Original Research Article

Serum Amino Transaminases Activities and De Ritis Ratio amongst Apparently Healthy Secretors and Non-Secretors of ABH Substances Dwelling in Uyo Urban, Akwa Ibom State, Nigeria.

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ABSTRACT

Aims: The current study was designed to determine a local reference range of serum Alanine (ALT) and Aspartate (AST) activities and De Ritis ratio amongst apparently healthy secretors and non-secretors of ABH substances within Uyo, Akwa Ibom~~, and~~ State, Nigeria, aged between 20 to 60 years for both genders.

Study design:the current study design was experimental and simple randomized sampling techniques was employed.

Place: the current study was carried out in Uyo, Akwa Ibom, ~~and~~ State, Nigeria.

Duration of the study: from 12th June to 6th December 2017.

Methodology: About 2ml of saliva and 5ml of Blood samples were collected from 400 subjects by standard procedures and both serum ALT and AST activities were determined by a spectrophotometric techniques using commercial kit reagent produced by Sigma-Aldrich Cooperation Limited Liability Company, USA while ABH secretor status determined by Haemagglutination Inhibition Method using Anti-H -Lectin kits produced by BIOTEC Laboratories Limited, Suffolk, United Kingdom.

Results: Statistically Chi-Squared (X^2) test shows significant differences (P <0.05) between the means serum AST and ALT levels according to gender ((X^2) = 4.99, P =0.025). No correlation found between serum ALT and AST activities with age and genders (r=0). De Ritis ratio = <1 according to gender, with no significant difference (P >0.05) between genders (t=2.44, P =0.1238,). Significant difference (P <0.05) between activities of ALT and AST in ABH secretor and non-secretors and sexes ((X^2) =5.1, P =0.0239,). Chi Squared shows no significant differences in the mean prevalence rate ratio between gender (X^2) = 3.2, df = 1, P = 0.07364, P >0.05). The prevalence rate ratio of ABH secretors and non-secretors was 4:1, according to ages and there was a significant difference (P <0.05) between male and female genders (t=2.44, P =0.1238,)

Conclusion: AST and ALT references ranges in male ABH secretors subjects were higher than in female non-secretors subjects respectively.

Keywords: “Reference range”, “ALT and AST activities", De Ritis ratio”, "Secretors” and “Non-Secretors of ABH substances".

1.1 INTRODUCTION

Serum transaminases (also called aminotransferases) are basically groups of enzymes in the human body that help in the catalysis of transamination reactions characterized by rapid reversible interconversion of amino acids and oxoacids involving transfer of the amino group (—NH2) of an α-amino acid to a carbonyl compound, commonly an α-keto acid (an acid with the general formula-RCOCOOH) **[1, 2].**Transaminases are ubiquitous in their cellular distribution and are non-plasma specific enzymes **[3]** because they do not have physiological function in the plasma. Evidences from the work of **[4]** have shown that transaminases are intercellular enzymes normally present in living cells and tissues of the hearts, livers parenchyma, pancreatic cells, spleens, lungs tissues, erythrocytes, bile, cerebrospinal fluid, salvia, kidneys, brain cells, plasma as well as sera of human being. In normal healthy state the concentration or activities of these enzymes in these cells and tissues are normally higher than in the plasma except in some specific pathological condition **[4].** The two aminotransferases of greatest clinical significance are:1) Aspartate transaminase (AST) or Aspartate aminotransferase (ASAT) formerly known as serum glutamic oxaloacetic transaminase -SGOT **(EC 2.6.1.1)**, is found in both cytosol and mitochondria in several organs such as the liver, cardiac muscle, skeletal muscle, kidney, brain, pancreas, lung, leukocytes, and erythrocytes but the concentration is highest in the hepatic parenchyma. It usually follows a decreasing concentration viz: heart, liver, cytoplasmic or soluble isoenzyme, and a mitochondrial isoform **[5].** 2) Alanine transaminase (ALT) or alanine aminotransferase ALAT, formerly serum glutamic pyruvic transaminase -SGPT **(EC 2.6.1.2)** is a cytosolic enzyme which is present predominantly in the liver and it is used as a clinical marker of hepatic damage and hepatotoxicity **[6, 7].** The two forms of ALT are ALT1 and ALT2 encoded by separate genes. There has been development of novel isoenzyme specific ALT1 and ALT2 antibodies and the expression of ALT1 found highest in liver, skeletal muscle and kidney and low levels in heart muscle but not detectable in pancreas. High ALT2 reactivity are detected in heart, skeletal muscle and no ALT2 expression found in liver or kidney **[8].**

Biochemical pathway and Mechanism of transaminase reaction.

In all amino transfer reactions, 2-oxoglutarate and l-glutamate is serving as one amino group acceptor and donor pair and Pyridoxal-5′-phosphate (P5′P) functions as coenzyme in the amino transfer reactions **[22]** and all amino acids are usually involved except Lysine and Threonine **[23].**These reactions are usually rapid and reversible, but the equilibra of ALT and AST favors the formation of L-alanine and L-aspartate. The α -keto gluterate and L-glutamate couples serve

as the amino group acceptor in the forward reaction and donor in the reverse reaction in all amino groups transfer reactions**[24, 25].** During transamination of ALT and AST, the serum pyridoxal –5 –phosphate (p-5-p) serving as the co-enzyme (forms a true prosthetic group) **[24]** binding to the apo-enzyme and accepts the amino group from the first substrate L-alanine or L-aspartate forming an enzyme-bound pyndoxamine-5pphosphate,resulting to the first reaction product pyruvate or oxaloacetate respectively **[23,24]** . The co-enzyme, in its amino form, then transfer the amino group to the second substrate (α-keto glutarate) to form the second product (L-glutamate) respectively, **[24] (as shown in figure 1-4)**.At the end the p-5-p is regenerated to its initial form via Schiff base **[26].** Generally, only a small amount of the co-enzyme is required to saturate the apoenzyme (karmen et al.,(1970)**[27].**The p-5-p is usually present in normal serum, but where it's deficient can be supplied (IFCC recommendations, (1976) **[28].**

Variation of normal range of serum amintransferases in normal state

The normal range of serum aminotransferases in healthy state is usually defined as the mean of the reference population plus 2 standard deviations (μ+2SD) which represents the serum values of approximately 95% of a normally distributed population under study **[1].** The normal values for aminotransferases in serum for normal healthy individuals has been known to vary considerably among laboratories, populations, races, genders and methods used by different manufacturers of reagents and equipment. Hence each laboratory has its own reference range for the serum transaminases depending on the mean and standard deviation of the resident population of the region concerned **[1].**

Challenges of establishing uniform and conventional normal range and secretor status

