ORIGINAL ARTICLE

Assessment of alanine amino transferase (ALT) and alkaline phosphatase (ALP) level amongst apparently healthy students of Niger Delta University

[1]Benedicta E. Kasia, [2]Ekere Enakeno Efemena & [2]Proph the Prophet

[1]Department of Chemical Pathology & [2]Department of Biochemistry,
Niger Delta University, Wilberforce Island, Bayelsa State

Correspondent Author:

Dr Benedicta E. Kasia,
Niger Delta University, Wilberforce Island, Bayelsa State
Email: kasia.benedicta@yahoo.co.uk

ABSTRACT

This study was conducted to estimate Alanine amino transaminase (ALT) and Alkaline phosphatase (ALP) amongst apparently healthy students of Niger Delta University. An analytical cross-sectional study on 102 individuals (55 males and 47 females) between ages 20-40 was conducted. Blood specimen was collected from 102 participant for measurement of ALT and ALP level using standard assay method. Result revealed that ALT level was higher in males (5.56±2.6) than in females (5.28±3.06) but was not statistically significant. ALT value of respondents between 20-30 years (5.44±2.86) was found higher than the ALT value of respondents between 31-40 years (5.33±1.15). When compared to the laboratory reference ranges for ALT (5-40U/L), a high number of respondents 61(60%) fell within the normal reference range (7.10±2.14) while 41 (40%)respondents were below the normal reference range (2.98±0.78). No high ALT level was recorded for any respondent. The female ALP level (19.47±7.98) was higher than the male ALP level (18.55±7.21) but was not statistically significant at p<0.05. ALP values of respondents between 31-40 years (20.00±7.54) was higher than the ALP value of respondents between 20-30 years (18.92±7.52). When compared to the laboratory reference ranges for ALP level (9-35U/L), a high number of respondents 95(93%) fell within the normal reference range (18.63±6.43), while 3(3%) respondents were below the normal reference range (8.25±0.00) and 4(4%) respondents were higher than the normal reference range (38.25±1.71). In conclusion, a normal ALT and ALP levels was observed amongst majority of the studied respondents which indicates low risk for hepatic injury among the respondents.

Keywords: Alanine amino transferase, Alkaline Phosphatase, Reference values

Introduction:

Alanine aminotransferase (ALT) and alkaline phosphatase (ALP) are the most widely used clinical biomarkers of hepatic health.[1] Alanine aminotransferase catalyses the transfer of an amino group from alanine to alpha-ketoglutarate in the alanine cycle to form pyruvate and glutamate. The ALT enzyme is found in serum and organ tissues, especially liver, although significant concentrations are also found in kidney, skeletal muscle, and myocardium. Lower levels of ALT are present in pancreas, spleen, and lung. Alanine aminotransferase is elevated in serum under conditions of significant cellular necrosis and is used as a measure of liver function.[2] Levels of ALT may be elevated in cases of hepatitis, congestive heart failure, liver or biliary duct damage, or myopathy. Leakage of ALT from the hepatocyte into the blood occurs following hepatocellular injury.[3] Elevations in ALT can be highly suggestive of liver injury but ALT is not liver-specific, due to extrahepatic sources. Although tests that measure the level of serum liver enzymes are commonly referred to as liver function tests, they usually reflect hepatocyte integrity or cholestasis rather than liver function, and a change in serum ALT and ALP level may be associated with a decrease in liver function in mass.[1,2,3]

Alkaline phosphatases (ALP) are a family of zinc metaloenzymes, with a serine at the active center. They release inorganic phosphate from various organic orthophosphates and are present in nearly all tissues. monoesters at basic pH values.[4] Alkaline phosphatase is a membrane-bound metalloenzyme that consists of a group of isoenzymes. It is present in mucosal epithelia of small intestine, proximal convoluted tubule of kidney, bone, liver and placenta. Each isoenzyme is a glycoprotein encoded by different gene loci. It is believed that all of the human ALP genes evolved from a single ancestral gene. In liver, alkaline phosphatase (ALP) is found in the microvilli of bile canaliculi and on the sinusoidal surface of hepatocytes. Average values of alkaline phosphatase vary with age and are relatively high in childhood and puberty and lower in middle age and higher again in old age.[6] The levels correlate with person's weight and inversely with the height of person. Highest levels of alkaline phosphatase occur in cholestatic disorders.[6] The bone isoenzyme may be involved in mammalian bone calcification while the intestinal isoenzyme is thought to play a role in the transport of phosphate into epithelial cells of the intestine.[4]

