International Journal of Medicine Research
ISSN: 2455-7404
www.medicinesjournal.com
Volume 3; Issue 3; July 2018; Page No. 15-18

Linearization of the calibration curve of the evaluation of transaminases activity by the colorimetric method

Mwelo Jan[1], Diyi L[2], Nzingula P[3], Mayangi M[4], Inkalaba KG[5], Mbinze KJ[6], Kodondi KK[7]

[1, 3, 5, 7] Laboratory of Biochemistry-Hematology, Faculty of Pharmaceutical Science, University of Kinshasa, DR Congo

[2] Research Institute in Sciences of Health, DR Congo

[6] Drugs Laboratory Analysis, Faculty of Pharmaceutical Science, University of Kinshasa, DR Congo

[4] Department of Basic Science, Faculty of Pharmaceutical Science, University of Kinshasa, DR Congo

Abstract

Objectives: this work has as objective to make linear the calibration curve obtained by the colorimetric method by introducing the regression equation that will be able to have an impact in the interpretation of the results, necessary to the diagnosis of hepatic and cardiac pathologies to a certain extent.

Equipment and methods: We worked with 43 serums blood. Transaminases were proportioned by the colorimetric method whose reading was achieved by means of a spectrophotometer. The various activities obtained underwent mathematical transformations by the method of square roots in order to make the curve linear.

Results: the results made it possible to obtain the regression equations. For the glutamate oxalo-acetate transaminase 0.016 X + 0.443 with R2= 0.995 and for the glutamate pyruvate transaminase: 0.022 X+ 0.443 with R2 = 0.982. The statistical analysis by the test of student (α= 0.05) revealed that there was no significant difference between the results in proportioning before linearization and after linearization.

Conclusion: We didn't note a difference between the colorimetric method passing by the calibration curve and the linearized method. However, if one wants to have values of transaminases which approach the true value, he would be desirable to carry out the linearization of the calibration curve obtained during colorimetric proportioning. We propose that the laboratories resort the straight regression lines found in this work. To the contrary case, we recommend the use of the enzymatic method.

Keywords: colorimetric method, transaminases activity, linearization, calibration curve

1. Introduction

Transaminases are important enzymes of the human organism whose the role is to transfer an amine group during many chemical processes in human organism [1]. They are present in all fabrics but their presence in blood with a high concentration is often linked to myocardium (infarction) or liver (necroses, hepatitis) attacks. In that case, the serum rates of transaminases are increased. One distinguishes two types of transaminases: ALAT (alanine transférase, SGPT) prevails in the livers and ASAT (aspartate transférase, SGOT) prevails in the cardiac muscles [2, 3, 4].

By what precedes we understand that the determination of the activity of transaminase in the hospital mediums makes it possible to characterize as well the affections hepatitises as cardiac [2, 5].

The proportioning of serum transaminases is a frequent biological regulation in general medicine. Several methods are used to evaluate the activity of transaminases. Among those, two methods are usually used at the laboratory: enzymatic method and colorimetric method. The use of the first doesn't pose any problem because it exploits specified enzymes. On the other hand second is likely to introduce an important skew.

Indeed, the determination of the activity of transaminases by the colorimetric method passes obligatorily by a calibration curve. The various activities are obtained by extrapolation in a system of axis of which the optical densities are in ordered and the activities in UI/l abscissa. The curve thus obtained is not a linear line [6]. So obtaining the activities of transaminases by extrapolation starting from this curve could give results with skew. This is why within the frame of our work, we judged important to linearize this calibration curve after data mathematical transformation's, in order to reduce appreciably skews and to approach the true values. The activities of transaminases will be obtained starting from the equation of the straight regression line (there = ax + b). With this intention, we go:

- To linearize the calibration curve of proportioning of transaminases by the colorimetric method.
- To check if there is difference between the results we got before linearization of the curve and those obtained afterwards, likely to influence diagnosis

We leave the assumption according to which the results got after linearization of the curve would be significantly different from those obtained without linearization of the calibration curve.

