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EVALUATION OF LIVER MARKER ENZYMES IN DIABETIC SUBJECTS IN THE UNIVERSITY OF PORT HARCOURT TEACHING HOSPITAL (UPTH)

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ABSTRACT

The activity levels of the liver marker enzymes; Aspartate and Alanine amino transferases (AST and ALT), Alkaline Phosphatase (ALP) and Gamma glutamyltransferase (GGT) in diabetic and non-diabetic subjects within the ages of 25 to 86 were assayed. This study was for two weeks. The results indicated that the plasma levels of AST and ALT from diabetic subjects increased significantly ($p < 0.05$ and $p < 0.001$) during the period of study while the plasma levels of ALP and GGT from the same subjects remained mostly constant and normal significantly ($p < 0.05$ and $p < 0.001$) during the period for the study. The findings in this study suggest that Diabetes mellitus does cause elevation of liver enzymes in humans.

Key words: Liver marker enzymes, enzymes activity levels, Diabetes Mellitus

INTRODUCTION

Diabetes mellitus prevalence estimation of more than 220 million people around the world, with 80% occurring in low- and middle-income countries, is expected, by experts, to increase to more than 400 million by the year 2030 (Wild *et al.*, 2004). Globally, as at 2010, an estimated 285 million people had diabetes, with type 2 making up about 90% of the cases (Williams, 2010). Diabetes mellitus; a metabolic disorder that is characterized by high blood glucose resulting from insulin resistance and relative insulin deficiency occurs throughout the world, the greatest increase in prevalence is, however, expected to occur in Asia and Africa, where most patients will probably be found by 2030 (Wild *et al.*, 2004). The increase in incidence has suggested an environmental (i.e., dietary) effect, but there is little understanding of the mechanism(s) at present, though there is much speculation some of it most compellingly presented (Wild *et al.,* 2004).

The liver is the largest gland of the human body. Its average weight is about 1.5kg (between 1.4-1.6kg) (Cotran *et al.,* 2005) and is a soft, pinkish brown, triangular organ. It is the largest internal organ in the human body.

If one has diabetes, the best defense against liver disease is: tight control of blood sugar levels, high cholesterol reduction, maintenance of a healthy weight, and avoidance of excessive consumption of alcohol (Maria, 2007). The National Institute of Health found that those that suffer from diabetes are twice as likely to suffer from a liver disease as well (Warner, 2010). Liver disease may cause or contribute to, be coincident with, or occur as a result of diabetes mellitus (Gavin and Anthony, 1999).

Of the four liver enzymes; AST, ALT, ALP, GGT; which reflect liver injury, AST and ALT are abundant in the liver and are mainly used for liver function test (LFT) and are usually elevated in diabetic patients or subjects (Paul, 2005).

The liver marker enzymes AST, ALT, ALP and GGT, are commonly measured clinically as part of diagnostic evaluation of hepatocellular injury to determine liver health. The measurement of the activity of the liver marker enzymes in blood plasma is an important diagnostic procedure to determine whether diabetics have suffered liver damage, be it acute or chronic. The objective was to assess the levels of the liver marker enzymes in diabetic subjects and to determine whether their liver was functional or not.

MATERIALS AND METHODS

The materials used include equipments such as centrifuge, water bath, spectrophotometer or BSA-3000 Chemical analyzer, pipette, lithium heparin tubes, human plasma and plain sterile tubes. Reagents such as 0.4g/ml sodium hydroxide solution and the reagent kits for ALT, AST, ALP and GGT (from Randox Laboratories Limited, United Kingdom) were also used.

Analysis

Plasma aspartate transaminase (AST) and Alanine transaminase (ALT) activities were assayed according to the method of Reitman and Frankel (1957). AST and ALT Activity Assay Kits were used. Alkaline phosphatase activity was assayed according to the method by Bessey, Lowry, and Brock in 1946. This method utilizes 4-nitro-phenyl phosphate which is a readily hydrolyzed, self-indicating ALP substrate. The Alkaline Phosphatase Assay Kit (Colorimetric) was used. It contains ALP Assay Buffer and ALP substrate. Gamma glutamyltransaminase activity was assayed according to the method by Szasz, G. in 1969. The Gamma Glutamyl Transferase (GGT) Activity Colorimetric Assay Kit was used. The components of the kit are GGT Assay Buffer, GGT substratem GGT positive control, pNA standard (2mM).

