 1-year treatment| 31.03±3.98  |           |             |                    | 0.293   |

Table 4: Correlation between the studied parameters in whole Gaucher patients

|                  | ALP         | GGT         | Age         | Duration of treatment |
|------------------|-------------|-------------|-------------|-----------------------|
| ALP r            | 0.643*      | -0.4*       | -0.345*     | 0.001                 |
| ALP p            | <0.001      | 0.001       | 0.004       |                       |
| GGT r            | -0.55*      | -0.736*     |             | <0.001               |
| GGT p            | <0.001      |             |             |                       |
| Age r            |             | 0.619*      | <0.001      |                       |
| Age p            |             |             |             |                       |

Table 5: ROC curve results for all studied parameters in patients comparing with control

| Parameters | AUC  | Sensitivity (%) | Specificity (%) | Cut-off value |
|------------|------|-----------------|-----------------|--------------|
| ALP        | 0.747| 68.7            | 65              | 176.34       |
| GGT        | 0.857| 74.6            | 85              | 31.75        |

Comparing with control subjects while the level of ALP in patients receiving treatment for more than one year showed a non-significant differences from that of control. These results also disagree with results obtained by Ciana and his coworkers, 2003 who found that there were non-significant differences observed in alkaline phosphatase levels between Gaucher patients and control subjects (Ciana et al., 2003). On the other hand, the more recent study reviewed that sometimes Gaucher patients may suffer from cholestasis with an increase in alkaline phosphatase and bilirubin levels (Stirnemann et al., 2017).

The other possible explanation of the non-significant increase in the activities of ALP enzyme in the first year of treatment is the fact that the treatment may cause an increase in the bone turnover that leads to an elevation in ALP levels of a bone origin that compensates the decrease in liver isozymes which is inconsistent with other previously published literature (Dussen et al., 2011; Giuffrida et al., 2012).

The results mentioned above and explanations proved by Receiver Operating Characteristic (ROC) curve data that illustrated in Tables 5 and 6 in which ALP activities showed a moderate AUC with poor sensitivity and specificity that caused by the slow decrease in its level in Gaucher patients and also caused by presence of more than ALP isozymes originate from different organs which involved in this.
Table 6: ROC curve results of ALP among all studied groups

|                  | Newly diagnosed | 3-5 months | 6-12 months | > 1-year treatment |
|------------------|-----------------|------------|-------------|--------------------|
| Control          | AUC 0.889       | 0.817      | 0.744       | 0.625              |
|                  | Sensitivity (%) | 88.9       | 72.2        | 90                 | 75                 |
|                  | Specificity (%) | 85         | 80          | 55                 | 55                 |
| Newly diagnosed  | AUC 0.593       | 0.672      | 0.789       |                    |
|                  | Sensitivity (%) | 77.8       | 77.8        | 88.9               |
|                  | Specificity (%) | 44.4       | 65          | 80                 |
| 3-5 months       | AUC             |            | 0.575       | 0.731              |
|                  | Sensitivity (%) |            | 61.1        | 83.3               |
|                  | Specificity (%) |            | 60          | 75                 |
| 6-12 months      | AUC             |            |             | 0.62               |
|                  | Sensitivity (%) |            |             | 55                 |
|                  | Specificity (%) |            |             | 80                 |

Table 7: ROC curve results of GGT among all studied groups

|                  | Newly diagnosed | 3-5 months | 6-12 months | > 1-year treatment |
|------------------|-----------------|------------|-------------|--------------------|
| Control          | AUC 0.994       | 1.00       | 0.815       | 0.710              |
|                  | Sensitivity (%) | 100        | 100         | 65                 | 70                 |
|                  | Specificity (%) | 95         | 100         | 95                 | 60                 |
| Newly diagnosed  | AUC             | 0.716      | 0.944       | 0.956              |
|                  | Sensitivity (%) | 77.8       | 88.9        | 88.9               |
|                  | Specificity (%) | 66.7       | 100         | 100                |
| 3-5 months       | AUC             |            | 0.981       | 0.983              |
|                  | Sensitivity (%) |            | 94.4        | 94.4               |
|                  | Specificity (%) |            | 95          | 95                 |
| 6-12 months      | AUC             |            |             | 0.59               |
|                  | Sensitivity (%) |            |             | 65                 |
|                  | Specificity (%) |            |             | 55                 |
disease. On the other hand, ALP activities can be considered as a very good parameter for the diagnosis confirmation as it increased in newly diagnosed untreated patients and showed a high AUC, sensitivity and specificity that persist to the first 5 months of treatment and become declined with continuous treatment. No previous studies tested the specificity and sensitivity of ALP activities measurement in Gaucher patients. Still, most of the studies agree with that ALP levels increase as result of a hepatobiliary and bone involvement and did not return to levels comparable to healthy children before at least one year of treatment (Dussen et al. 2011; Giuffrida et al. 2012; linari and castaman 2015; Smid et al. 2016)

Gamma Glutamyl Transferase (GGT) enzyme considered as a second marker for the hepatobiliary involvement that occurs in Gaucher patients and its manner of elevation showed to be parallel to that of ALP except that it didn’t affect by bone involvement (Hall and Cash, 2012; Badrick and Turner, 2016)

