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Estimating the Plasma and Serum Activity Levels of Aspartate Aminotransferase and Alanine Aminotransferase, in Live Animals Using Regression Model

[1]V.M. Ahur, [2]P.O. Agada and [1]S.A. Saganuwan

[1]Department of Veterinary Physiology, Pharmacology and Biochemistry, College of Veterinary Medicine, University of Agriculture, Makurdi, Nigeria

[2]Department of Mathematics/Statistics/Computer Science, College of Science, University of Agriculture, Makurdi, Nigeria

Corresponding Author: Victor Masekaven Ahur, Department of Veterinary Physiology, Pharmacology and Biochemistry, College of Veterinary Medicine, University of Agriculture, Makurdi, Nigeria Tel: (234) 08065728084

ABSTRACT

There is a challenge in estimating the plasma/serum activity levels of aspartate aminotransferase (AST) and alanine aminotransferase (ALT) concentrations using continuous absorbance values determined from previous experiments by the Randox Company. Reitman and Frankel determined the activity levels of AST over a discrete range of absorbance values (0.02, 0.17) with a step size of 0.01 and an activity level of ALT over a discrete range of absorbance values (0.025, 0.500) with a step size of 0.025, making the estimation a difficult task. The work provides an AST and ALT absorbance regression models for estimating the plasma/serum activity levels of AST and ALT. The validity of the AST and ALT absorbance regression models is confirmed by their respective values of coefficients of determination (R^2) at 99.65 and 99.44% suggesting the reliability of the model. Hence, the model can be used to determine the plasma/serum activity levels of AST and ALT in live animals.

Key words: Regression, absorbance, activity level, enzymes

INTRODUCTION

There has been rapid increase in knowledge of hepatocyte injury which in turn had increased the number of tests available for measurement of liver function parameters (Dufour *et al*., 2000). Most enzymes reside within cells, where they function in various phases of intermediary metabolism and only small quantities of them are present in the serum (Murray *et al*., 2006). Pronounced myocardial infarction and acute hepatitis, can cause cellular damage and some of the intracellular content will escape into extracellular fluid and eventually reach the serum in high concentrations (Sallie *et al*., 1991; Khadr *et al*., 2007; Singh *et al*., 2011; Alisi *et al*., 2011). It these situations the diagnostic values of determinations of plasma or serum enzymes have so far been used (Murray *et al*., 2006). In the asymptomatic or sub-clinical stage of hepatitis, elevated concentrations of serum enzymes may be the sole indicator of the presence of disease (Adolph and Lorenz, 1982). In chronic diseases such as hepatic cirrhosis, biliary obstruction or pancreatitis, the concentrations of enzymes in plasma or serum may be elevated during periods of progression of the disease (Hultcrantz *et al*., 1986).

Serum or plasma enzyme levels have been always considered as markers for monitoring degree of chemically induced liver damage (Lin and Wang, 1986; Ngaha *et al*., 1989; Hukkeri *et al*., 2002). The enzyme L-alanine aminotransferase (L-ALT), L-aspartate aminotransferase (L-AST), alkaline phosphatase (ALP), total and direct bilirubin, gamma glutamyl transpeptidase (GGT), sorbitol dehydrogenase (SDH) and Lactate dehydrogenase (LDH) are often used in assessing the integrity of the liver cells (Ngaha *et al*., 1989; Obi *et al*., 1998; Dufour *et al*., 2000; Hukkeri *et al*., 2002). Among the commonly used enzymes for hepatic damage are L-aspartate aminotransferase and L-alanine aminotransferase (Hukkeri *et al*., 2002; Chakrabarki, 2006). Both are found in the liver and are intracellular enzymes necessary for amino acid production (Murray *et al*., 2006). They are however released into the systemic circulation when there is serious tissue damage or cell death (Siegers *et al*., 1985; Obi *et al*., 2001).

Since the values of serum and plasma activity levels of AST and ALT are deduced from Reitman and Frankel table over a discrete range of absorbance values, there is need for redirection of attention towards estimating AST and ALT in live animals using regression model with a view to having more precise and accurate results in addition to saving time.

MATERIALS AND METHODS

The kits for analyzing serum or plasma AST and ALT were bought from Randox (Germany) and the absorbance-activity levels of the kits as estimated from previous research works were enclosed in the pack that carried the kits. The previous estimated absorbance-activity levels were analyzed in two days thereby arriving at relevant regression model that can be used for extrapolation of activity level from absorbance. The experiment was done in the Computer Science Laboratory of the Department of Mathematic/Statistic/Computer, College of Science, University of Agriculture, Makurdi, Nigeria.