Blood Sample Collection

Blood sample were collected at the phlebotomy unit of the Chemical Pathology Department of the University of Port Harcourt Teaching Hospital (UPTH) from confirmed diabetic patients as they came for their routine fasting blood glucose test.

3ml of blood samples were collected by venipuncture into lithium heparin tubes and labeled properly. Altogether, 30 diabetics made up of 15 male and 15 female subjects, and 10 controls made up 5 male and 5 female subjects were used for the study.

Sample Preparation

The samples were centrifuged at 2000g for 10 minutes and the supernatant (plasma) was separated into plain sterile tubes for analysis. The age range of the subjects was from 25 to 86 years.

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Statistical Analysis
Significant differences were determined using Student's t-test. The t-test was used to compare the means of the diabetic subjects and the normal subjects for each of the enzymes. Values were considered significantly at p = 0.05 and p = 0.001.

RESULTS
The results obtained for the plasma mean levels of the liver enzymes in diabetic male subjects under different age brackets are shown in Table 1.

The results obtained for the plasma mean levels of the liver enzymes in normal male subjects (control) under different age brackets are shown in Table 2. The results obtained for the plasma mean levels of the liver enzymes in diabetic female subjects under different age brackets are shown in Table 3. The results obtained for the plasma mean levels of the liver enzymes in normal female subjects (control) under different age brackets are shown in Table 4.

Table 1: Mean Level of AST in Diabetic Male Subjects in different age brackets.

Age Bracket	AST Level, X	Mean, $\bar{X}$	X - $\bar{X}$	(X - $\bar{X}$)^2
25 – 41	23U/l	22.2U/l	0.8U/l	0.64(U/l)^2
43 – 51	19U/l	22.2U/l	-3.2U/l	10.24(U/l)^2
53 – 59	19U/l	22.2U/l	-3.2U/l	10.24(U/l)^2
60 - 68	26U/l	22.2U/l	3.8U/l	14.44(U/l)^2
70 - 74	24U/l	22.2U/l	1.8U/l	3.24(U/l)^2
				38.80(U/l)^2 = ∑ (X - $\bar{X}$)^2

$$Standard\ Deviation, S = \sqrt{\frac{\sum (X - \bar{X})^2}{(n-1)}}$$

Where
X = each level or value
$\bar{X}$ = the mean or average of the values
n = the number of values
∑ = summation (the sum across the values).

n = 5; n – 1 = 5 – 1 = 4

$$\therefore S = \sqrt{\frac{38.80\ (U/l)^2}{4}} = S = \sqrt{9.70(U/l)^2}$$

= 3.11U/l

Approximately, S = 3 U/l

Table 2: Mean Level of ALT in Diabetic Male Subjects in different age brackets.

Age Bracket	ALT Level, X	Mean, $\bar{X}$	X - $\bar{X}$	(X - $\bar{X}$)^2
25 – 41	18U/l	21.4U/l	-3.4U/l	11.56(U/l)^2
43 – 51	25U/l	21.4U/l	3.6U/l	12.96(U/l)^2
53 – 59	15U/l	21.4U/l	-6.4U/l	40.96(U/l)^2
60 - 68	26U/l	21.4U/l	4.6U/l	21.16(U/l)^2
70 - 74	23U/l	21.4U/l	1.6U/l	2.56(U/l)^2
				89.20(U/l)^2 = ∑ (X - $\bar{X}$)^2

$$Standard\ Deviation, S = \sqrt{\frac{\sum (X - \bar{X})^2}{(n-1)}}$$

Where

X = each level or value

$\bar{X}$ = the mean or average of the values

n = the number of values

$\sum$ = summation (the sum across the values).

n = 5; n – 1 = 5 – 1 = 4

$$\therefore\ S = \sqrt{\frac{89.20\ (U/l)^2}{4}} = S = \sqrt{22.30(U/l)^2}$$

= 4.72U/l

Approximately, S = 5 U/l

Table 3: Mean Level of ALP in Diabetic Male Subjects in different age brackets.