GGT doesn’t include as common as ALP in the profile of adult or pediatric liver function tests (LFT) and due to some shortcomings in adults mainly, GGT reliability was limited as it affected by various factors and drugs since it is induced by several drugs that make the interpretation of test results difficult beside its high sensitivity to minor hepatic injury that lead to an abnormal result in adult patients who had no primary hepatobiliary disease. However, in pediatric practice, GGT has significant advantages over ALP. The GGT superiority owned to that ALP activity in childhood is age-dependent, leading to a difficulty in result interpretation. There is also a significant incidence of raised serum ALP bone isoenzyme activity due to vitamin D deficiency, which doesn’t affect the activities of the GGT enzyme. High serum ALP in childhood was observed frequently and invariably as a result of bone isoenzyme that might be liberated due to active osteoclast cells. An elevated GGT activity assumed to be easier to interpret in children as they received the drug in a more controlled manner than adults. Thus, GGT can be used as a supportive marker for ALP in situations where plasma ALP is increased and it is obvious whether the increased activity is caused by bone or liver isoenzymes (Cabrera-Abreu and Green, 2002; Hall and Cash, 2012; Badrick and Turner, 2016), the current study, the activities of GGT in Gaucher patient were significantly higher than those of controls that confirm the results obtained by ALP in that Gaucher patients have hepatobiliary involvement which causes the elevation of these two enzymes as illustrated in Table 1. but, the elevation of GGT showed to be more sensitive and specific than ALP levels as demonstrated in Table 5 which clarify that the activities of GGT showed a higher AUC, sensitivity and specificity than those of ALP that is owned to that it has no bone origin that may affect its specificity and sensitivity as in the case of ALP (Badrick and Turner, 2016).

Additionally, previous literature reported that GGT is a glycoprotein-enzyme which predominantly linked to cell membranes in tissues and organs that possess a high secretion or absorption capacity. This assumes that beyond its predominantly hepatocytic localization it is also present in several other organs such as spleen (Hannuksela et al., 2007; Balan et al., 2018) which is also involved in Gaucher disease and several researches reported that the spleen indices such as hemoglobin and platelets level become improved within the first 6 months in addition to splenomegaly that showed to be improved within the first 12 months which is parallel to the hepatobiliary improvement (Pastores et al., 2004; Arikan-Ayyildiz et al., 2011). So, both liver and spleen showed to response nearly at the same time, which might be the cause of significant GGT reduction within the first 12 months as listed in the current research data.

Furthermore, sub grouping the patients in accordance with the duration of receiving treatments revealed an interesting point that prove the superiority of GGT on ALP as a prognostic and follow-up marker as it increased significantly in patients did not receive treatment and remain elevated in the first 5 months, after that the activities become decreased significantly and reach a levels comparable and non-significantly differ from that of controls after 6 months of treatment and declined slightly after one year of treatment which can provide an information about how long the hepatobiliary functions require to reverse and return to the normal values and can be used in combination with ALP to determine the time required for bone to be improved as its concluded from the significant reduction of ALP activities after one year of treatment. So, we can conclude from the combination of GGT and ALP that using ERT cause an improvement in hepatobiliary functions within 6-12 months whereas bony involvement become improved after more than one year of treatment with ERT these deductions are in agreement with several previous reports (Arikan-Ayyildiz et al., 2011; Shemesh et al., 2015).

ROC curve results illustrated in the Table 7 highlights the role of GGT as a confirmation marker for the diagnosis of Gaucher disease beside other
laboratory investigations as it showed a high AUC with excellent sensitivity and specificity when newly diagnosed patients compared with controls. Moreover, ROC curve results support the assumption that GGT activities can provide a powerful marker for prognosis and follow-up patients receiving ERT for Gaucher disease as it showed high AUC values and excellent sensitivity and specificity in patients receiving treatment for more than a year in comparison with those receiving ERT for less than 6 months. Ultimately, results of the present study revealed that both studied markers; ALP and GGT were positively and significantly correlated with each other and both of them were negatively and significantly correlated with the duration of receiving treatments that confirm the results mentioned above in that these markers decline as a result of an improvement in the liver spleen and bone with a variations in the time required for these organs to respond properly which is also in agreement with previous literature (Arikan-Ayyildiz et al., 2011; Shemesh et al., 2015). Levels of ALP activities showed a significant negative correlation with the age of children subjected to the current study which is consistent with previous literature that owned the high activities of ALP enzyme to the elevated rate of bone turnover in children (Otero et al., 2011; Turan et al., 2011). GGT showed a similar manner of correlation with age despite previous work deduce that the level of GGT increased with age (Bussler et al., 2018). The possible explanation of this conflict results is the duration of treatment; older children mainly received ERT for a longer time as they diagnosed before the younger ones which cause a significant reduction in the levels of both ALP and GGT which lead eventually to the significant negative correlation between age of patients and both ALP and GGT activities.

**CONCLUSION**

In the end, we can conclude that ALP and GGT can be used as supportive diagnostic markers beside the other investigations with superiority for GGT in the monitoring the response of liver and possibly spleen to treatment whereas combining ALP with GGT can provide a powerful tool for the assessment of the bone improvement.

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