Simple regression model: A simple linear regression model is given as follows (Arua *et al*., 2000):

$$Y = \beta_0+\beta_1X+\varepsilon \quad (1)$$

where, Y is the dependent variable, X is the independent variable, β_0 and β_1 are regression parameters and ε is the random error. The objective is to minimize ε as much as possible so that the regression model accounts for a good percentage of the total variation in the data. SSS) equals the sum of squares of regression (SSR) plus the sum of squares of error (SSE) written:

$$SST = SSR+SSE \quad (2)$$

So that:

$$\frac{SSR}{SST} = 1-\frac{SSE}{SST} \quad (3)$$

where, SSR/SST = R^2 the coefficient of determination, is the ratio of the model explained variation to the total variation and SSE/SST is the proportion of the total variation unexplained by the model. These values expressed in percentage, can be used quickly to validate the model.

Quadratic regression model: A regression model can be non-linear; a simple form of this is the quadratic regression model (Arua *et al*., 2000):

$$Y = \beta_0+\beta_1X+\beta_2X^2+\varepsilon \quad (4)$$

where, Y is the dependent variable, X is the independent variable, β_0, β_1 and β_2 are regression parameters and ε is the random error. The coefficient of determination (R^2) still applies in validating the model in order to measure how well it fits the data.

Modelling the relationship between the activity levels of AST and ALT in plasma/serum and absorbance: The simple steps of fitting a regression model into a scattered plot of data using the Microsoft Excel data sheet is being used. The regression model equation and coefficient of determination (R^2) are displayed on the graph. This process is carried out for determining the regression equations and coefficient of determination (R^2), respectively that can be used to relate activity levels of AST and ALT and absorbance. The Randox-Reitman and Frankel AST and ALT activity level tables used in fitting the respective regression models are given below (Table 1, 2).

AST-absorbance regression model: The data in Table 1 is typed into the Excel data sheet and a quadratic trend line:

$$y = 2491.2x^2+36.516x+7.0661$$

is seen to be a line of best fit for the scattered plot. This is true by judging from the value of R^2 measuring 99.65% model explained variation (Fig. 1).

Table 1: Randox-Reitman and Frankel aspartate aminotransferase (AST) activity level

Absorbance	AST activity level (IU L^{-1})
0.02	7
0.03	10
0.04	13
0.05	16
0.06	19
0.07	23
0.08	27
0.09	31
0.10	36
0.11	41
0.12	47
0.13	52
0.14	59
0.15	67
0.16	76
0.17	89

Table 2: Randox-Reitman and Frankel alanine aminotransferase (ALT) activity level

Absorbance	ALT activity level (IU L^{-1})
0.025	4
0.050	8
0.075	12
0.100	17
0.125	21
0.150	25
0.175	29
0.200	34
0.225	39
0.250	43
0.275	48
0.300	52
0.325	57
0.350	62
0.375	67
0.375	72
0.400	77
0.425	83
0.475	88
0.500	94

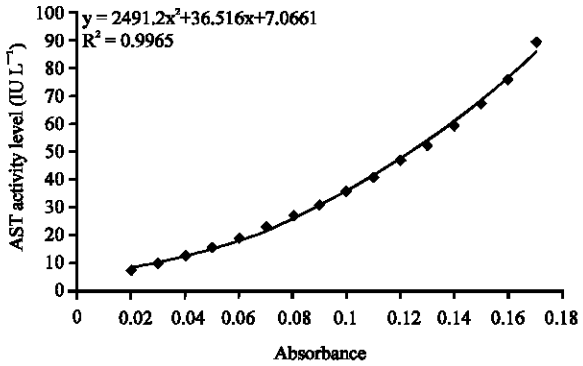

Fig. 1: Aspartate aminotransferase (AST) absorbance regression model

ALT-absorbance regression model: The data in Table 2 is typed into the Excel data sheet and a simple regression model trend line:

$$y = 192.81x-3.2901$$

is seen as the line of best fit for the scattered plot. This is also true judging from the value of R^2 measuring 99.44% model explained variation (Fig. 2).