Age Bracket	ALP Level, X	Mean, $\bar{X}$	X - $\bar{X}$	$(X - \bar{X})^2$
25 – 41	27U/l	219 U/l	51U/l	$2601(U/l)^2$
43 – 51	169U/l	219 U/l	-50U/l	$2500(U/l)^2$
53 – 59	290U/l	219 U/l	71U/l	$5041(U/l)^2$
60 - 68	179U/l	219 U/l	-40U/l	$1600(U/l)^2$
70 - 74	187U/l	219 U/l	-32U/l	$1024(U/l)^2$
				$12766(U/l)^2 = \sum (X - \bar{X})^2$

$$Standard\ Deviation, S = \sqrt{\frac{\sum (X - \bar{X})^2}{(n-1)}}$$

Where

X = each level or value

$\bar{X}$ = the mean or average of the values

n = the number of values

$\sum$ = summation (the sum across the values).

n = 5; n – 1 = 5 – 1 = 4

$$\therefore\ S = \sqrt{\frac{12766(U/l)^2}{4}} = S = \sqrt{3191.5(U/l)^2}$$

= 56.49U/l

Approximately, S = 56U/l

Table 4: Mean Level of GGT in Diabetic Male Subjects in different age brackets.

Age Bracket	GGT Level, X	Mean, $\bar{X}$	X - $\bar{X}$	$(X - \bar{X})^2$
25 – 41	32U/l	27.8 U/l	4.2U/l	$17.64(U/l)^2$
43 – 51	18U/l	27.8 U/l	-9.8U/l	$96.04(U/l)^2$
53 – 59	34U/l	27.8 U/l	6.2U/l	$38.44(U/l)^2$
60 - 68	27U/l	27.8 U/l	-0.8U/l	$0.6(U/l)^2$
70 - 74	28U/l	27.8 U/l	0.2U/l	$0.04(U/l)^2$
				$152.80(U/l)^2 = \sum (X - \bar{X})^2$

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$$Standard\ Deviation, S = \sqrt{\frac{\sum (X - \bar{X})^2}{(n-1)}}$$

Where

X = each level or value

$\bar{X}$ = the mean or average of the values

n = the number of values

$\sum$ = summation (the sum across the values).

n = 5; n – 1 = 5 – 1 = 4

$$\therefore\ S = \sqrt{\frac{152.80(U/l)^2}{4}} = S = \sqrt{38.20(U/l)^2}$$

= 6.18U/l

Approximately, S = 6U/l

Table 5: Mean Level of AST in Non-diabetic Male Subjects (control) in different age brackets.

Age Bracket	AST Level, X	Mean, $\bar{X}$	X - $\bar{X}$	$(X - \bar{X})^2$
25 – 41	7U/l	8 U/l	-1U/l	$17.64(U/l)^2$
43 – 51	11U/l	8 U/l	3U/l	$96.04(U/l)^2$
53 – 59	7U/l	8 U/l	-1U/l	$38.44(U/l)^2$
60 - 68	7U/l	8 U/l	-1U/l	$0.6(U/l)^2$
				$12(U/l)^2 = \sum (X - \bar{X})^2$

$$Standard\ Deviation, S = \sqrt{\frac{\sum (X - \bar{X})^2}{(n-1)}}$$

Where

X = each level or value

$\bar{X}$ = the mean or average of the values

n = the number of values

$\sum$ = summation (the sum across the values).

n = 4; n – 1 = 4 – 1 = 3

$$\therefore\ S = \sqrt{\frac{12(U/l)^2}{3}} = S = \sqrt{4(U/l)^2} = 2U/l$$

$\therefore$ Standard Deviation, S = 2U/l

Table 6: Mean Level of ALT in Non-diabetic Male Subjects (control) in different age brackets.

