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DETERMINATION OF THE ACTIVITIES OF AST, ALT AND ALP IN JUVENILE HYPERTENSIVE PATIENTS

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INTRODUCTION

1.1 BACKGROUND INFORMATION

Hypertension (HTN), which is referred to as condition in which the force of the blood against the artery walls is too high, is a known risk factor for coronary artery disease (CAD) in young adults, and the presence of childhood hypertension may contribute to the early development of CAD. Hypertension is one of the major causes of premature death globally. Hypertension increases with high sodium and low potassium intake, obesity, alcohol consumption, physical inactivity, and unhealthy diet as modifiable risk factors. The prevalence and rate of diagnosis of hypertension in children and adolescents appear to be increasing (Sorof *et al*., 2004). This is due in part to the increasing prevalence of juvenile hypertension as well as growing awareness of this disease. There is evidence that juvenile hypertension can lead to adult hypertension.

In pre-industrial societies, BP levels had narrow distributions with mean values that changed little with age and averaged around 115/75 mmHg (Page *et al*., 2014), a value that probably represents the normal (or ideal) BP for humans. However, in most contemporary societies, systolic BP levels rise steadily and continuously with age in both men and women. Other factors, such as genetic predisposition or adverse intrauterine environment (such as gestational hypertension or pre-eclampsia), have small but definite associations with high BP levels in adulthood. Even modest rises in mean population BP lead to large increases in the

absolute number of people with hypertension (Rose and Day, 2010). As economic development progresses, hypertension initially affects those with a high socioeconomic status, but at later stages of economic development, the prevalence of hypertension and its consequences are greatest in those with lower socioeconomic status; this phenomenon is seen both within and between countries. Further, the speed of change prevalence of hypertension since 2000 to 2010 has been much more rapid than in previous epidemiological transitions (Rose and Day, 2010).

There is an increasing correlation between liver dysfunction and high blood pressure. The liver plays an important role in many metabolic functions such as protein synthesis, blood clotting, cholesterol biosynthesis, glucose, and iron metabolism. The liver enzymes such as alanine aminotransferase (ALT), aspartate aminotransferase (AST), γ-glutamyl transferase (GGT), and alkaline phosphatase (ALP) are routinely screened for evaluation of liver function (Rahman *et al*., 2020). The levels of ALT and AST are increased in the plasma due to numerous medical conditions including nonalcoholic fatty liver disease (NAFLD) as the most common cause of elevated liver enzymes (Armstrong *et al*., 2012). GGT is a marker of alcohol consumption, but it is also related to the fat precipitation in the liver (fatty liver).

The interrelationship between liver dysfunction and the development of hypertension is being increasingly recognized. The liver is a vital organ in metabolism that plays numerous roles included synthesis, degradation, storage, and biotransformation of bio-molecules in the human body (Corless and Middleton, 2020). The liver enzymes alanine and aspartate aminotransferase (ALT and AST), γ-glutamyltransferase (GGT), and alkaline phosphatase (ALP) have been widely used as a good marker of liver health (Hanley *et al*., 2004). The elevated levels of ALT, AST, and GGT reflect an excess fat deposition in the liver, a condition termed as nonalcoholic fatty liver disease (NAFLD). These enzymes are suggested to have substantial clinical and

epidemiological significance as convenient surrogate markers of NAFLD and related liver dysfunction. Some epidemiological studies have demonstrated an association of ALT and GGT with metabolic syndrome, CVD and type 2 diabetes (Jiang *et al*., 2013; Sattar *et al*., 2004). In previous studies, CVD has been demonstrated as a leading cause of death in NAFLD, with higher rates coinciding with increased liver-related mortality throughout follow-up investigations (Ong *et al*., 2008). An association between higher serum GGT levels and hypertension has been reported in some longitudinal and cross-sectional studies (Lee *et al*., 2003; Bonnet *et al*., 2017; Stranges *et al*., 2005). However, most of the previous studies assessed the relationship that included only one or two hepatic enzymes and their findings were inconsistent. The epidemiological data concerning the extent of elevated liver enzymes in the Nigerian hypertensive individuals are not available yet. To address these issues, a cross-sectional study was conducted to examine the association of the liver enzymes with hypertension in juvenile patients.