Model validation: Beside the good R^2 values for both models, the Paired-Samples t-test ran from the SPSS statistical software, shows P-values of 0.995 and 0.998 for the AST-absorbance and the

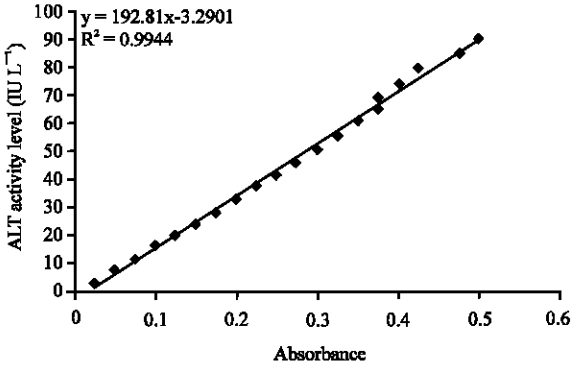

Fig. 2: Alanine aminotransferase (ALT) absorbance regression model

Table 3: Actual aspartate aminotransferase (AST) and alanine aminotransferase (ALT) activity levels and their corresponding model estimates

Actual AST activity level (IU L^{-1})	AST-absorbance model estimate (IU L^{-1})	Actual ALT activity level (IU L^{-1})	ALT-absorbance model estimate (IU L^{-1})
7	8.79278	4	1.5299
10	10.40348	8	6.3499
13	12.51242	12	11.1699
16	15.11960	17	15.9899
19	18.22502	21	20.8099
23	21.82868	25	25.6299
27	25.93058	29	30.4499
31	30.53072	34	35.2699
36	35.62910	39	40.0899
41	41.22572	43	44.9099
47	47.32058	48	49.7299
52	53.91368	52	54.5499
59	61.00502	57	59.3699
67	68.59460	62	64.1899
76	76.68242	67	69.0099
89	85.26848	72	69.0099
		77	73.8299
		83	78.6499
		88	88.2899
		94	93.1099

ALT-absorbance models, respectively, indicating that there is no significant difference between the actual AST and ALT activity levels and their corresponding, computerized model estimates at 1% level of significance (Table 3, 4).

Computerized version of the AST-absorbance and the ALT-absorbance regression models: The Turbo Pascal for windows version 1.5 is used in writing a program for computing the activity level of AST and ALT in Serum given an absorbance value or a set of absorbance values (discrete or continuous). A simple flowchart which pictorially depicts the workings of the computer program is given in Fig. 3. Interested readers may consult the correspondence author for the adopted computer program used.

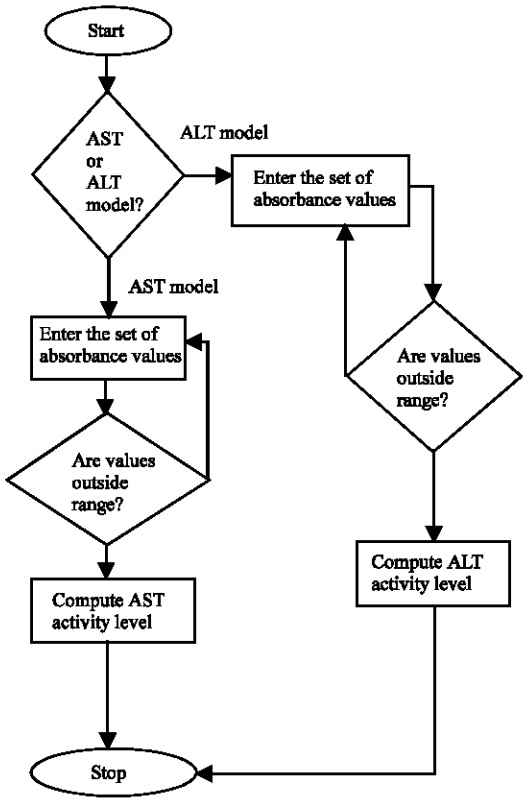

Fig. 3: A program flowchart depicting the workings of the computerized version of the models

Table 4: Paired-samples T-test for comparing the actual aspartate aminotransferase (AST) and alanine aminotransferase (ALT) activity levels with their corresponding, model estimates

				99% Confidence interval of the difference				
	Mean	SD	SEM	Lower	Upper	t-value	df	Sig. (2-tailed)
Actual value of AST-model Estimate of AST	0.00310	2.08065	0.46525	-1.32794	1.33414	0.007	19	0.995
Actual value of ALT-model Estimate of ALT	0.00107	1.47496	0.36874	-1.08550	1.08764	0.003	15	0.998

RESULTS

The Microsoft Excel AST-absorbance and ALT-absorbance regression models (Fig. 1, 2), the table of actual AST and ALT activity levels and their corresponding model estimates (Table 3), the paired-samples t-test table for comparing the actual AST and ALT activity levels with their corresponding model estimates (Table 4) and the program flowchart depicting the workings of the computerized version of the model (Fig. 3) are clearly displayed in this section.