Age Bracket	ALT Level, X	Mean, $\bar{X}$	X - $\bar{X}$	$(X - \bar{X})^2$
25 – 41	2U/l	1.5U/l	0.5U/l	$0.25(U/l)^2$
43 – 51	2U/l	1.5U/l	0.5U/l	$0.25(U/l)^2$
53 – 59	1U/l	1.5U/l	-0.5U/l	$0.25(U/l)^2$
60 - 68	1U/l	1.5U/l	-0.5U/l	$0.25(U/l)^2$
				$1.00(U/l)^2 = \sum (X - \bar{X})^2$

$$Standard\ Deviation, S = \sqrt{\frac{\sum (X - \bar{X})^2}{(n-1)}}$$

Where
X = each level or value
$\bar{X}$ = the mean or average of the values
n = the number of values
$\sum$ = summation (the sum across the values).

n = 4; n – 1 = 4 – 1 = 3

$$\therefore\ S = \sqrt{\frac{1.00(U/l)^2}{3}} = \quad S = \sqrt{0.33(U/l)^2}$$

= 0.57U/l

Approximately, the Standard Deviation, S = 1U/l

Table 7: Mean Level of ALP in Non-diabetic Male Subjects (control) in different age brackets.

Age Bracket	ALP Level, X	Mean, $\bar{X}$	X - $\bar{X}$	(X - $\bar{X}$)^2
25 – 41	118U/l	178.25U/l	-60.25U/l	3630.06(U/l)^2
43 – 51	181U/l	178.25U/l	2.75U/l	7.56(U/l)^2
53 – 59	269U/l	178.25U/l	90.75U/l	8235.56(U/l)^2
60 - 68	145U/l	178.25U/l	-33.25U/l	1105.56(U/l)^2
				12978.74(U/l)^2 = $\sum$ (X - $\bar{X}$)^2

$$Standard\ Deviation, S = \sqrt{\frac{\sum (X - \bar{X})^2}{(n-1)}}$$

Where
X = each level or value
$\bar{X}$ = the mean or average of the values
n = the number of values
$\sum$ = summation (the sum across the values).

n = 4; n – 1 = 4 – 1 = 3

$$\therefore S = \sqrt{\frac{12978.74(U/l)^2}{3}} = S = \sqrt{4326.25(U/l)^2}$$

= 65.77U/l

Approximately, the Standard Deviation, S = 66U/l

Table 8: Mean Level of GGT in Non-diabetic Male Subjects (control) in different age brackets.

Age Bracket	GGT Level, X	Mean, $\bar{X}$	X - $\bar{X}$	(X - $\bar{X}$)^2
25 – 41	22U/l	31U/l	-9U/l	81(U/l)^2
43 – 51	28U/l	31U/l	-3U/l	9(U/l)^2
53 – 59	54U/l	31U/l	23U/l	529(U/l)^2
60 - 68	20U/l	31U/l	-11U/l	121(U/l)^2
				740(U/l)^2 = $\sum$ (X - $\bar{X}$)^2

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$$Standard\ Deviation, S = \sqrt{\frac{\sum (X - \bar{X})^2}{(n-1)}}$$

Where
X = each level or value
$\bar{X}$ = the mean or average of the values
n = the number of values
$\sum$ = summation (the sum across the values).

n = 4; n – 1 = 4 – 1 = 3

$$\therefore\ S = \sqrt{\frac{740(U/l)^2}{3}} = S = \sqrt{246.7(U/l)^2}$$

= 15.71U/l

Approximately, the Standard Deviation, S = 16U/l

Table 9: Mean Level of AST in diabetic Female Subjects in different age brackets.

Age Bracket	AST Level, X	Mean, $\bar{X}$	X - $\bar{X}$	$(X - \bar{X})^2$
37 – 45	24U/l	25.4U/l	-1.4U/l	$1.96(U/l)^2$
47 – 48	23U/l	25.4U/l	-2.4U/l	$5.76(U/l)^2$
49 – 53	19U/l	25.4U/l	-6.4U/l	$40.96(U/l)^2$
54 – 60	25U/l	25.4U/l	-0.4U/l	$0.16(U/l)^2$
61 - 68	36U/l	25.4U/l	10.6U/l	$112.36(U/l)^2$
				$161.20(U/l)^2 = \sum (X - \bar{X})^2$

$$Standard\ Deviation, S = \sqrt{\frac{\sum (X - \bar{X})^2}{(n-1)}}$$

Where
X = each level or value
$\bar{X}$ = the mean or average of the values
n = the number of values
$\sum$ = summation (the sum across the values).