Clinical Significance of Alanine Amino Transferase (ALT)

ALT is commonly measured clinically as part of liver function tests and is a component of the AST/ALT ratio. When used in diagnosis, it is almost always measured in international units/liter (U/L). While sources vary on specific reference range values for patients, 0-40 IU/L is the standard reference range for experimental studies. Significantly elevated levels of ALT (SGPT) often suggest the existence of other medical problems such as viral hepatitis, diabetes, congestive heart failure, liver damage, bile duct problems, infectious mononucleosis, or myopathy, so ALT is commonly used as a way of screening for liver problems. Elevated ALT may also be caused by dietary choline deficiency.[7] However, elevated levels of ALT do not automatically mean that medical problems exist. Fluctuation of ALT levels is normal however, in that it is often reflected by biochemical abnormalities of liver enzymes.[1]

Clinical Significance of Alkaline Phosphatase (ALP)

Alkaline phosphatase plays an integral role in metabolism within the liver and development within the skeleton. Due to its widespread prevalence in these areas, its concentration in the bloodstream is used by diagnosticians as a biomarker in helping determine diagnoses such as hepatitis or osteomalacia[4] The levels of this enzyme in the blood depend on factors such as age, gender, blood type. Abnormal levels of alkaline phosphatase in the blood could indicate issues relating to the liver, gall bladder or bones. Kidney tumours, infections as well as malnutrition has also shown abnormal level of alkaline phosphatase in blood.[9] Liver and bone diseases are the most common causes of pathological elevation of ALP levels, although ALP may originate from other tissue, such as the placenta, kidneys or intestines, or from leukocytes. The third trimester of pregnancy (placenta origin) and adolescence (bone origin) are associated with isolated increase in serum ALP levels.[10] Elevation of ALP with prolonged itching is related with Hepatitis A presenting with cholestasis.[11] Other diseases like infiltrative liver diseases, abscesses, granulomatous liver disease and amyloidosis may cause a rise in ALP. Mildly elevated levels of ALP may be seen in cirrhosis, hepatitis and congestive cardiac failure. Transient hyperphosphataemia in infancy is a benign condition characterized by elevated ALP levels of several folds without evidence of liver or bone disease and it returns to normal level by 4 months. ALP has been found to be elevated in peripheral arterial disease, independent of other traditional cardiovascular risk factors.[4] Some conditions or diseases such as hypophosphatasia, postmenopausal women receiving estrogen therapy because of osteoporosis, men with recent heart surgery, malnutrition, magnesium deficiency, hypothyroidism, severe anaemia, children with achondroplasia and cretinism, children after a severe episode of enteritis, pernicious anaemia, aplastic anaemia, chronic myelogenous leukaemia, Wilson's disease may lead to reduced levels of alkaline phosphatase.[9]

. Liver Function and Dysfunction

The liver is a large, complex organ that is well designed for its central role in carbohydrate, protein and fat metabolism. It is the site where

waste products of metabolism are detoxified through processes such as amino acid deamination, which produces urea.[1]

*Liver Function and Dysfunction

The liver is a large, complex organ that is well designed for its central role in carbohydrate, protein and fat metabolism. It is the site where waste products of metabolism are detoxified through processes such as amino acid deamination, which produces urea.[1] In conjunction with the spleen it is involved in the destruction of spent red blood cells and the reclamation of their constituents. It is responsible for synthesizing and secreting bile and synthesizing lipoproteins and plasma proteins, including clotting factors. It maintains a stable blood glucose level by taking up and storing glucose as glycogen (glycogenesis), breaking this down to glucose when needed (glycogenolysis) and forming glucose from non-carbohydrate sources such as amino acids (gluconeogenesis).

Liver dysfunction is the inability of the liver to perform its primary functions such as detoxification and aiding in digestion. Several factors are responsible for liver dysfunction (damage to the liver) and they include; metabolic disorders such as obesity, diabetes and high triglycerides.[12] Other causes of liver failure are alcoholism, illness, pharmaceuticals (drugs), chemotherapy, pregnancy, poor diet, pesticides and heavy metals, viral infections, excess vitamin A, etc. Symptoms of liver failure include; abnormal metabolism of fats, digestive problems, nutrient mal-absorption, blood sugar problems, neurological effects, weakened immune system, hormonal imbalances.[12] Other symptoms include; bad breath, skin rashes, itchy skin (pruritus), offensive body odour, dark circles under the eyes, yellow discoloration of the eyes, red swollen itchy eyes (allergic eyes), acne rosacea, brownish spots on the skin (liver spots), red palms and soles which may also be itchy and inflamed, flushed facial appearance. Diseases associated with liver disorders are life threatening and most times the only remedy is a complete liver transplant [12]