Since the discovery of tissues cellular enzymes **[9, 10]** extensively studies have been carried out on the reference ranges for the differences diagnosis, prognosis, monitoring and treatment of patients with acute and chronic intra and extra hepatic, cardiac, diseases, muscles dystrophy and progressive dermatomyosities. Although, extensive studies on the references of these enzymes have been marked with the quest for optimal analytic methodology of greater specificity, sensitivity and reproducibility, the reference range of these enzymes in both blacks and Caucasians subjects have never been investigated in respect to their secretion and non-secretion status of ABH substances **[11].** Besides, over the past few decades clinicians have believed that the reference ranges of these tissues enzymes as quoted on western literatures may be specific or definitive only for normal ABH secretors and non-secretors Caucasian

populations and only serves as a guide for other normal populations since there are differences in life styles , diets, diseases conditions, genetic variation, environmental and occupational factors which may alter the activities of these enzymes in both subjects even in normal state **[11].**Several studies in the past, based on blood donors (after exclusion of people with viral hepatitis) had suggested an upper limit of normal for the ALT level between 40 to 50 U/L.Later on, due to increasing prevalence of patients with metabolic disorders like metabolic syndrome and non-alcoholic fatty liver disease, there has been a debate on revision of upper limit of normal for serum aminotransferases **[12, 13, 14].**The idea that subjects with metabolic abnormalities must be excluded while evaluating the upper limit of normal for serum ALT levels came forth from the findings of **[15].**Subsequently many authors have re-evaluated the upper limit of normal of ALT values in specific populations, including adults and adolescents and observed similar results **[16, 17, 18, 19, 20].**However, there is concern regarding the cost effectiveness and unclear benefits of implementing lower value of upper limit of normal**[21].**This means that there may be differences between published values and actual reference values of these enzymes in these subjects. Studies on secretion status of ABH substances have also contributed in variation of the normal values of transaminases even in normal healthy states due to the fact that non-secretion status of ABH substances are more susceptible to a lot of pathological condition than secretors and even in normal states.

2.3 Biochemical, physiological and Pathological factors responsible for changes in serum ALT & AST levels.

Under normal condition, almost all the ALT and AST activities are intercellular, since they are non-plasma specific enzymes **[3]**. It is only in some pathological conditions that these enzymes can be released from the cells or tissues into the circulating plasma. Some of these specific pathological conditions include the following:

a) AST levels in Liver Diseases: In liver diseases such as acute hepatocellular and extra hepatocellular diseases, serum AST levels are higher than normal. These values are particularly increased to about 10 to 1000 times the normal range, in most cases of acute viral hepatitis and hepatocellular type of toxic hepatopathy as in iproniazid jaundice of carbotetrachloride poisoning [10].In shock and severe anoxia levels of serum AST may be elevated to 20-fold or more [24]And In extrahepatic obstruction of intra-hepatic cholestasis the value from normal to 10 fold elevations [24]In gall stone obstruction which is presumed to be a manifestation of ascending cholangitis and parenchyma hepatic injury, higher values may also be seen from normal to 8

fold elevations [29]In fatty metamorphosis of the liver, alcoholic or obsessed patient the levels are normal only slightly elevated one-fold **[30]**.Whereas in liver cirrhosis the values are usually elevated though rarely more than four folds. In deeply jaundice alcoholic with steatorrheanecrosis (alcoholic hepatitis) serum AST levels are usually elevated less than five folds. Serum AST levels up to 10-folds in alcoholic patients may reflect coincidence delirium tremens or alcohol myopathy. In fatty liver of pregnant women serum AST levels are usually elevated, but to less than 10-folds **[31]**.In active post necrosis, cirrhosis (chronic active hepatitis) serum AST levels may be low as in liver cirrhosis or as high as those seen in viral hepatitis 3 to 20 fold Serum AST values from normal to fivefold elevation are seen in patients with carcinoma or lymphoma of liver **[10]** Generally, if serum AST values are elevated to more than 100-fold, this may suggest the presence of acute or sub-acute hepatic necrosis, while lesser values than this may reflect chronic hepatic diseases. The diagnostic value of serum AST should then be tested against other biochemical parameters and clinical features such as serum bilirubin, ALT, alkaline phosphatase, creatine kinase and lactate dehydrogenase **[24]**. ALT levels in Liver Diseases .On the other hand serum ALT levels are high in acute hepatocellular diseases and lower in obstructive jaundice and chronic diseases, but negligible in extra-hepatic diseases. These levels usually reflect acute hepatic diseases with more specificity and sensitively than those of AST **[32]**.In most cases of viral hepatic or hepatocellular types of toxic hepatopathy serum ALT levels may be higher than those of AST. Conversely, serum ALT values are lower than serum AST values in cases of liver cirrhosis, fatty metamorphosis of the liver delirium tremens, hepatic carcinoma and myocardial infarction **[33, 34]** .

B) De Ritis ratio or ALT/AST ratio :The differences in serum AST and ALT levels in several forms of hepatic diseases have led to the usefulness of De Ritis ratio or ALT/AST ratio in clinical diagnosis ~~(Deritis, Coltorti, and gusti, (1956)~~ **[35]** .The AST/ALT ratio have also added little diagnostic value to ALT levels may be above 300 fold elevations. Values within these ranges are usually due to acute hepatic necrosis (viral or toxic) and uncommonly due to infectious mononucleosis or choked cholithiasis. The latter two conditions will yield a ALT/AST ratio different from that of hepatitis **[10 ,35]**. Furthermore, in hepatitis with serum AST values exceeding 1000 folds, the ALT/AST ratio is usually lower compared to AST values below 1000 folds. When AST values are below 300 folds the relative elevations of the two enzymes are of differential value. Hence in hepatitis infectious mononucleosis and obstructive jaundice with serum AST in this range, the ALT/AST ratio is usually 1.0 or below. In infiltrative diseases like cirrhosis or steatonecrosis (alcoholic hepatitis) ALT/AST ratio is usually above 1.0. This

information is of considerable assistance in distinguishing acute viral or toxic hepatitis from steatonecrosis **[30]** .Conversely, the alcoholic apparent cirrhosis with ALT/AST ratio equals to 1.0 indicates complications of acute pancreatitis with obstruction of the common duct or a drug-induced hepatic injury **[36]**.In delirium tremens, the AST/ALT ratio is higher than 1.0 indicating myocardial infarction or muscle diseases. In general, although the determination of serum ALT or AST levels are useful in following the course of hepatitis, measurement of AST or ALT or both is of considerable value in the recognition of early hepatitis and lesser value in distinguishing it from obstructive jaundice. Measurements of both parameters are extremely useful in distinguishing acute hepatitis from cirrhosis or streato necrosis. Measurement of either or both enzymes is also useful in early detection of hepatic damage due to hepato-toxic agent, while AST is useful in detection of metastasis of the liver **[37].**