n = 5; n – 1 = 5 – 1 = 4

$$\therefore S = \sqrt{\frac{161.20(U/l)^2}{4}} = S = \sqrt{40.30(U/l)^2}$$

= 6.35U/l

Approximately, the Standard Deviation, S = 6U/l

Table 10: Mean Level of ALT in diabetic Female Subjects in different age brackets.

Age Bracket	ALT Level, X	Mean, $\bar{X}$	X - $\bar{X}$	$(X - \bar{X})^2$
37 – 45	19U/l	21.2U/l	-2.2U/l	$4.84(U/l)^2$
47 – 48	24U/l	21.2U/l	2.8U/l	$7.84(U/l)^2$
49 – 53	25U/l	21.2U/l	3.8U/l	$14.44(U/l)^2$
54 – 60	16U/l	21.2U/l	-5.2U/l	$27.04(U/l)^2$
61 - 68	22U/l	21.2U/l	0.8U/l	$0.64(U/l)^2$
				$54.80(U/l)^2 = \sum (X - \bar{X})^2$

$$Standard\ Deviation, S = \sqrt{\frac{\sum (X - \bar{X})^2}{(n-1)}}$$

Where
X = each level or value
$\bar{X}$ = the mean or average of the values
n = the number of values
$\sum$ = summation (the sum across the values).

n = 5; n – 1 = 5 – 1 = 4

$$\therefore\ S = \sqrt{\frac{54.80(U/l)^2}{4}} = S = \sqrt{13.70(U/l)^2}$$
= 3.70U/l

Approximately, the Standard Deviation, S = 4U/l

Table 11: Mean Level of ALP in diabetic Female Subjects in different age brackets.

Age Bracket	ALP Level, X	Mean, $\bar{X}$	X - $\bar{X}$	(X - $\bar{X}$)^2
37 – 45	180U/l	179.6U/l	0.4U/l	0.16(U/l)^2
47 – 48	164U/l	179.6U/l	-15.6U/l	243.36(U/l)^2
49 – 53	144U/l	179.6U/l	-35.6U/l	1267.36(U/l)^2
54 – 60	210U/l	179.6U/l	30.4U/l	924.16(U/l)^2
61 - 68	200U/l	179.6U/l	20.4U/l	416.16(U/l)^2
				2851.20(U/l)^2 = $\sum$ (X - $\bar{X}$)^2

$$Standard\ Deviation, S = \sqrt{\frac{\sum (X - \bar{X})^2}{(n-1)}}$$

Where
X = each level or value
$\bar{X}$ = the mean or average of the values
n = the number of values
$\sum$ = summation (the sum across the values).

n = 5; n – 1 = 5 – 1 = 4

$$\therefore\ S = \sqrt{\frac{2851.20(U/l)^2}{4}} = S = \sqrt{712.80(U/l)^2}$$
= 26.70U/l

Approximately, the Standard Deviation, S = 4U/l

Table 12: Mean Level of GGT in diabetic Female Subjects in different age brackets.

Age Bracket	GGT Level, X	Mean, $\bar{X}$	X - $\bar{X}$	(X - $\bar{X}$)^2
37 – 45	29U/l	24.4U/l	4.6U/l	21.16(U/l)^2
47 – 48	15U/l	24.4U/l	-9.4U/l	88.36(U/l)^2
49 – 53	19U/l	24.4U/l	-5.4U/l	29.16(U/l)^2
54 – 60	29U/l	24.4U/l	4.6U/l	21.16(U/l)^2
61 - 68	30U/l	24.4U/l	5.6U/l	31.36(U/l)^2
				191.20(U/l)^2 = $\sum$ (X - $\bar{X}$)^2

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$$Standard\ Deviation, S = \sqrt{\frac{\sum (X - \bar{X})^2}{(n-1)}}$$

Where
X = each level or value
$\bar{X}$ = the mean or average of the values
n = the number of values
∑ = summation (the sum across the values).

n = 5; n – 1 = 5 – 1 = 4

$$\therefore S = \sqrt{\frac{191.20(U/l)^2}{4}} = S = \sqrt{47.80(U/l)^2}$$

= 6.91U/l

Approximately, the Standard Deviation, S = 7U/l

Table 13: Mean Level of AST in Non-diabetic Female Subjects (control) in different age brackets.