Biomarkers of Hepatocellular Injury

Alanine aminotransferase (ALT) is regarded as a reasonably specific indicator of liver disease .[13] Damaged hepatocytes leak their ALT into the extracellular space and ultimately plasma, so that ALT activity will be increased in animals with damaged hepatocytes when compared to those with normal hepatocytes.[7] Among the traditional markers of hepatocellular injury, ALT is considered to be a sensitive and translatable indicator of hepatocellular injury in the common preclinical species. However, there may be instances of hepatic injury where ALT activity is not elevated due to inhibitory factors such as Vitamin B12 deficiency, or interference by the presence of pyridoxal-5'-phosphate inhibitors such as isoniazid or lead. Due to the none specificity of ALT to hepatocellular injury, ancillary clinical chemistry and histopathology tests are often used to help interpret ALT values.[7]

.When elevated ALT levels are found in the blood, the possible underlying causes can be further narrowed down by measuring other enzymes. For example, elevated ALT levels due to hepatocyte damage can be distinguished from bile duct problems by measuring alkaline phosphatase. Also, myopathy-related elevations in ALT should be suspected when the aspartate transaminase (AST) is greater than ALT; the possibility of muscle disease causing elevations in liver tests can be further explored by measuring muscle enzymes, including creatine kinase.[13] Clinicians are often more confused in differentiating liver diseases and bony disorders when they see elevated ALP levels and in such situations measurement of gamma glutamyl transferase becomes useful as it is raised only in cholestatic disorders and not in bone diseases.

The enzyme alkaline phosphatase is an important serum analyte and its elevation in serum is correlated with the presence of bone, liver, and other diseases.[9] Therefore Alanine aminotransferase (ALT) and alkaline phosphatase (ALP) are the most widely used clinical biomarkers of hepatic health.[1]

Justification of the Study: Majority of adults including students in the health sector are either not aware of their current health status or have poor health seeking behaviour. Disturbances in biochemical metabolism predispose most people to high risk of morbidity and mortality either from

hepatic, renal or other systemic illnesses with resultant debilitating effects. These conditions would have been prevented by early detection via screening. Therefore this study aims to determine the baseline levels of alanine amino transferase and alkaline phosphatase of apparently healthy students of the College of Health Sciences, Niger Delta University. This will not only be useful in assessing morbidities like hepatic failure, intestinal, muscular or bony disorders but will create awareness as to modifying lifestyles and engaging in preventive measures aimed at retarding and forestalling complications that may arise.

Methodology:
Participants: This was a cross-sectional study carried out amongst the students of the College of Health Sciences, Niger Delta University, Bayelsa State from August to November 2019.The study was approved by our institutional research and ethics committee after explaining the objectives and benefits of the study. Written and informed consent was sought and obtained from each participant before the study. A total of 102 apparently healthy students of the Niger Delta University Bayelsa state, aged within 20-40years were recruited into the study. This was done due to the proximity of investigators and easy contact to the referral hospital for further treatment of cases that may be discovered during survey. No participant was under any form of medication likely to influence any of the parameters under investigation. A general examination was carried out on each participant to rule out fever or jaundice. Structured questionnaire was used in the collection of selected demographic data which includes age, sex and history of alcohol abuse / medications. Pregnant individuals and those with acute / chronic diseases were excluded from the study.

Sample collection: An overnight fast and the avoidance of caloric and caffeinated drink was advised, after which about 3mls of venous blood was collected into a lithium heparin anticoagulant bottle. The blood sample was centrifuged at 3500rpm and supernatant plasma collected. The plasma samples was stored at 2-8^{0}C until ready for analysis. All analysis was done within 24hrs of sample collection at room temperature. The preanalytical, analytical and post analytical phases of analysis were controlled throughout the study.

Method: Plasma Alanine transaminase and Alkaline Phosphatase were determined manually by Colorimetric and Kinetic method respectively.

Statistical Analysis: The statistical analysis of raw data obtained was done using SPSS version 23 software application. The frequency of risk factors was analysed by tables. Comparison of groups was done by one way ANOVA and results were expressed as mean ± Standard Deviation (SD). Probability or P-value will be set at 0.05 or 5% confidence interval.