1.2 RESEARCH QUESTIONS

How do we determine the activity of AST, ALT and ALP in juvenile hypertensive patients?

1.3 MECHANISM AND PATHOPHYSIOLOGY OF JUVENILE HYPERTENSION

BP is determined by several parameters of the cardiovascular system, including blood volume and cardiac output (the amount of blood pumped by the heart per minute) as well as the balance of arterial tone that is affected by both intravascular volume and neurohumoral systems (discussed in the following sections). The maintenance of physiological BP levels involves a complex interplay of various elements of an integrated neurohumoral system that includes the

renin-angiotensin-aldosterone system (RAAS), the role of natriuretic peptides and the endothelium, the sympathetic nervous system (SNS) and the immune system. Malfunction or disruption of factors involved in BP control in any of these systems can directly or indirectly lead to increases in mean BP, BP variability or both, over time resulting in target organ damage (for example, left ventricular hypertrophy and CKD) and CVD outcomes. The pathophysiological mechanisms responsible for hypertension are complex and act on a genetic background. Primary hypertension involves multiple types of genes; some allelic variants of several genes are associated with an increased risk of developing primary hypertension and are linked in almost all cases to a positive family history. This genetic predisposition, along with a host of environmental factors, such as high Na+ intake, poor sleep quality or sleep apnoea, excess alcohol intake and high mental stress, contribute to the development of hypertension. Finally, the probability of developing hypertension increases with aging, owing to progressive stiffening of the arterial vasculature caused by, among other factors, slowly developing changes in vascular collagen and increases in atherosclerosis (Steppan *et al*., 2011). Immunological factors can also play a major part, especially on the background of infectious or rheumatological diseases such as rheumatoid arthritis. The mosaic theory of hypertension describes its multifaceted pathophysiology (Harrison, 2013).

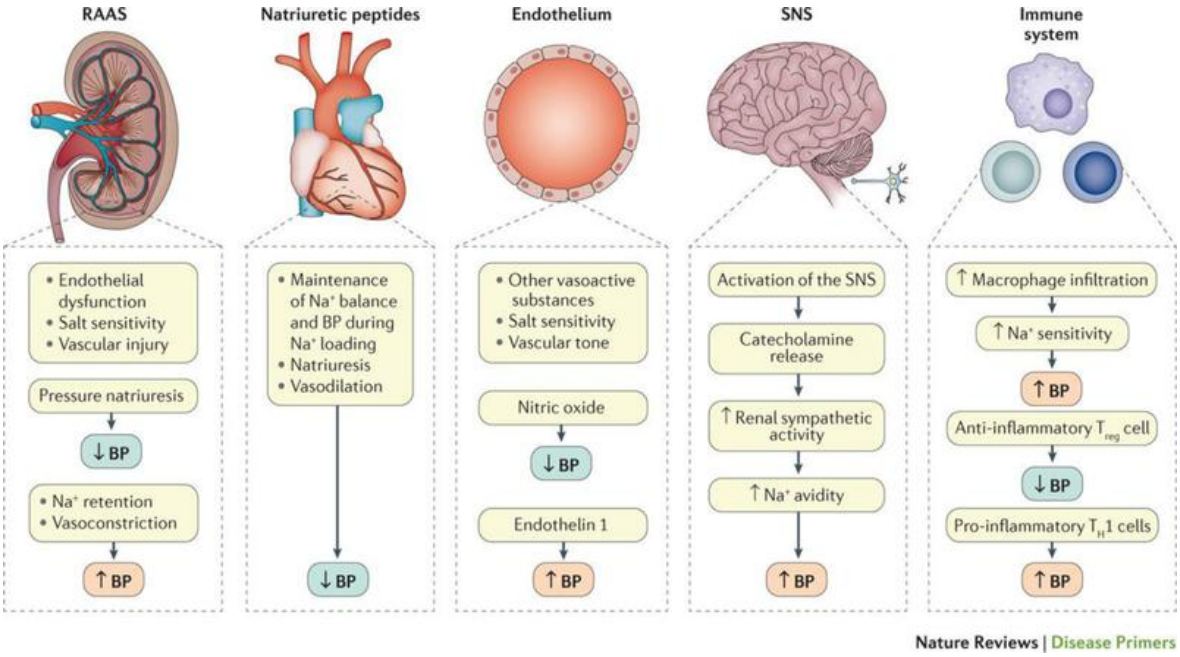

Figure 1.1: The main neuroendocrine systems involved in the regulation of blood pressure (Hall, 2018).