Age Bracket	AST Level, X	Mean, $\bar{X}$	X - $\bar{X}$	$(X - \bar{X})^2$
37 – 45	7U/l	10.3U/l	-3.3U/l	$10.89(U/l)^2$
49 – 53	14U/l	10.3U/l	3.7U/l	$13.69(U/l)^2$
54 – 60	10U/l	10.3U/l	-0.3U/l	$0.09(U/l)^2$
				$24.67(U/l)^2 = \sum (X - \bar{X})^2$

$$Standard\ Deviation, S = \sqrt{\frac{\sum (X - \bar{X})^2}{(n-1)}}$$

Where
X = each level or value
$\bar{X}$ = the mean or average of the values
n = the number of values
∑ = summation (the sum across the values).

n = 3; n – 1 = 3 – 1 = 2

$$\therefore S = \sqrt{\frac{24.67(U/l)^2}{2}} = S = \sqrt{12.34(U/l)^2}$$

= 3.51U/l

Approximately, the Standard Deviation, S = 4U/l

Table 14: Mean Level of ALT in Non-diabetic Female Subjects (control) in different age brackets.

Age Bracket	ALT Level, X	Mean, $\bar{X}$	X - $\bar{X}$	$(X - \bar{X})^2$
37 – 45	6U/l	6.3U/l	-0.3U/l	$0.09(U/l)^2$
49 – 53	6U/l	6.3U/l	-0.3U/l	$0.09(U/l)^2$
54 – 60	7U/l	6.3U/l	-0.7U/l	$0.49(U/l)^2$
				$0.67(U/l)^2 = \sum (X - \bar{X})^2$

$$Standard\ Deviation, S = \sqrt{\frac{\sum (X - \bar{X})^2}{(n-1)}}$$

Where
X = each level or value
$\bar{X}$ = the mean or average of the values
n = the number of values
∑ = summation (the sum across the values).

n = 3; n – 1 = 3 – 1 = 2

$$\therefore \quad S = \sqrt{\frac{0.67(U/l)^2}{2}} = S = \sqrt{0.34(U/l)^2}$$

= 0.58U/l

Approximately, the Standard Deviation, S = 1U/l

Table 15: Mean Level of ALP in Non-diabetic Female Subjects (control) in different age brackets.

Age Bracket	ALP Level, X	Mean, $\bar{X}$	X - $\bar{X}$	(X - $\bar{X}$)^2
37 – 45	90U/l	137.3U/l	-47.3U/l	2237.29(U/l)^2
49 – 53	139U/l	137.3U/l	1.7U/l	2.89(U/l)^2
54 – 60	183U/l	137.3U/l	45.7U/l	2088.49(U/l)^2
				4328.67(U/l)^2 = ∑ (X - $\bar{X}$)^2

$$Standard\ Deviation, S = \sqrt{\frac{\sum (X - \bar{X})^2}{(n-1)}}$$

Where
X = each level or value
$\bar{X}$ = the mean or average of the values
n = the number of values
∑ = summation (the sum across the values).

n = 3; n – 1 = 3 – 1 = 2

$$\therefore S = \sqrt{\frac{4328.67(U/l)^2}{2}} = S = \sqrt{2164.34(U/l)^2}$$

= 46.52U/l

Approximately, the Standard Deviation, S = 47U/l

Table 16: Mean Level of GGT in Non-diabetic Female Subjects (control) in different age brackets.