Results
The result of the present study are presented in table 1, 2, 3 and 4. Table 1 shows the demographic characteristics of studied participants. A total of 102 respondents which comprises of 55 males and 47 females were interviewed. The age groups was between 20-40 years. Majority of the respondents 99(97%) were aged between 20-30 years which comprises of 53(53.54%) for males and 46(46.46%) for females. Only 3(3%) of respondents were between 31-40 years which comprises of 1(33.33%) for male and 2 (66.67%) for female.

Table 1: Demographic characteristics of studied participants

		AGE GROUP	
GENDER	N (%)	20-30 N (%)	31-40 N (%)
MALE	55 (53.92)	53 (53.54)	1 (33.33)
FEMALE	47 (46.08)	46 (46.46)	2 (66.67)
TOTAL	102 (100)	99(97.0)	3(3.0)

Table 2 shows the comparison of gender of respondents with ALT and ALP levels. The male ALT value was found higher than the female ALT value with a mean ± standard deviation of 5.56 ±2.6 (male) and 5.28 ±3.06 (female) respectively. The p-value was 0.56, which shows that the result was not statistically significant. The ALP values in females was higher than the male ALP value with a mean ± standard deviation of 19.47 ±7.98 (female) and 18.55 ±7.21 (male) respectively. P-value was 0.54, which shows that the result was not statistically significant.

Table 2: Comparison of gender of respondents with Alanine transaminase (ALT) and Alkaline phosphatase (ALP) levels

Parameters	Gender	Mean ±SD	SEM	P-value	Remark
ALT (U/L)	Male (n)	5.56±2.64	0.35	0.56	NS
	Female (n)	5.28±3.04	0.45		
ALP (U/L)	Male (n)	18.55±7.21	0.97	0.54	NS
	Female (n)	19.47±7.98	1.16		

Key: SD= Standard deviation, SEM= Standard Error of Mean, S=statistically significant ($p<0.05$,) NS = Not statistically significant

Table 3: Comparison of Age group distribution with ALT and ALP level

Parameter	Age group	Mean ±SD	SEM	P-value	Remark
ALT (U/L)	20-30	5.44±2.86	0.20	0.94	NS
	31-40	5.33±1.15	0.66		
ALP (U/L)	20-30	18.92±7.52	0.75	0.80	NS
	31-40	20.00±7.54	4.35		

Key: SD=Standard deviation, SEM Standard Error of Mean, S=statistically significant ($p<0.05$), NS = Not statistically significant

Table 3 shows the comparison of age group of respondents with plasma ALT and ALP levels. The ALT value of respondents between 20-30 years was found higher than the ALT value of respondents between 31-40 years with a mean ± standard deviation of 5.44±2.86 (20-30 years) and 5.33±1.15 (31-40 years) respectively. The p-value was 0.94, which shows that the result was not statistically significant. Contrary to this, the ALP values of respondents between 31-40 years was higher than the ALP value of respondents between 20-30 years with a mean ± standard deviation of 20.00±7.54 (31-40) and 18.92±7.52 (20-30) respectively. P-value was 0.80, this also was not statistically significant.

Table 4: Comparison of ALT and ALP level with laboratory reference ranges

Parameters		N	Mean ±SD	SEM	P-value	Remark
ALT (µ/L)	Normal	61	7.10±2.14	0.28		
	Low	41	2.98±0.78	1.56	0.94	NS
	High	-	-	-		
Total		102				
ALP (µ/L)	Normal	95	18.63±6.43	0.97		
	Low	3	8.25±0.00	1.60	0.07	NS
	High	4	38.25±1.71	1.18		
Total		102				

Reference ranges: ALT =5-40U/L ALP = 9-35U/.L

Table 4 Shows the comparison of ALT and ALP level with the laboratory reference ranges. The laboratory reference ranges for this study was 5-40U / L for ALT and 9-35U / L for ALP level. When compared to the laboratory reference ranges for ALT, a high number of respondents 61(60%) fell within the normal reference range with a mean ± Standard deviation of 7.10±2.14, while 41(40%) respondents were below the normal reference range with a mean ± Standard deviation of 2.98±0.78. No high ALT level was recorded for any respondent. P-value was 0.94, which shows that the result was not statistically significant. In terms of ALP about 95(93%) of respondents fell within the normal reference range with a mean ± Standard deviation of 18.63±6.43, while 3(3%) respondents were below the normal reference range with a mean ± Standard deviation of 8.25±0.00 and 4(4%) of respondents were higher than the normal reference range with a mean ± Standard deviation of 38.25±1.71. However, the result was not statistically significant(p=0.07)

Discussion

Plasma enzymes like the alanine aminotransferase and alkaline phosphatase serve diagnostic purposes in health and diseases. The estimation of ALT and ALP enzymes are used as index of hepatic damage most times especially when there is increased activity in measurable quantities.