The obtaining of a linear regression line for reported values of absorbance against AST activity level is suggestive of linear correlation between the two parameters. That is as the absorbance levels of the plasma or serum increases, AST level also increases given 0.9965% coefficient of determination pointing to content validity and reliability of the model (Fig. 1). Although, the regression line could only account for 0-89 international unit per liter (IU L^{-1}) activity level of AST

against an absorbance range level of 0.02-0.17, then what happens to the activity level of the absorbance above 0.17 is another challenge. For the plasma or serum that has absorbance above 0.18, such plasma or serum can be diluted to enable the relative activity level being read. Nevertheless, the obtaining of linear line of regression for reported values (0-0.5) of absorbance against ALT activity level is a clear indication of linear correlation between absorbance and ALT activity level (Fig. 2). But if absorbance is higher than 0.5 then there is need for dilution of plasma or serum which its activity is intended to be measured. The coefficient of determination for absorbance-ALT activity level is 0.9944 also signifying both the content validity and reliability that may likely be observed in plasmas and sera collected from various animals of different genetic backgrounds. However, there are variations between actual AST and ALT activity levels and their corresponding model estimates (Table 3). For instance at absorbance level of 0.02 the actual AST activity level is 7 and the estimated AST level is approximately 8.8 with difference of 1.8 which may not be statistically significant. At absorbance level of 0.17 the AST activity level is 85.3 given a difference level of 3.7. But differences in the AST levels at range between 0.02-0.17 are not as high as at 0.02 and 0.17 absorbance levels. Generally the differences between actual AST and estimated AST levels is not statistically significant ($p>0.01$). But the actual ALT level of 4 at 0.025 is higher than estimated ALT level of 1.5. At this level, the difference is significant. But at 0.5 level of absorbance the actual ALT levels is 93.1 given slight difference of 0.9 (Table 3). However, the overall difference between the actual ALT and estimated ALT is statistically not significant ($p>0.01$) signifying the content and predictive validities as well as reliability of the model. Although the program flowchart depicting the workings of the computerized version of the models has similar patterns of operation, one good thing about the results produced by the program is that all the values entered within the range invariably limiting the problems that may likely be encountered if the values were to be outside the range. The mean, standard deviation, standard error of mean, confidence interval between lower and upper limits of actual AST and ALT and estimated AST and ALT fall within the normal range as shown by 0.995 and 0.998, respectively (Table 4).

As shown in Fig. 1 and 2, the AST-absorbance and ALT-absorbance regression models have been successfully formulated. These models were validated by comparing the actual AST and ALT activity levels with their corresponding model estimates (Table 4) using the paired-sample T-test. The respective p-values of 0.995 and 0.998 suggest no significant difference between the actual values of AST and ALT activity levels and those of their corresponding model estimates. It is important to mention that these models can be used in estimating the activity level of AST for given discrete or continuous absorbance values in the range (0.02-0.17) and an activity level of ALT for given discrete or continuous absorbance value in the range (0.025-0.500) with high degree of accuracy as shown over 99% of the total variation as indicated by their respective coefficient of determination (R^2).

DISCUSSION

The obtaining of the regression line that could account for 0-89 IU L^{-1} activity level of AST against an absorbance range level of 0.02-0.17 is corroborated by the finding of Balouchzadeh *et al.* (2011) indicating that the activity level of normal rats is within the range of 8.79 and 85.27 IU L^{-1}. So they concluded that the activity of AST above 85.27 IU L^{-1} in rat is practically significant. This increase can be caused by toxicants such as ethanol. However the authors have also reported that the activity level of ALT (93.10 IU L^{-1}) is practically significant confirming the accuracy and precision of our regression model. Although, Balouchzadech *et al.* (2011) reported ALT activity level of 22.67±2.62 IU L^{-1} in normal rats and 52.33±3.71 IU L^{-1} in the