Sodium is a crucial regulator of blood volume: high serum sodium concentration promotes fluid (water) retention, thereby increasing blood volume and BP. When dietary sodium increases in normotensive individuals, compensatory haemodynamic changes occur to maintain constant BP. These changes include reduction in renal and peripheral vascular resistance and increased production of nitric oxide (a vasodilator) from the endothelium. However, if the effect of nitric oxide is impaired or absent, an increase in BP occurs. Endothelial dysfunction is a risk factor for the development of salt sensitivity and subsequent hypertension. Chronic high salt ingestion can result in endothelial dysfunction, even in salt-resistant individuals (Feng *et al*., 2017), and also affects the gut microbiota, with resultant changes that contribute to increased salt sensitivity and the development of hypertension (Feng *et al*., 2017). Thus, the gut microbiota appears to contribute to salt sensitivity of BP and the pathogenesis of hypertension.

The Renin-Angiotensin-Aldosterone System (RAAS) has wide-ranging effects on BP regulation, mediating Na+ retention, pressure natriuresis (that is, the mechanism whereby increases in renal perfusion pressure (the gradient between renal arterial and venous blood pressure) lead to decreased Na+ reabsorption and increased Na+ excretion), salt sensitivity, vasoconstriction, endothelial dysfunction and vascular injury, and plays an important part in the pathogenesis of hypertension (Hall and Hall, 2018). The RAAS is present at the cellular level in many organs, but its most crucial role is to help regulate pressure-volume homeostasis in the kidney, where it maintains perfusion in volume depleted states (that is, when there is a reduction in extracellular fluid volume as a result of sodium and fluid loss) and is suppressed in volume expanded (fluid overload) conditions. Renin and its precursor pro-renin are synthesized and stored in the juxtaglomerular cells of the kidney and are released in response to various stimuli. The main function of renin is to cleave angiotensinogen to form angiotensin I. Angiotensin-converting enzyme (ACE) cleaves angiotensin I to form angiotensin II, which is at the center of the pathogenetic role of the RAAS in hypertension (Sighn and Williams 2020).