The results obtained from measurement of these parameters under study were done using similar analytical methods and units of measure as those in literature with quality control measures assured.

From the present study, it was observed that ALT level was higher in males (5.56±2.6) than in females (5.28±3.06) but was not statistically significant. This is in keeping in study by Kasia et al[13] who assessed the health status of hospital staff here in Bayelsa state where the male ALT values were higher. Similar study by Solomon et al[15] showed significantly elevated ALT levels in 112 of 3547 participants (3.3%), males than in females (p<0.001) probably due to high haemoglobin levels in male adolescents. This is also comparable with a study by Liu *et al.*, (2014)[7] on blood donors without a diagnosis of hepatitis from London, in which the ALT level was much higher in males than females. Other researchers have attributed the gender-based differences in ALT levels to the hormonal differences between males and females by Poustchi *et al.*[15] and higher muscle mass in males as suggested by Saathoff et al.[16] The higher ALT value of respondents between 20-30 years is contrary to study by Sami *et al*[17] where no statistically noteworthy association was observed between elevated levels of ALT and age.

This study revealed that majority of the respondents 61(60%) had within normal reference value for ALT which is in keeping with Akinosun et al[18] that reported high percentage of normal findings amongst healthy University College Hospital Staff and Petrol attendants within Ibadan metropolis. This is also comparable to study by Kasia et al[13] report of 84% of normal ALT values amongst healthy staff of Niger Delta University Teaching Hospital, Okolobiri.

From the present study, the female ALP level was higher than the male ALP values but was not statistically significant. This is contrary to previous report by Nargis *et al*[19] which showed a high serum ALP levels in males than females. Maniolo et al[20] compared other variables including ALP in white and black population and also identified higher mean values for ALT, ALP, Gamma glutamyl transferase and total bilirubin in both black and white males when compared to their female counterparts. These findings suggest some racial similarities in these parameters.

This study showed that the ALP values of respondents between 31-40 years was higher than the ALP value of respondents between 20-30 years. This is not in line with a study conducted by Turan *et al*[21] on serum alkaline phosphatase levels in healthy children, which showed that higher ALP levels were noted at 10-11 years in girls (p=0.02) and at 12-14 years in boys (p<0.001) and starts declining after age 12 and 14 in girls and boys, respectively. It is also worthy of note that average values of alkaline phosphatase vary with age and are relatively high in childhood and puberty and lower in middle age and higher again in old age as observed by Thapa and Anuj.[6]

From our study we observed that a high number of respondents 95(93%) fell within the normal reference range. This finding is in keeping with another by Enemchukwu et al[22] where within normal values for ALP, ALT, Aspartate Transaminase and total bilirubin were reported for healthy hospital staff in Abia state as against elevated values in malaria, typhoid and malaria-typhoid co-infected participants.

This paper was limited by low turnout of participants which reduced the sample size, this could be explained based on lack of interest or poor health-seeking behaviour of students towards their health status. However, the result of this study is tenable anywhere.

Conclusion

The health status of the studied participants was good as revealed via normal ALT and ALP levels amongst majority of the students which indicates low risk for hepatic injury among them.

Recommendation

Establishment of reference range for age and sex should be advocated to increase our understanding and thus the need for future assessment.

REFERENCES

1. Sharma, U., Pal, D.,Prasad, R. Alkaline phosphatase: an overview. *Indian Journal of Clinical Biochemistry*.2014; 29: 269-278.

2. Giannini EG, Testa R, Savarino V. Liver enzyme alteration: a guide for clinicians. *Canadian Medical Association Journal*.2015; 172: 367–379.

3. Ruhl, C. E., Everhart, J. E. Diurnal Variation in Serum Alanine Aminotransferase Activity in the United States Population. *Journal of Clinical Gastroenterology*.2013; 47:165-190

4. Whyte, M. P. Physiological role of alkaline phosphatase explored hypophosphatasia. *Annals of the New York Academy of Sciences,* 2010; 1192:190–200

5. Thapa, B. R. and Anuj, W. Liver Function Tests and their Interpretation, *Indian Journal of Pediatrics,* 2017; 74: 663-671.

6. Liu, Z., Que, S., Xu, J. and Peng, T. Alanine Aminotransferase-Old Biomarker and New Concept: A Review. *International Journal of Medical Sciences,* 2014; 11: 925-935.