b) Cardiac Diseases: Serum AST levels in cardiac diseases such as myocardial infarction are higher than normal within the first 12 hours. After this time as the myocardial infarction progresses there is persistent rise from 3 to 5 days but high abnormal values of serum AST and ALT will occur in the second day.However, severe angina pains may be present for sometimes before necrosis occurs **[24]**.When serum AST and ALT levels are elevated in absence of electrocardiographic changes of where electrocardiography failed to show pathology, the enzymes diagnosis may be the earliest due to acute cardiac necrosis proceeding Erythrocyte Sedimentation Rate and Leukocyte counts. Serum levels of AST may also be elevated in pulmonary infarction particularly when associated with their failure. Minimal elevations may occur in acute pericarditis and no change in acute Rheumatic carditis. Extra-cardiac factors causing rise in AST levels such as hepatic diseases or muscle necrosis are usually distinguishable clinically by simultaneous determination of the ALT and AST levels. However in most cases serum ALT if effected minimally or not at all by myocardial infarction. Elevated serum ALT and AST levels are usually associated with severe heart failure, shock and acute hepatic diseases and re-infarction, although re-infarction values will be smaller and transient. Elevations of serum ALT level has also been reported on surgical procedures involving myocardial walls and inter ventricular septum.Generally, the limitations of serum AST as a diagnostic modality in myocardial infarction is primarily related to the fact that the rise in its level may be seen in a large varieties of diseases affecting heart muscles than ALT which is more specific and sensitive to the liver cells.Other Factors such as drugs induction and therapy like ascorbic acid, cholinergic,codeine salicylate, guanelhidine, hydralazine, isoniazid, ampicillin, oral contraceptives, meperidine, morphine and tolbutamide will increase the serum levels of

these enzymes, when administered, **[24]** Anticoagulants may be associated with increases in serum ALT and AST activities and this effect is independent of prothrombin time **[38].**Haemoglobinopathy disoders resulting in excess hemolysis of the red blood cells will increase serum ALT and AST activities by 2% with each 10mg/dl of Hemoglobin **[24]** .In addition degradation and excretory pathways and processes will help to remove these enzymes from the plasma at the end of their biological half **[39].** Consequently rapid disappearance from the plasma is usually observed **[40]** and since the half-life of serum AST is less than that of serum ALT (2 days and 6.3 days respectively) it is expected that the disappearance of AST from plasma will be more rapid than the disappearance of serum ALT**[41 ,42,43] .**Finally, dietary factors and severe deficiency of vitamin B6, will affect the plasma level of p-5-p and consequently causing decrease in serum ALT and AST activities **[22, 23].**

h) Contribution and influence of the Secretion of ABH Substances in the variation of normal ranges of AST/ALT levels

The concept of secretor status is a concept which reveals and explains why some individuals in the population have the ability or the inability to secrete soluble ABH blood group substances into their body secretions. The fact that A and B substances were present in the saliva of most A and B individuals was first discovered by Lehrs and Putkonen in 1930 **[44].** Later Schiff, (1934) **[45]** suggested that the presence or absence of the blood group substance in the saliva was determined by a special gene, the secreting gene which he designated as ***Se*** (for secretor) ; the non-secreting gene was designated as ***se***, and ***Se*** was supposed to be the dominant member of the pair. In recent times based on the secreting gene,the secretor status of individuals in the population can be determined as Secretors or non –secretors ~~(D'Adamo,2001~~**).[46]**

Definition of secretors and non-secretor: The term secretor is applied to those individuals in the population with genotype ***(Se/Se or Se/se***) who are able to secret **H**-substance with or without **A** or **B-**substance and does not take into account the presence of Lewis or any other blood group substance in their saliva. Approximately 80 percent of individuals in the population are secretors, ***(Se/ se or Se,/se)*** and they secret **H-**substance irrespective of their **ABO** blood group .Thus the saliva of group **O** secretors contain **H** and that of group **A** and **B** secretors contain **A,B** and **H** and **AB** secretor contain **A, B** and **H**. ~~(Morgans and Watkins 1948)~~.[47]The amount of **A** substance in saliva of group **A** secretor follows a log normal distribution .The amount of **A** in saliva appears to vary independently of one another, although there is a significant correlation between the ratio of **A** to **H** amongst sibs **[48]** ~~(Clark , 1959).~~ **ABH**

secretion is controlled by two alleles, ***Se*** and ***se***. ***Se*** is dominant and ***se*** is recessive (or amorphic).On the other hand individuals who do not secrete their blood type antigen into their secretions are termed 'non-secretors'. They do not secret **A, B** or **H** either. About 15-20 percent of the individuals in the population are non-secretors. The Genetic basis & characteristics of Secretors status. The ***Se*** locus is located on chromosome 19 at 19q13.3. It contains two exons that span about 25 kb of genomic DNA. The **Se** locus encodes a specific fucosyltransferase that is expressed in the epithelia of secretory tissues, such as salivary glands,the gastrointestinal tract,and the respiratory tract ~~(D' Adamo, 2001)~~ [46].The enzyme it encodes, catalyzes the production of H antigen in bodily secretions."Secretors" have at least one copy of the Se gene that encodes a functional enzyme—their genotype is Se/Se or Se/se. They secrete H antigen which, depending on their ABO genotype, is then processed into A and / or B antigens. Non-secretors are homozygous for null alleles at this locus (se/se). They are unable to produce a soluble form of H antigen and hence do not produce A and B antigens ~~(Mollicone, 1995).~~ [49]Secretor Status and Pathology Records on disease susceptibility among secretors & non-secretors states have been well studied over the pasts decades. The FUT2 gene encodes the enzyme α (1, 2) fucosyltransferase, which determines expression of blood-group antigens on mucosal epithelial cell surfaces and in secretions. Homozygotes for a specific gene stop mutation in FUT2 (non-secretors) and cannot produce this enzyme and thus are unable to express blood group antigens. Non-secretor status is therefore associated with a decreased risk of several infections than secretors. Some of these infections include digestive system diseases, oral pathology like throat and oesophageal cancers, duodenal ulcers, peptic ulcers diseases, gastritis, respiratory system diseases like chronic obstructive pulmonary disease (COPD), habitual snoring, bleeding times, diabetes and metabolic problems syndrome X ,heart disease, variety of autoimmune diseases including ankylosing spondylitis , reactive arthritis, psoriatic arthropathy, Sjogren's syndrome, multiple sclerosis' Grave's disease .Bacterial urinary tract infections, fungal infections, Relationship between HIV I & 2 infection, alcoholism ~~(D'Adamo , 2001)~~ **[46].**

THE PURPOSE OF THE STUDY

General objective or Aim: Primarily aimed at establishing a true reference range of serum ALT and AST activities and De Ritis Ratio in apparently healthy ABH secretor and non-secretor subjects drawn from urban dwellers of Oyu, Akwa Ibom state,Nigeria

Specific Objectives

1) Establish the ABH secretor and non-secretor status in apparently healthy subjects drawn from urban dwellers.
2) Establish of a true reference range of serum ALT and AST activities and De Ritis ratio in apparently healthy drawn from urban dwellers.
3) Determine whether there is any statistically significance correlation between the variables, ages and genders.
4) Determine the frequency of distribution and the prevalence rate of ABH secretor and non-secretor status in apparently healthy subjects drawn from urban dwellers and the secretor status ratio.