2. Equipment and Methods

Patients

We worked with 43 serums of the people who attend the laboratory of biochemistry-hematologic of Faculty of pharmaceutical Science of the University of Kinshasa. The total blood taken in a dry tube was centrifuged (centrifugal machine sigma) with 3000 turns during 5 minutes and then elutriated. Evaluation of the activity of transaminases were proportioned by the colorimetric method whose reading was achieved by means of a spectrophotometer (Spectronic 200). The various activities were found by extrapolation on the calibration curve which takes a curvilinear form [6].

Mathematical transformations of the results

There exist several processes of mathematical transformation aiming at returning a curve linear such as those using the mathematical logarithms, opposite, the roots squares etc. [7, 8]. In our case, we chose the method using the squares roots. Then the data transformation having been used for the development of calibration curve by mathematical equations in order to obtain a linear straight regression line of the form there = ax + B. The activities were obtained starting from this new curve. The oxalo-acetic acid reacts with 2.4 dinitro-phenyl hydrazine to form Hydrazone. The intensity of colouring of formed hydrazone is directly proportional to the produced quantity of oxaloacétate, therefore directly proportional to the activity of GOT [9].

The intensity of colouring of formed hydrazone is directly proportional to the produced quantity of pyruvic acid, therefore directly proportional to the activity of GPT [9, 10].

Comparison of the results by a statistical test

The two series of results were compared by statistical methods in particular the test of student after checking of the homoscedasticity.

The results got after linearization of the curve were compared by the test of student (comparison of two averages) with those obtained by the method of reference in order to conclude with a significant difference or not between the two methods. To test the statistical assumption, one Formula One a worthless assumption (H0) and an alternative assumption (H1). The problem consists in using the results of the sample to reject H0 or not. One calculates the variable with this intention "T". If T calculated is lower than T tabular with the threshold of significance chosen, we keep the assumption i.e. we consider the difference observed is no significant; on the other hand if T calculated is higher than T tabular, it there is rejection of the worthless assumption, we say that the difference observed is real [11, 12].

3. Results

3.1 Result of the linearization

Linearization of the calibration curve of the GOT

The graphs of calibration curve GOT before and after linearization are mentioned below:

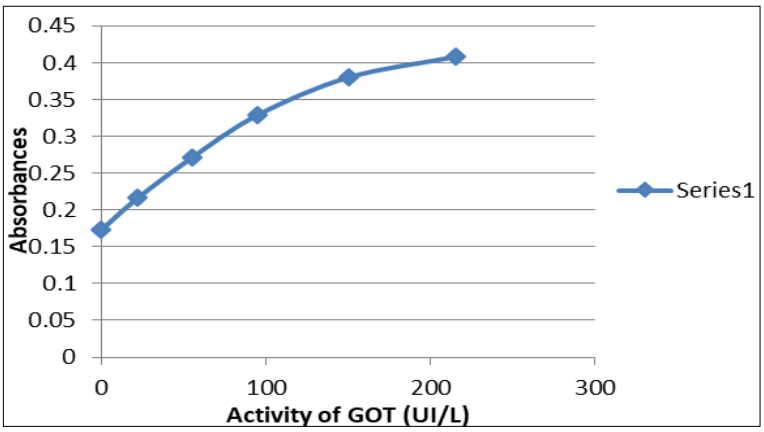

Fig 1: Calibration curve GOT before Linearization

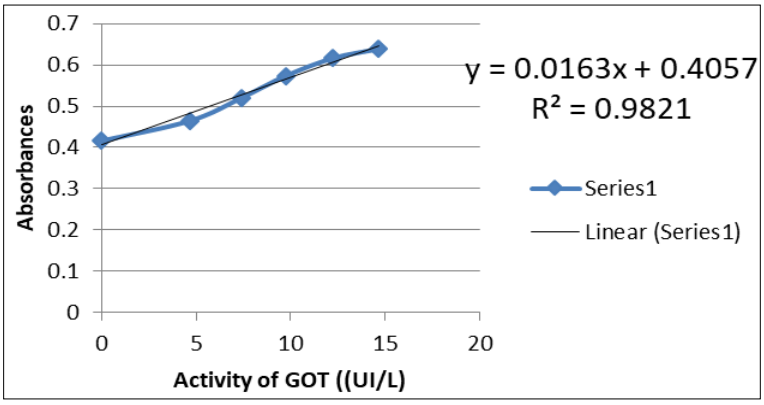

Fig 2: Calibration curve GOT after Linearization

The linearization of this curve made it possible to obtain a line of the form Y= Ax+b is Y= 0,0163X+0,4057 with R2=0,9821 Linearization of the calibration curve of the GPT

The graphs of calibration curve GPT before and after linearization are mentioned below:

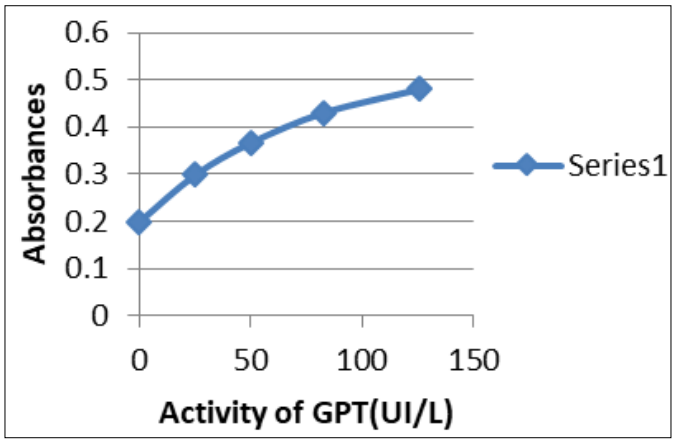

Fig 3: Calibration curve GPT before Linearization

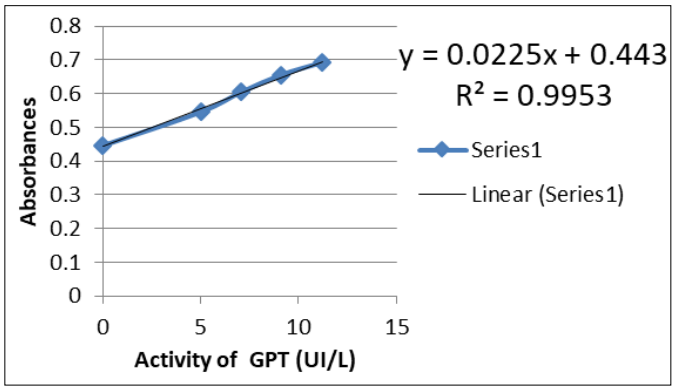

Fig 4: Calibration curve GPT after Linearization

3.2 Comparison of the results between the two methods

The results found after linearization of the curve were compared with those found before linearization to see whether there would be no significant difference between the two methods. The selected statistics are that of student. Before its application, a test of homoscedasticity was carried out whose results are included in table I

Table 1: Results of the homoscedasticity

	GOT	GPT
$F_{calculed}$	1,19	1,2
$F_{tabular}$	1,67	1,67

F calculated < Ftabular then the variances are homogeneous; we can apply the test of student. The résults of data analysis by the test of student are included in table II.