7. Akuyam, S. A., Abubakar, A., Lawal, N., Yusuf, R., Aminu, S. M., and Hassan, A. Assessment of biochemical liver function tests in relation to age among steady state sickle cell anemia patients. *Nigeria Journal of Clinical Practice* 2017*;* 20: 1428-1433.

8. Kirti, R., Sanchi, D. and Rachita, R. Brief review on alkaline phosphatases-an overview *International Journal of Microbiology and Bioinformatics,*2012; 2:1-4.

9. Lallès, J. P. Intestinal alkaline phosphatase: novel functions and protective effects. *Nutrition Reviews*.2014; 72: 82-94.

10. Bilski, J., Mazur-Bialy, A., Wojcik, D., Zahradnik-Bilska, J., Brzozowski, B., Magierowski, M., Mach, T., Magierowska, K., Brzozowski, T. Role of Intestinal Alkaline in Inflammatory Disorders of Gastrointestinal Tract. *Mediators of Inflammation* 2017; 22: 907-1001.

11. Ghouri, N., Preiss, D., and Sattar, N. Liver enzymes, non-alcoholic fatty liver disease, and incident cardiovascular disease: a narrative review and clinical perspective of prospective data. *Hepatology* 2010; 52: 1156-1161.

12. Lorenzo, C., Hanley, A., Rewers, M., and Haffner, S.The association of alanine aminotransferase within the normal and mildly elevated range with lipoproteins and apolipoproteins: the Insulin Resistance Atherosclerosis Study. *Diabetologia. 2013;* 56:746-757.

13. B. E. Kasia, O. E. Kunle-Olowu, U.B.A. Mrakpor. Health status and measurement of some liver function parameters of staff of Niger Delta University Teaching Hospital, Okolobiri, Bayelsa State. Port Harcourt Medical Journal 2014;8:67-73

14. Solomon, C., Yeh, J., Chang, M., Liao, Y., , L., Neoh, C., , T., Wang, J. Gender difference of Alanine Aminotransferase elevation may be associated with higher haemoglobin levels among male adolescents. *PLoSOne.* 2010; 5: 13-69.

15. Poustchi, H., George, J., Esmaili, S., Esna-Ashari, F., Ardalan, G., and Sepanlou, S. G. Gender differences in healthy ranges for serum alanine aminotransferase levels in adolescence. *PLoS One.* 2011; 6: 21178.

16. Saathoff ES, Schneider P, Kleinfeldt V, Geis. S, Haule D, Maboko L, Samky E, et al Laboratory Reference values for healthy adults from southern Tanzania. *Trop Med Int Health* 2008;13: 612-625

17. Sami, H. A., Baig, M., Bashawri, J., Aashi, M. M., Shaibi, F. K. Alqarni, D. A. Prevalence and Association of Elevated Liver Transaminases in Type 2 Diabetes Mellitus Patients in Jeddah, Saudi Arabia. *Cureus.*2019; 11, 51-66.

18. Akinosun OM, Arinola OG, Salimonu LS. Immunoglobulin classes and liver function tests in Nigerian petrol attendants. *Indian Journal of Occupational and Environmental Medicine* 2006; 10:58-61

19. Nargis, W., Ahamed, B. U., Hossain, M. A., Biswas, S. M., and Ibrahim, M. Pattern of Elevated Serum Alkaline Phosphatase (ALP) Levels in Hospitalized Patients: A Single Centre Study. *Pulse* 2013; 6: 1-23

20. Manolio TA, Burke GL, Savage, PJ. Jacobs DR Jr, Sidney S, Wagenknecht LE, Allman RM et al. Sex and race related differences in liver associated serum chemistry tests in young adults in the CARDIA study. *Clin Chem* 1992; 38:1853-1859.

21. Turan, S., Topc, B., Gökçe, B., Güran, T., Atay, Z., Omar, A., Akçay, T.and Bereket, A. Serum alkaline phosphatase levels in healthy children and evaluation of alkaline phosphatase z-scores in different types of rickets. *Journal of Clinical Research Pediatrics and Endocrinology* 2011; 3: 7-11

22. Enemchukwu BN, Ibe CC, Udedi SC, Iroha A, Ubaoji KI and Ogundapo SS. Liver function assessment in malaria, typhoid and malaria-typhoid co-infection in Aba, Abia State, Nigeria. *Pakistan Journal of Biological Sciences 2014; 17: 860-863.*