Significance of the study. At the time of the commencing this study, it was on record that no such study have been carried out in this area before now. Hence, the findings are hoped to help for differential diagnosis and monitoring of treatments in patients with liver diseases cardiac diseases, muscles dystrophy and dermatomyosities in which the levels of these enzymes are altered and their secretor status ratio.

2. STUDY SETTING

Study area: The study area is Uyo urban and it is in one of the major oil producing state of Nigeria. The town became the capital of the state on September 23, 1987 following the creation of Akwa Ibom State from Cross River State. The University of Uyo resides in this town. Uyo town is the capital city of Akwa Ibom state, located southeastern Nigeria. Uyo lies on the road from Oronto Ikot Ekpene. A collecting station for palm oil and kernels, it is also a local trade centre (yams, cassava [manioc], palm produce) and area is inhabited mainly by the Ibibio people. The town has a brewery and a textile mill. It is the site of the University of Uyo (since 1991). ~~(Encyclopedia Britannica,2020)~~ **[50]**.

Geographically Oyu lies on Latitude: 5.0333 Lat (DMS) 5° 1' 60N, Longitude: 7.9266 ,Long (DMS) 7° 55' 36E and Elevation (Feet): 830 Zip Code: 520261.**[50]**. Uyo Local Government is a creation of statute of the Federal Government of Nigeria. It is by creation one of the 31 Local Government Councils that make up Akwa Ibom State, which is one of the 36 State of the Federation of Nigeria.The Population of Uyo town by NPC (in 2016 EST.) is 440,000.**[51]**

Study Design: experimental and **Duration of study**: The work was carried out in Chemical Pathology Unit, Department of Medical Lab Science,University of Calabar ,Nigeria ~~between December 2009 and December 2010.~~ from 12th June to 6th December 2017.

a)Calculation of sample size.The Formula of Cochran, 1977, for calculating the sample size (S) was adopted in this study and is given by **[52]**: $S= t^2p (1-p)/ e^2$, Where t= t value (The alpha level used in determining sample size in most educational research studies is either .05 **[53]** . In Cochran's formula, t value for alpha level of .05 is 1.96 for 95% confidence level for sample sizes above 120. p= prevalence rate in percentage (%) of secretors and non-secretors of ABH substances in Oyu urban Akwa Ibom State ,Nigeria and in this case it is taken to be 0.5 or 50% since nobody had ever worked on this population **[54,55]** .While e = tolerance error or confidence interval expressed as decimal and it is taken to be 0.05. Therefore $S = (1.962)^2 (0.5(1-0.5)/(0.05)^2= (1.962)^2 (0.5)^2 / (0.05)^2= 384.16$= approximately 400 samples scheduled to be sampled. However , only 300 subjects for AST and 303 for ALT were actually used due to cases of loss data and specimen during the study and inadequate reagents .

b) **Criteria for Selection of apparently healthy individuals:** About 400 samples from apparently healthy individuals of both sexes, and aged 20years and above were selected from Uyo Urban dwellers in Akwa Ibom State.

c) **Ethical Approvals**:

These were sought and obtained from the management of the Ethical Committee, University of Calabar Teaching Hospital,Calabar, Cross Rivers State Nigeria and the Research Ethical Committee, Centre for Clinical Governance, Research & Training Ministry of Health Calabar, and Cross Rivers State, Nigeria. Oral informed consent was obtained from the participants and same was approved by the Local ethical committee in the study area concerned administrative approval from Uyo Urban ,Akwa Ibom State, Nigeria

d)**Informed and written consent**:These were also sought and obtained from these donors before inclusion in the study.

e)**Administration of questionnaire**:The harmless nature and advantage of the research was also explained to each subjects in the form of counselling in which a questionnaire was administered on each of the subjects to obtain more medical information about clinical history. After counselling, informed consent forms were filled and signed by these subjects screening and collection of samples started.

Collection and treatment of blood samples: About 5ml of blood samples from apparently healthy

individuals of both sexes, were collected by veinu -puncture into a clean, dried blood tubes. The samples were allowed to stand at room temperature for about 2 hours to clot and retract. They were then spun and stored in a deep freezer frozen. All haemolysed samples were excluded.

Collection of Saliva Samples for determining of ABH Secretor Status: Two millilitres of saliva was collected from the mouth of these subjects using a new commercial Whole-Saliva Collection Device before transferring into a clean, dried and labeled 16 x 100mm centrifuge Pyrex tubes.

Methods for the determination of ABH secretor status : Saliva test by Haemagglutination inhibition method using Anti-H (Lectin) (Ulex europaeus) (produced by BIOTEC Laboratories Ltd Suffolk , UK.) The procedure of each test was strictly followed as outlined in the Manufacturer Technical Manual.

Methods used for assaying serum ALT/AST levels: In this work, the colorimetric method of Reitman and Frankel (1957) technique as updated by World Health Organisation Laboratory Manual (Lab/86.3, page 75 –78, (1986) publication was employed **[56, 57]** using a commercial kit reagent produced by Sigma- Aldrich Cooperation Limited Liability Company, USA. The amount of oxaloacetate pyruvate product as result of transamination by AST and ALT respectively was estimated by coupling with 2-4-KNPH to give a brown colour complex –hydro zone in an alkaline solution, which was then read colorimetrically using a green filter or spectrophotometrically at a wavelength of 515nm. A proper use of incubation time and temperature were employed at 37.0^{c} for 30 min. and 1hr for ALT and AST respectively. The procedure of test was strictly followed as outlined in the Manufacturer Technical Manual(**see figure 5)**

Statistical analysis. The raw data base of the results for both sexes were subjected to statistical analysis .Data were represented with frequency (f) and percentages (%) while continuous data were expressed as mean and standard deviations. One sample Kolmogorov-Smirnov test was used to assess the normality of the data. All data were normally distributed; hence, parametric procedure was used. Statistical Package for Social Students software version 24 (supplied by SPSS International company Chicago, United State of America) was used for the statistical analysis of the data .Comparison of the parameters and variables between the samples were performed using independent t-test while comparison among various age groups and genders were analyzed using one way analysis of variance (ANOVA). Association between variables was analyzed using the Chi Squared X^2 test, Fischer's exact test and coefficient of correlation (*r*). Prevalence rate formulae and coefficient of variance were used. Alpha value of 0.5 was used. A two tailed *p*-value of <0.05 was considered indicative of a statistically significant difference. Data were expressed as plus or minus two standard deviation.

3.RESULTS:

The results obtained for this work are displaced in **Tables 1, 2, 3, 4 and 5** as shown below.