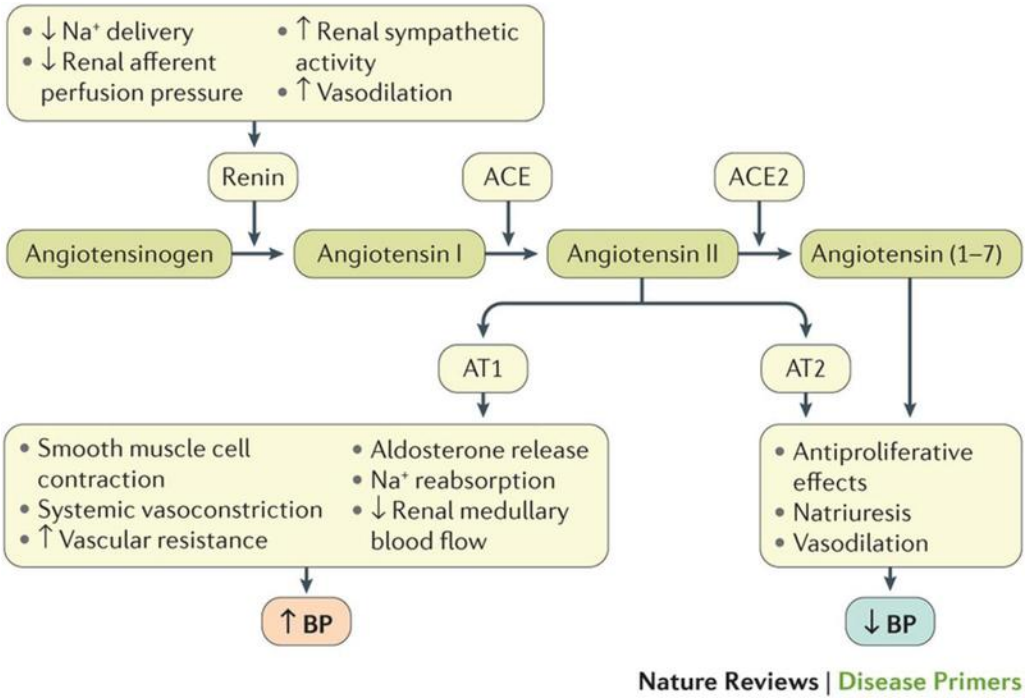

Figure 1.2: Role of the renin-angiotensin-aldosterone system in the regulation of blood pressure (Hall and Hall, 2018).

Aldosterone plays a crucial part in hypertension by binding to the mineralocorticoid receptor, it induces non-genomic effects (that is, without directly modifying gene expression) that include activation of the amiloride-sensitive sodium channel, commonly known as the epithelial sodium channel (ENaC) and result in the stimulation of renal Na+ reabsorption in the cortical collecting duct36. Aldosterone also has many non-epithelial effects that contribute to endothelial dysfunction, vasoconstriction and hypertension (Zhou and Bubien, 2001).

Inflammation makes an important contribution to the genesis of hypertension and related target organ damage. Inflammation is associated with increased vascular permeability and release of potent mediators, such as reactive oxygen species, NO, cytokines and metalloproteinases. Cytokines mediate the formation of neo-intima (a new or thickened layer of

arterial intima), thereby decreasing the lumen diameter of resistance vessels (small arteries and arterioles highly innervated by autonomic nerves and the primary vessels involved in the regulation of BP), and promoting vascular fibrosis, leading to increased vascular resistance and stiffness. Cytokines also affect renal tubular function by increasing local synthesis of angiotensinogen and angiotensin II, as well as promoting sodium and volume retention in hypertension (Harrison and Bernstein, 2018). Matrix metalloproteinases stimulate the degradation of the extracellular matrix, allowing infiltration of immune cells through the vessel wall into the interstitium of the affected organs, promoting apoptosis and enhancing collagen synthesis and matrix deposition, leading to target organ damage (Harrison and Bernstein, 2018).

1.4 Juvenile Patient Education

Hypertension is a lifelong disorder. For optimal control, a long-term commitment to lifestyle modifications and pharmacologic therapy is required. Therefore, repeated in-depth patient education and counseling not only improves compliance with medical therapy but also reduces cardiovascular risk factors.

One of the various strategies to decrease cardiovascular disease risk include the prevention and treatment of obesity: An increase in body mass index (BMI) and waist circumference is associated with an increased risk of developing conditions with high cardiovascular risk, such as hypertension, diabetes mellitus, impaired fasting glucose, and leftventricular hypertrophy (Bombelli *et al*., 2011).

1.5 Aspartate Transaminase (AST)

AST, also known as aspartate aminotransferase, catalyzes the reversible transfer of an α-amino group between aspartate and glutamate and, as such, is an important enzyme in amino

acid metabolism. AST is found in the liver, heart, skeletal muscle, kidneys, brain, red blood cells and gall bladder. Serum AST level, serum ALT (alanine transaminase) level, and their ratio (AST/ALT ratio) are commonly measured clinically as biomarkers for liver health. Aspartate transaminase catalyzes the interconversion of aspartate and α-ketoglutarate to oxaloacetate and glutamate.

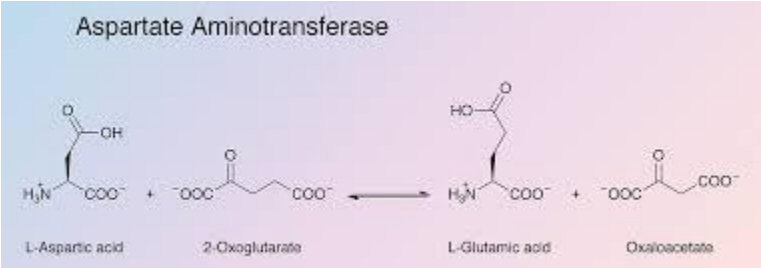

Figure 1.3: Action of Aspartate Aminotransferase (Berg *et al*., 2016).