Table II. Results of the analysis of the data by the test of student

Parameters	Activity of GOT en UI/L		Activity of GPT en UI/L	
	Méthod without linearization	Méthod with linearization	Méthod without linearization	Méthod with linearzation
Average	105,85	92,84	17,024	12,823
Variance	1176,96	983,62	359,09	299,097
T calculeted	1,8		1,073	
Ttabular α=0,05)	1,9867		1,9867	

4. Discussion

Our work consisted in linearizing the calibration curve obtained during the proportioning of transaminases by the colorimetric method. We initially proportioned the samples by the colorimetric method which passes obligatorily by the calibration curve. The activities of transaminases are obtained

by extrapolation starting from the aforementioned curve. Having noted that the calibration curve was not linear to allow a good extrapolation free from any skew, we operated mathematical transformations of the data of the curve by the method of square roots in order to obtain a linear right with an equation of the form there = ax + B allowing to determine the various activities [8, 13].

Thus, the activities of transaminases obtained were compared with those of the calibration curve not linearized to note if there were no significant differences between the two methods. We chose the test of student which compares to the average two population subjected under investigation. The application of this test requires as a preliminary to check the homogeneity of the variances. The application of the test of homoscedasticity for GOT and GPT showed that the two variances were homogeneous (Fcalculé<Ftabulaire) [7, 11]. Thus the two methods could be compared by the test of student.

The application of this test of Student showed that Tcalculted for GOT/GPT was lower than T tabular for the two methods. Indeed, T calculated being lower than T tabular for the two methods, one does not reject the worthless assumption. Therefore, the two developed methods have equal averages.

One can conclude that there is no significant difference between the colorimetric method without linearization and the colorimetric method with linearization of the calibration curve.

What enables us to accept the worthless assumption [11, 12]. Have regard of these results, it is necessary to come to a conclusion about non the significant difference between the two methods.

5. Conclusion

The purpose of our work was to check if the results of the proportioning of transaminases by the colorimetric method using the calibration curve were different after linearization from the calibration curve by the mathematical equations.

The analysis of the results by the test of student showed that there were no significant differences between the two methods. Thus during the proportioning of transaminases by the colorimetric method using the calibration curve, it is not necessary to linearize the aforementioned curve.

However, if one wants to have values of transaminases which approach the true value, he would be desirable to carry out the linearization of the calibration curve obtained during colorimetric proportioning. We propose that the colorimetric laboratories using the method use the straight regression lines found in this work. To the contrary case, we recommend the use of the enzymatic method.

6. References

1. Rawn DJ. Traité de biochimie. Ed Deboek, 458-468
2. Bouvenot, Devul Der B, Guikkevin C, Queneau P, Schaeffer A. pathologie médicale 4: Gastro enterologie, hépathologie, hématologie, Masson, Paris, 1995, 238-278.
3. Beaudeux JL, Genevieve D. Biochimie médicale-marqueurs actuelles et perpectives/ Lavoisier, 2èdition, 2011, 601.
4. Eugène C, Contentin L, Beaulieu S. Les hépatites virales. Elsevir Masson, Paris, 2ème édition, 2004, 194.
5. Bernard S. Biochimie clinique, instrument et technique de laboratoire Maloire, Paris, 1985, 187-188.
6. Manuel de procedure de dosage colorimetrique des transaminases. Laboratoire Biomerieux/
7. Jacques Kruh, Biochimie I. Biologie cellulaire et moléculaire. Herman, Paris, 1995, 155-199
8. Hans Ulrich et Gawehn K. Principes de l'analyse enzymatique, Technique et Documentation, Paris, 1979, 17-98.
9. Muray, Bender, Bothan, Rosdwel, Kennlly. Biochimie Harper, ed Deboek, 2011, 59.
10. Boulanger P, Polonouski J, Taueau F, Mandel P, Biserrte G. Biochimie médicale: fascicule R: les constituants de l'organisme vivant. Masson, Paris, 1997, 345.
11. Schwart: méthodes statistiques à l'usage de médecin et de biologiste. Flammarion, Paris, 1963, 163-171.
12. Rudaz S, et Rozet E. Guidelines and validation issues in pharmaceutical analysis. Departement of analytical chemistry. Institute of Pharmacy. Cours, Université de Genève, 2010.