Table 1: Distribution of apparently healthy urban dwellers recruited based on gender and age group

Parameter	**Number of Female subjects recruited**		**Number of Male subjects recruited**		**Total number of Female + Male subjects recruited**		*P* Value
Age range/yrs	**Frequency**	**(%)**	**Frequency**	**(%)**	**Frequency**	**(%)**	***P*=0.05**
20-30	41	(15.24%)	30	(17.13%)	71	(32.37%)	**(*p*> 0.05)***
31-40	51	(38.9%)	101	(58.72%)	152	(50.16%)	
41-50	20	(7.43%)	20	(11.42%)	40	(18.85%)	**(*p*> 0.05)***
51-60	19	(13.13%)	21	(11.14%)	40	(18.27%)	
Total 400	**131.0**	**(43.23%)**	**172.0**	**(56.76%)**	**303.0**	**(100%)**	***(p* <.05)****

** Significant difference, *no significant difference

A total of 400 subjects were recruited but only **303 subject**'s samples were actually analysed **for this study** T**there were four age groups which ranges from 20 to 60 years for both genders with total number of 172(57.33%) and 131(43.23%) from males and female subjects respectively.**

The age group one had the highest frequency of distribution and percentage of 51 (38.9%) for female, 101 (17.13%) for male and 152 (50.1%) for total respectively.

Table: 2: shows frequency distribution, percentages, Serum AST activities and secretion status of ABH substances among male and female secretors and non-secretor apparently healthy urban dwellers

Parameter	Female Mean Value Serum			Male Mean Value Serum			Female + Male Mean Value Serum			*P* Value
	AST ± 2 SD IU/L)	f	(%)	AST± 2 SD IU/L)	f	(%)	AST ± 2 SD IU)	f	(%)	P=0.05
Total Range	28.02±19.25 8.7 7----47.27		128(47.67 %)	25.29±20.45 04.75---45.74	172	(57.33%)	26.96±19.85 07.1-----46.54	300	(100%)	(p> 0.05)*
Secretor of ABH substances	18.10±14.03		103 (80.46%)	16.22±10.11	134	(77.91%)	17.16±12.07	237	(79.00%)	(p> 0.05)*
Range	4.07-----32.13			6.11-----26.33			5.09---29.23			
Non-secretor of ABH substances	04.32±01		25 (19.53%)	06.38±8.11	38	(20.34%)	10.70±9.11	63	(21%)	(p<0.05)**
Range	2---5.32			0-------14.49			1.59-----19.81			
CV (%)	37.18			40.29			38.72			(p> 0.05)*

** Significant difference, *no significant difference,

- **Out of the** 400 subjects recruited for the study,only 300 **samples** were **collected for determining** of **the ABH secretor status and Aspartate amino transaminases (AST) activities 172(57.33%) and 128(47.67%) were from males and females subjects.**
- **The mean value of frequency of distribution and prevalence rate of ABH secretor were 133(22.35%) and 99(77.34%) for male and female subjects respectively**
- **The frequency of distribution and prevalence rate of ABH non-secretor were 39(22.67%) and 29(22.35%) male and female subjects respectively.**
- **The total mean of frequency of distribution and prevalence rate were 232(77.33%) for ABH secretor and 68 (22.18%) for non-secretors respectively.**
- **The mean values of means AST activities with the ABH secretor status for females, males, and total were 18.10±14.03± 2 SD IU/L, 16.22±10.11± 2 SD IU/L and 17.16±12.07± 2 SD IU/L at 37⁰c respectively secretor and 04.32±01± 2 SD IU/L, 06.38±8.11± 2 SD IU/L and 10.70±9.11± 2 SD IU/L for non-secretors respectively**
- **The mean values of means AST activities without the ABH secretor status for males, females, and total were 25.29±20.45± 2 SD IU/L,28.02±19.25± 2 SD IU/L and 26.96±19.85± 2 SD IU/L at 37⁰c respectively**
- **The corresponding coefficient of variation (CV) were 39.50%, 34.34% and 36.82% for females ;males, and total females ;males, and total respectively.**

AST activities with ABH secretor status were higher in secretor than in non-secretors in both sexes (p<0.05

Table 3: shows frequency distribution, percentages, Serum ALT activities and secretion status of ABH substances among male and female secretors and non-secretor apparently healthy urban dwellers

Parameter	Female MeanValue Serum		Male Mean Value Serum		Female + Male Mean ValueSerum		*P* Value
	ALT ± 2 SDIU/L)	F %	ALT ± 2 SDIU/L)	F %	ALT± 2 SD IU)	F %	*P*=0.05
Total	22.42 ±17.04	131(82.70%)	22.60 ±18.22	172(80.8%)	22.76 ±17.63	303 (100%)	(*p*> 0.05)*
Secretor of ABH substance Range	17.44±10.12	108(82.44%	18.17±10	138(80.23%)	17.05±11	246(81.69%)	(*p*<0.05)**
Non-Secretor of ABH substances Range	05.44±10.44	23(17.44%)	10.10±11.17	34(19.76%)	15.54±21.61	57(18.30%)	(*p*> 0.05)*
CV (%)	37.18		40.29		38.72		(p> 0.05)*

** Significant difference, *no significant difference,

- Out of the 400 subjects recruited for tis study, only 303 samples were actually collected for determining the ABH secretor status and serum Alanine Amino Transaminases (ALT) levels,172(57.33%) and 131(43.23%) were from males and female subjects respectively.
- The frequency of distribution and prevalence rate of ABH secretor were 138 (80.80%) and 108 (82.70%) for male and female subjects respectively.
- The frequency of distribution and prevalence rate of ABH non- secretor were 34(19.20%) and 23(17.30%) for male and female subjects respectively.
- The total mean frequency of distribution and prevalence rate were 247(81.69%) for ABH secretors and 57 (18.30%) for non-secretor respectively.
- The mean values of serum ALT activities without the ABH Secretor Status for females ;males, and total were 17.44±10.12± 2 SD IU/L ,18.17±10, 17.05±11± 2 SD IU/L at 370c respectively
- and 05.44±10.44± 2 SD IU/L, 10.10±11.17± 2 SD IU/L, and 15.54±21.61± 2 SD IU/L± 2 SD IU/L for ABH non secretor respectively.
- The mean values of serum ALT activities with the ABH Secretor Status for males, females, and total were 22.92±17.04± 2 SD IU/L, 22.60±18.22± 2 SDIU/L and 22.76±17.63± 2 SD IU/L at 370c respectively and ALT activities were higher in ABH secretor than in non-secretors in both sexes (p<0.05).
- The corresponding coefficient of variation (CV) were 37.18%, 40.29%, and 38.72% for females ;males, and total respectively.
- ALT activities with ABH Secretor status were higher in ABH secretor than in non-secretors in both sexes (p<0.05).

Table 4 Shows the De Ritis Ration or Serum ALT/AST activities and ABH Secretor / ABH non –secretor prevalence rate ration in apparently healthy urban dwellers of Oyu according to secretor and gender

Parameter	Female Mean Value	Male Mean Value	Total Mean Value	Remarks 95.5 Limit
AST /ALT ratio or De Ritis ratio	0.885	0.807	8.844	**<1**
ABH Secretor / ABH non – secretor Prevalence ratio Ratio for AST	4.12	3.83	3.76	
ABH Secretor / ABH non – secretor prevalence rate Ratio for ALT	4.66	4.20	4.46	
t=test	**1.48**	**1.326**	**1.79**	**(p>0.05)***

***no significant difference, The DERITIS Ratio or AST/ ALT ratio was less than one in both sexes.**

T=test was negative .There was no relationship between mean values of the male and female

Table 5: Summary of the comparison of all the parameters used for serum ALT/AST activities in apparently healthy urban dwellers for both sexes and ages.