rats administered 15% ethanol per 100 g b.wt. But Nikkon *et al.* (2008) reported AST of 14.00±0.82 IU L^{-1} and ALT of 12.25±0.50 IU L^{-1} for rats fed normal diet whereas AST (19.50±0.58 IU L^{-1}) and ALT (18.00±0.82 IU L^{-1}) were recorded for rats administered chloroform fraction of *Duranta repens* stem in rats. Conversely, Patrick-Iwuanyanwu and Wegwu (2008) reported high levels of AST in normal rats (121±0.01 IU L^{-1}) and carbon tetrachloride induced liver damage rats (182±0.02 IU L^{-1}) with ALT values of 38.6±0.03 and 149.00±0.04 IU L^{-1}, respectively. The values of AST reported are outside the range of values of our regression line indicating damage of hepatocyte. In another report, ethanol intoxicated rats showed AST values of 76.17±3.36 IU L^{-1} and ALT values of 60.96±3.12 IU L^{-1}, respectively (Hamed, 2011). Although rats administered sodium dichromate at dose level of 0.625 mg kg^{-1} body weight produced increased AST (220.20±0.51 IU L^{-1}) and ALT (45.70±2.65 IU L^{-1}) in comparison with the control group administered vehicle having AST and ALT values of 210.50±9.26 and 42.60±2.90 IU L^{-1} respectively (Vihol *et al.*, 2012). Saganuwan (2006a) examined the effects of sulphadimidine on haematological and biochemical parameters of Nigerian mongrel dogs and reported sulphadimidine pre-administration level of AST (34.0 IU L^{-1}) and post-administration level (18.33 IU L^{-1}) whereas the pre-administration and post-administration levels of ALT were 20.00 IU L^{-1} and 8.60 IU L^{-1} respectively. The values of AST before (190.50±31.32 IU L^{-1}) and after (190.50±31.32 IU L^{-1}) administration of ceftriaxone were not within the range absorbance values of regression model indicating that the model may not be used with such high value (Saganuwan, 2006b). Saganuwan *et al.* (2008) reported 14.00±2.76 IU L^{-1} and 16.71±2.14 IU L^{-1} for control and experimental mice administered 160 mg kg^{-1} b.wt of potassium permanganate, respectively. However, ALT values for control and experimental mice administered oral potassium permanganate were 6.43±2.44 and 7.57±1.81 IU L^{-1}, respectively. In another study, AST values for control (29.78±3.48 IU L^{-1}) and experimental (21.56±2.86 U L^{-1}) are within the range of regression model. The AST values for control (5.75 IU L^{-1}) and experimental (15.4 IU L^{-1}) *Cyprinus carpio* (fish) administered curacron agree with our report that AST and ALT regression models can be used to extrapolate the values of AST and ALT from absorbance. Since the control and experimental values of ALT from *Cyprinus carpio* are 0.68 and 13.84 IU L^{-1}, respectively (Joseph and Raj, 2011). But at the absorbance of 0.02, the extrapolation of both the ALT and AST values are impossible. Therefore, 0.68 IU L^{-1} value cannot be extrapolated from our regression model for ALT and AST. Perhaps, such values can be deduced after dilution of samples. So the discrepancies in the values of normal and experimental animals may be due to their environmental, genetic, physiological, pathological and nutritional factors. If the two constants 'a' and 'b' that define a linear regression equation are calculated, then quantitatively described are the rates of AST and ALT activity levels (Y) with a change in absorbance level (X). That is dependence of Y on X, must not automatically assume a biological cause-and-effect relationship. Causal relationships are concluded only with some insight into natural phenomenon being investigated and may not be declared by statistical testing alone. Indeed, it is often necessary to determine the inter-relationship between the two variables under study and other variables for an observed dependence may, in-fact be due to the influence of one or more additional variables.

Therefore, a linear regression function is mathematically nothing more than a straight line forced to fit through a set of data points and it may not all describe a natural phenomenon such as AST and ALT (Zar, 2008). Since the line that best fits a joint distribution of observations is taken to be the equation that yields the smallest overall error of estimate, a good descriptive statistic like this one should consider all the observed values in order to capture error associated with our prediction equation by adding all of the prediction errors (Frank and Althoen, 1995). Standard

deviations of AST (2.08) and ALT (1.47) are less so much so that many of the reported values of AST and ALT standard deviations are above our values. Likewise, standard error of means of AST (0.465) and ALT (0.369) are very minimal, all indicating the minimization of errors, validity, precision and reliability of the regression model. Other factors that may affect the AST and ALT values of animals are toxicant, sex, age, pedigree, the sample size of the experimental animals and ratio of male to female animals per group. A range of 8-12 animals of same sex or both sexes in ratio of female 4-6 and male 4-6 (Saganuwan, 2012) with a total number of not less than 30 animals can reduce both standard deviation and standard error of means of all the observations (Frank and Althoen, 1995). The larger the sample size the more the test is powered. The wavelength of spectrophotometer used to measure the activity level of AST can be deduced from regression line. The formula being opposite over adjacent which is tangent. In the present study opposite is 68 IU L^{-1} divided by adjacent which is 0.12 gives 567 nm wavelength with resultant tangent of 0.5.

CONCLUSION

The challenge being faced in estimating the activity levels of plasma and serum Aspartate Aminotransferase (AST) and Alanine Aminotransferase (ALT) concentrations in plasma and serum using continuous absorbance values as determined from experiments has been removed. The computerized version (Fig. 3) of these models can be used to estimate plasma and serum levels of AST and ALT.

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