Age Bracket	GGT Level, X	Mean, $\bar{X}$	X - $\bar{X}$	(X - $\bar{X}$)^2
37 – 45	8U/l	18.7U/l	-10.7U/l	114.49(U/l)^2
49 – 53	14U/l	18.7U/l	-4.7U/l	22.09(U/l)^2
54 – 60	34U/l	18.7U/l	15.3U/l	234.09(U/l)^2
				370.67(U/l)^2 = ∑ (X - $\bar{X}$)^2

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$$Standard\ Deviation, S = \sqrt{\frac{\sum (X - \bar{X})^2}{(n-1)}}$$

Where

X = each level or value

$\bar{X}$ = the mean or average of the values

n = the number of values

$\sum$ = summation (the sum across the values).

n = 3; n – 1 = 3 – 1 = 2

$$\therefore S = \sqrt{\frac{370.67(U/l)^2}{2}} = S = \sqrt{185.34(U/l)^2}$$

= 13.61U/l

Approximately, the Standard Deviation, S = 14U/l

Table 17: Comparison of the Mean Values for the Four Sets of Subjects for AST

	Diabetic male subjects	Non-diabetic male subjects	Diabetic female subjects	Non-diabetic female subjects
Mean Values, X	22.2U/l $^{+1.8U/l}$	8U/l $^{+1U/l}$	25.4U/l $^{-1.4U/l}$	10.3U/l $^{-1.3U/l}$
Standard Deviation, S	3U/l $^{+1U/l}$	2U/l $^{+2U/l}$	6U/l $^{-2U/l}$	4U/l $^{-0U/l}$

$t = (X_1 – X_2)/s$

Where t = test for significance

X_1 = Mean value of the diabetic male and female subjects

X_2 = Mean value of the non-diabetic male and female subjects

S= Standard Deviation mean value of all the subjects

X_1 = ((22.2+25.4)U/l)/2 = (47.6U/l)/2 = 23.8U/l = 24U/l

X_2 = ((8+10.3)U/l)/2 =(18.3U/l) /2 =9.15U/l = 9U/l

S = ((3+2+6+4)U/l)/4 = (15U/l)/4 =3.75U/l = 4U/l

$\therefore$ t = ((24-9)U/l) /4U/l = (15U/l)(4U/l)= 3.75

The calculated t value of 3.75 shows that the difference between the mean values is highly significant. Clearly, the diabetic subjects' values for AST are elevated when compared to that for the non-diabetics.

Table 18: Comparison of the Mean Values for the Four Sets of Subjects for AST

	Diabetic male subjects	Non-diabetic male subjects	Diabetic female subjects	Non-diabetic female subjects
Mean Values, X	21.4U/l $^{-0.4U/l}$	1.5U/l $^{+2.5U/l}$	21.2U/l $^{-0.2U/l}$	6.3U/l $^{-2.3U/l}$
Standard Deviation, S	5U/l $^{-2U/l}$	1U/l $^{+2U/l}$	4U/l $^{-1U/l}$	1U/l $^{+2U/l}$

$t = (X_1 – X_2)/s$

Where t = test for significance

X_1 = Mean value of the diabetic male and female subjects

X_2 = Mean value of the non-diabetic male and female subjects

S= Standard Deviation mean value of all the subjects

X_1 = ((21.4+21.2)U/l)/2 = (42.6U/l)/2 = 21.3U/l = 21U/l

X_2 = ((1.5+6.3)U/l)/2 =(7.8U/l) /2 =3.9U/l = 4U/l

S = ((5+1+4+1)U/l) /4 = (11U/l)/4 =2.75U/l = 3U/l

$\therefore$ t = ((21-4)U/l) /3U/l = (17U/l)(3U/l)= 5.7

The calculated to value of 5.7 shows that the difference between the mean values is highly significant. Clearly, the diabetic subjects' values for ALT are elevated when compared to the non-diabetic subjects' (control) values for ALT.