Parameter	Female Mean Value	Male Mean Value	Female + Male Mean Value	Remarks
Serum ALT ± 2 SD /(IU/L)	22.42 ±17.04	22.60 ± 18.22	22.76 ±17.63	95.5 limit (p> 0.05*
CV (%)	37.18	40.29	38.72	
R with age	0.012	0.011	-	
N	131.0	172.0	303.0	
Serum AST± 2 SD (IU/L)	25.89 ± 20.45	28.02 ± 19.25	26l96 ± 19.85	95.5 Limit (p>0.05)*
CV (%)	39.50	34.34	36.82	
R with age	0.013	0.012	-	
N	128.0	172.0	300.0	
Serum ALT/AST Ratio	0.88S	0.807	8.844	<1
Mean age value	34 +7.6	38.7 + 86	36.6 + 81	95.5 limit (p>0.05)*

** Significant difference, *no significant difference

Statistical analysis of these result shows that the means values of serum AST levels were significantly higher than the mean values of serum ALT levels in all study groups.(p<0.05). There was no correlation between serum ALT and AST activities with age for both genders.

The AST/ ALT ratio was less than one in both sexes.

The male mean values of serum of ALT activities were slightly higher than the female ALT mean values .However this difference were not statistically significant (p>0.05).

ALT activities were higher in ABH secretor than in non-secretors in both sexes (p<0.05). AST activities were higher in ABH secretor than in non-secretors in both sexes (p<0.05).

Statistical analysis of these result shows that the means values of serum AST levels were significantly higher than the mean values of serum ALT levels in all study groups.(p<0.05). There was no correlation between serum ALT and AST activities with age for both genders.

The AST/ ALT ratio was less than one in both sexes. The male mean values of serum of ALT activities were slightly higher than the female ALT mean values .However this difference were not statistically significant (p>0.05).

ALT activities were higher in ABH secretor than in non-secretors in both sexes (p<0.05).

AST activities were higher in ABH secretor than in non-secretors in both sexes (p<0.05).

3. **DISCUSSION:** All the subjects on whom this study was conducted were mostly civil servants, and some business men who were apparently healthy and aged between 20 years and above. They were screened using the inclusive and exclusive criteria (Table 1).

The results in (Table 2) of this study shows that out of the 300 samples collected for determining the ABH secretor status and Aspartate amino transaminases (AST) activities 172(57.33%) and 128(47.67%) were from males and females subjects The mean value of frequency of distribution and prevalence rate of ABH secretor were 134 (77.91%) and 103(80.46%) for male and female subjects respectively while the frequency of distribution and prevalence rate of ABH Non-secretor were 38(20.34%) and 25(19.53%) male and female subjects respectively. The total mean of frequency of distribution and prevalence rate were 237(79.00%) for ABH secretor and 63 (21%) for non-secretors respectively. These finding are perfectly in line with the works of **[46, 58, 59]**.The mean values of AST activities before determining the ABH secretor status for males, females, and total were 25.29±20.45(±2SD IU/L),28.02±19.25(±2 SD IU/L) and 26.96±19.85 (±2 SD IU/L) at 37^{o}C respectively. Statistically these results show significance difference between the means values of serum AST levels amongst male and female subjects ($p<0.05$).These results are in line with those quoted in literature for western population by **[60,61].** The mean values of means AST activities after determining the ABH secretor status for females, males, and total were 18.10±14.03(±2SD IU/L), 16.22±10.11(±2 SD IU/L) and 17.16±12.07(±2 SD IU/L) at 370c respectively for secretor and 04.32±01(±2SD IU/L), 06.38±8.11(+2SD IU/L) and 10.7±09.11(+2 SD IU/L) for non-secretors respectively .The serum AST activities with ABH secretor status were higher in secretor than in non-secretors in both sexes ($p<0.05$).The corresponding coefficient of variation (CV) were 39.50%, 34.34% and 36.82% for females; males, and total females, males, and total respectively.

Results in this study also shows that out of the 303 samples collected for determining ABH secretor status and serum Alanine Aminotransferases (ALT) levels,172(56.76%) and 131(43.23%) were from males and female subjects respectively. The frequency of distribution and prevalence rate of ABH secretors were 138 (80.80%) and 108 (82.70%) for male and female subjects respectively, while the frequency of distribution and prevalence rate of ABH non-secretors were 34(19.76%) and 23(17.44%) for male, female subjects respectively and total mean frequency of distribution and prevalence rates were 246(81.69%) for ABH secretors and 57 (18.30%) for non-secretor respectively. These finding are perfectly in line with works of **[46, 58, 59]**.

The results in (**Table 3**) shows that the mean values of serum ALT activities after determining the ABH secretor status for females, males, and total were 17.44±10.12 (±2 SD IU/L) , 18.17±10, 17.05±11(±2SDIU/L) at 37^{0}C respectively and 05.44±10.44(±2SD IU/L), 10.10 ± 11.17 (±2 SD IU/L), and 15.54±21.61(±2 SD IU/L) at 37.0c for ABH non-secretor respectively and serum ALT activities were higher in ABH secretor than in non-secretors in both sexes ($p<0.05$).These mean values and reference value for serum ALT levels are in line with published data but that of serum AST levels appears slightly higher ($p>0.05$) than the reference value quoted **[57].**This increase may not be clinically significant, the reasons may obviously be due to differences in life style, diets, diseases conditions, occupational and environment factors**[23, 62]**

The mean values of serum ALT activities before determining the ABH Secretor Status for males, females, and total were 22.92±17.04(±2 SD IU/L), 22.60±18.22(±2 SD IU/L) and 22.76±17.63(±2 SD IU/L) at 37^0 C respectively .There was no significance difference between the mean value for male and female subjects which are also in line with that quoted in literatures by **[63]** for western populations). The corresponding coefficient of variation (CV) were 37.18%, 40.29%, and 38.72% for females; males, and total respectively. The male mean value of serum ALT and AST activities before determining ABH secretor status were slightly higher than the female mean value of serum ALT and AST activities, *(p*>0.05). This is also in line with those reported by **[24].** There was no correlation between serum ALT and AST activities with age for both genders **(Table 4).**This is also in line with the findings of **[64, 65, 66 & 67])**.The mean value of serum AST /ALT Ratio in both sexes were all less than 1, this agrees with **[68, 69]** report. The prevalence rate ratio of the ABH secretors and non-secretor was 4:1 which is in line with the report of **[46]**.

5. SUMMARY OF THE FINDINGS

In the **Table 5** shows the summary of the recruitment of subjects and all the results obtained for this work were entered to show how they are interrelated.

6. CONCLUSION

These findings have been shown that the levels of serum ALT and AST activities in these subjects studied fall slightly above the upper limit of the normal range of those quoted in literatures for other populations. The serum ALT activities and AST activities with ABH Secretor status were statistical significantly higher in ABH secretors than in non-secretors in both sexes (p<0.05).The prevalence rate ratio of ABH secretors and non-secretors established for the first time was 4:1

6.AVAILABILITY OF DATA AND MATERIALS: Datasets generated and analysed in this study are available from the corresponding author on request-

7.ABBREVIATIONS

Anti-A= Antibody A, **Anti-B**=Antibody B, **Anti-H** =Antigen H Substances, **A-** = Blood group A Rh D Negative , **A+** =Blood group A Rh D Positive, **AB-** = Blood group AB Rh D Negative, **AB+** =Blood group AB Rh D Positive, **ABO Group** = A, B, & O Blood Group System, **ANOVA**= Analysis of Variance, **ALT** =Alanine transaminase , **ALAT**=alanine aminotransferase, **AST**=spartate transaminase, **ANTI-H**= Antigen H Substances, **ASAT**= Aspartate aminotransferase= **AIDS** =Acquired Immune Deficiency Syndrome, **EMTCT**= Elimination of Mother-To-Child Transmission, **IU /L**=International Unit /Litre, **NH2**=amino group,

FCT=Federal Capital Territory, **FMOH**= Federal Ministry of Health, **PR**=Prevalence rate, **HIV**=Human Immuno-deficiency Virus, **SGOT**=serum glutamic oxaloacetic transaminase, SGPT=serum glutamic

pyruvic transaminase, ***P* >0. 05**=*P*-values indicating the Level of no significance, ***P* < 0. 05**= *P*-values indicating the Level of significance, **Se,Se** =Pair of dominant alleles for the secreting genes controlling secretors, **Se,se**=Pairs of recessive alleles for the secreting genes controlling non-secretors , **SD**= Standard Deviation of the Mean, **SPSS**=Statistical Package for Social Science, **t**=Student independent test, **UCTH**= University Of Calabar Teaching Hospital, UN=United Nations, **UNAIDS**=United Nations Joint Programme on HIV/AIDS **UNICEF**=United Nations Children Emergency Fund, USAID=United States Agency for International Development, **WHO**=World Health Organization, **CV**=Coefficient Of Variation **NAFLD**=Non-alcoholic Fatty Liver Disease, **AFLD**=Alcoholic Fatty Liver Disease, ***r***=Correlation coefficient.

COMPETING INTERESTS DISCLAIMER:

Authors have declared that no competing interests exist. The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

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9.DECLARATION BY THE AUTHORS Authors' contributions : Conception of study: FJN , 2)Design of study: FJN and IIEN, 3) Sample analysis: FJN, EWO, UOA, IIEN, 4)Data analysis: FJN , EWO,UOA ; IIEN, 5) Statistical analysis: FJN , EWO,UOA , IIEN, 6) Initial manuscript draft: FJN , EWO,UAO , IIE, 7) All authors read and approved the final manuscript.

11.ETHICS APPROVAL AND CONSENT TO PARTICIPATE:

This study was approved by HealthResearch Ethical Committee (HREC) of the University of Calabar Teaching Hospital. Oral informed consent was obtained from the participants and same was approved by the ethics committe .

12.CONSENT FOR PUBLICATION: Not applicable.