Table 19: Comparison of the Mean Values for the Four Sets of Subjects for ALP

	Diabetic male subjects	Non-diabetic male subjects	Diabetic female subjects	Non-diabetic female subjects
Mean Values, X	219U/l $^{-20U/l}$	178.25U/l $^{-39.8U/l}$	179.6U/l $^{+19.8U/l}$	137.3U/l $^{+20.7U/l}$
Standard Deviation, S	56U/l $^{-7U/l}$	66U/l $^{-17U/l}$	27U/l $^{+22U/l}$	47U/l $^{+2U/l}$

$$t = (X_1 - X_2)/s$$

Where t = test for significance

X_1 = Mean value of the diabetic male and female subjects

X_2 = Mean value of the non-diabetic male and female subjects

S= Standard Deviation mean value of all the subjects

$$X_1 = ((219+179.6)U/l)/2 = (398.6U/l)/2 = 199.3U/l =\underline{\underline{199U/l}}$$

$$X_2 = ((178.25+137.3)U/l)/2 =(315.55U/l) /2 =157.78U/l = \underline{\underline{158U/l}}$$

$$S = ((56+66+27+47)U/l) /4 = (196U/l)/4 = \underline{\underline{49U/l}}$$

$$\therefore t = ((199-158)U/l) /49U/l = (41U/l)(49U/l)= \underline{\underline{0.84}}$$

The calculated to value of 0.84 shows that the difference between the mean values is not very significant. Clearly, the diabetic subjects' values for ALP are mostly not elevated when compared to that for the non-diabetics (control).

Table 20: Comparison of the Mean Values for the Four Sets of Subjects for GGT

	Diabetic male subjects	Non-diabetic male subjects	Diabetic female subjects	Non-diabetic female subjects
Mean Values, X	27.8U/l $^{-1.8U/l}$	31U/l $^{-6U/l}$	24.4U/l $^{+1.6U/l}$	18.7U/l $^{+6.3U/l}$
Standard Deviation, S	6U/l $^{+5U/l}$	16U/l $^{-5U/l}$	7U/l $^{+4U/l}$	14U/l $^{-3U/l}$

$$t = (X_1 - X_2)/s$$

Where t = test for significance

X_1 = Mean value of the diabetic male and female subjects

X_2 = Mean value of the non-diabetic male and female subjects

S= Standard Deviation mean value of all the subjects

$$X_1 = ((27.8+24.4)U/l)/2 = (52.2U/l)/2 = 26.1U/l =\underline{\underline{26U/l}}$$

$$X_2 = ((31+18.7)U/l)/2 =(49.7U/l) /2 =24.85U/l = \underline{\underline{25U/l}}$$

$$S = ((6+16+7+14)U/l) /4 = (43U/l)/4 = 10.75U/l = \underline{\underline{11U/l}}$$

$$\therefore t = ((26-25)U/l) /11U/l = (1U/l)(11U/l)= \underline{\underline{0.09}}$$

The calculated to value of 0.09 shows that the difference between the mean values is almost not significant. Clearly, the diabetic subjects' values for GGT are almost not elevated when compared to the non-diabetic subjects' (control) values for GGT.

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DISCUSSION

The results from this study have shown that diabetes mellitus affect the liver since there was a significant difference between the mean values for the diabetics and control when compared. Report showed that the normal range of values for AST is from 0-12U/l of plasma at 37°C, for men and women; that for ALT is from 0-12U/l of plasma at 37°C for both sexes; that for ALP is from 98-279U/l of plasma, at 37°C, for both sexes; while that for GGT is from 11-50U/l of plasma, at 37°C, for men and from 7-32°U/l of plasma, at 37°C, for women.

Almost all the AST and ALT values for the diabetic subjects were above the normal range while most of the ALP and GGT values for the same subjects were within the normal range, which implies that the activity of AST and ALT in the diabetic subjects was mainly above their normal levels while the activity of ALP and GGT in the same subjects was mainly within their normal levels.

The findings in this report indicated variation in the levels of AST, ALT, ALP and GGT between the male and female diabetic subjects when compared; the levels of the males were higher, significantly, than those of the females. This variation could have arisen from environmental and nutritional factors, and also the physiological differences between the males and the females, for example; hormonal difference and genetic make-up.

The study of the determination of AST, ALT, ALP and GGT levels in diabetic subjects showed that the liver was affected by diabetes mellitus as most of the levels of the liver marker enzymes were above the normal range and very few of the levels of ALP and GGT were below the normal range.

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