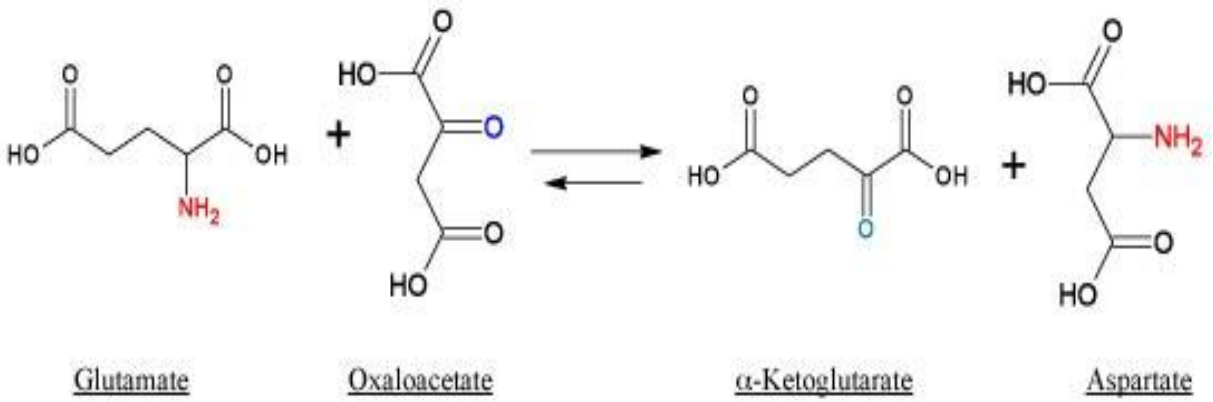

Figure 1: showing the principle and Mechanism of transaminase reaction

(Tietz et al., 1987, Matinez-Carrion and Peterson, 1970, karmen et al., 1970

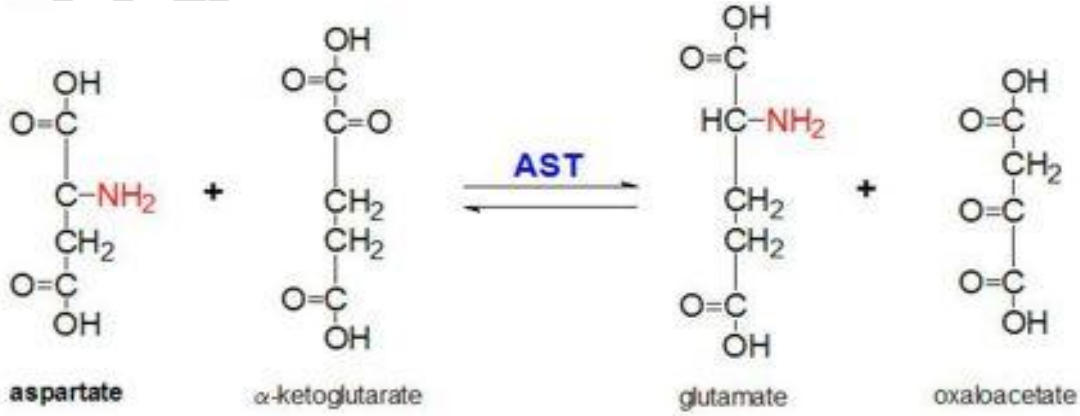

Figure 2: showing the Mechanism of aspartate amino transaminase reaction

(Tietz et al., 1987, Matinez-Carrion and Peterson, 1970, karmen et al., 1970

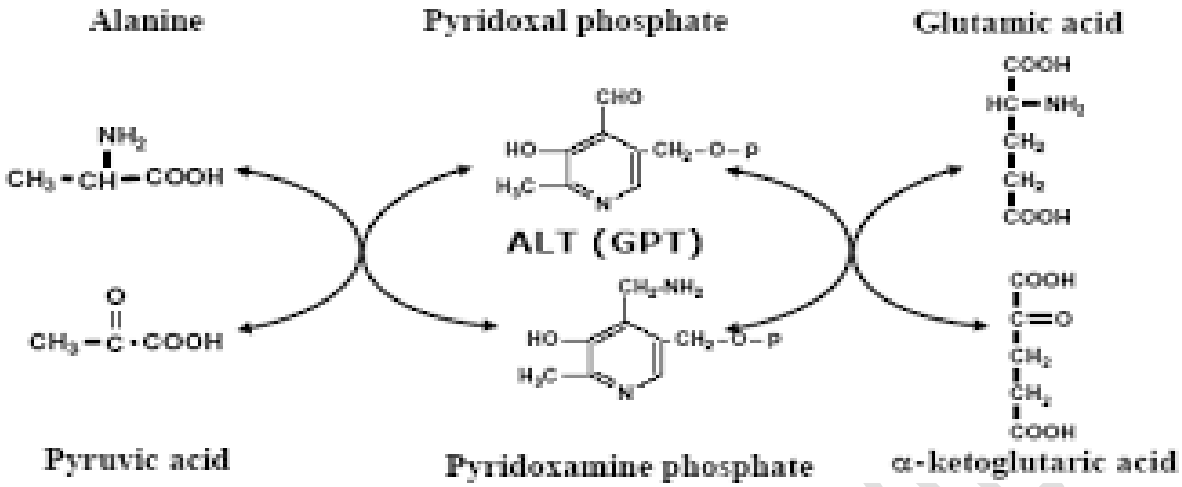

Figure 3: showing of the Mechanism of alanine amino transaminase reaction

(Tietz et al., 1987, Matinez-Carrion and Peterson, 1970, karmen et al., 1970

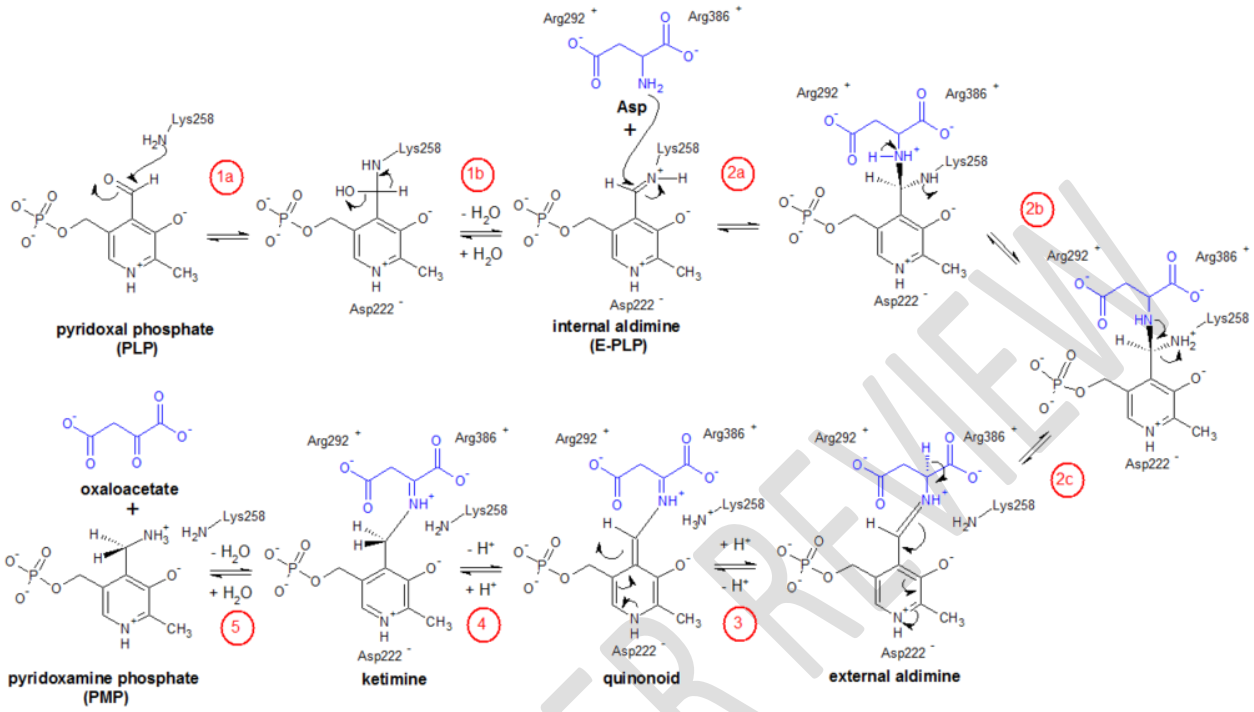

Figure 4: Showing the steps and stages in mechanism of transamination reaction

1. **Internal aldimine formation**:

First, the ε-amino group of Lys258 forms a Schiff base linkage with the aldehyde carbon to generate an internal aldimine **(1a, 1b).**

2. **Transaldimination**:

The internal aldimine then becomes an external aldimine when the ε-amino group of Lys258 is displaced by the amino group of aspartate. This transaldimination reaction occurs via a nucleophilic attack by the deprotonated amino group of Asp and proceeds through a tetrahedral intermediate. **(2a, 2b, 2c)**. As this point,The carboxylate groups of Asp are stabilized by the guanidinium groups of the enzyme's Arg386 and Arg 292 residues.

3. **Quinonoid formation**:

The hydrogen attached to the a-carbon of Asp is then abstracted (Lys258 is thought to be the proton acceptor) to form a quinonoid intermediate.

4. **Ketimine formation**:

The quinonoid is reprotonated, but now at the aldehyde carbon, to form the ketimine intermediate.

5. **Ketimine hydrolysis**:

Finally, the ketimine is hydrolyzed to form PMP and oxaloacetate.This mechanism is thought to have multiple partially rate-determining steps. However, it has been shown that the substrate binding step (transaldimination) drives the catalytic reaction forward.

(Tietz et al., 1987, Matinez-Carrion and Peterson, 1970, karmen et al., 1970).

Calculation

Amount of oxaloacetate

$$= \frac{AT - ATB \quad X \quad 66.7}{AS - ASB}$$

$$\text{Amount of pyruvate} = \frac{AT - ATB \quad X \quad 133}{AS - ATB}$$

N/B: **AT** = Absorbance of Test samples

ATB = Absorbance of Test blank

As = Absorbance of standard

ASB = Absorbance of standard blank.

Figure 5 showing the formula for the calculation of the concentration of Amount of oxaloacetate and Amount of pyruvate formed during transamination reaction (from method of Reitman and Frankel (1957) technique as updated by W. H. O. Laboratory Manual (Lab/86.3, page 75 – 78, (1986) publication)