AST is similar to alanine transaminase (ALT) in that both enzymes are associated with liver parenchymal cells. The difference is that ALT is found predominantly in the liver, with clinically negligible quantities found in the kidneys, heart, and skeletal muscle, while AST is found in the liver, heart (cardiac muscle), skeletal muscle, kidneys, brain, and red blood cells. As a result, ALT is a more specific indicator of liver inflammation than AST, as AST may be elevated also in diseases affecting other organs, such as myocardial infarction, acute pancreatitis, acute hemolytic anemia, severe burns, acute renal disease, musculoskeletal diseases, and trauma.. However, the use of AST for such a diagnosis is now redundant and has been superseded by the cardiac troponins (Gaze, 2007). Abnormal liver function test with raised aspartate aminotransferase (AST) are commonly seen in primary care setting. Chronic alcohol consumption, drugs, non-alcoholic steatohepatitis (NASH) and chronic viral hepatitis are common causes associated with raised AST.

1.6 Alanine Transaminase (ALT)

ALT, also known as alanine aminotransferase, is found in plasma and in various body tissues but is most common in the liver and catalyzes the two parts of the alanine cycle. Serum ALT level, serum AST (aspartate transaminase) level, and their ratio (AST/ALT ratio) are routinely measured clinically as biomarkers for liver health. The half-life of ALT in the circulation approximates 47 hours. Aminotransferase is cleared by sinusoidal cells in the liver (Giannini *et al*., 2005). ALT catalyzes the transfer of an amino group from L-alanine to α-ketoglutarate, the products of this reversible transamination reaction being pyruvate and L-glutamate (Yang *et al*., 2009). ALT (and all aminotransferases) require the coenzyme pyridoxal phosphate, which is converted into pyridoxamine in the first phase of the reaction, when an amino acid is converted into a keto-acid.

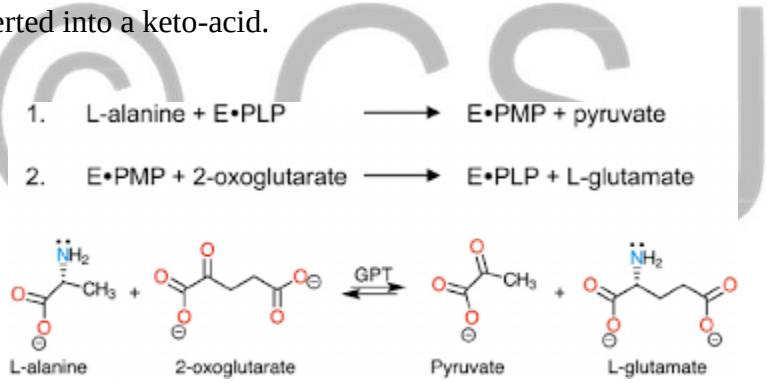

Figure 1.4: Action of Alanine aminotransferase (Ghouri *et al*., 2010)

ALT is commonly measured clinically as part of liver function tests and is a component of the AST/ALT ratio. Alanine aminotransferase (ALT) is an enzyme found predominantly in the liver but also in other tissues such as the kidneys, heart, and muscle cells. An increase in ALT serum levels indicates definite liver cell injury due to many causes. When liver cells are damaged, they release ALT into the bloodstream. High levels of ALT in the blood may be a sign of a liver

injury or disease. Some types of liver disease cause high ALT levels before symptoms of the disease manifest. So, an ALT blood test may help diagnose certain liver diseases early.

1.7 Alkaline Phosphatase (ALP)

The enzyme alkaline phosphatase (ALP, alkaline phenyl phosphatase) is a phosphatase with the physiological role of dephosphorylating compounds. Alkaline phosphatase is found in the periplasmic space of *E. coli* bacteria. This enzyme is heat stable and has its maximum activity at high PH. In humans, it is found in many forms depending on its origin within the body. It plays an integral role in metabolism within the liver and development within the skeleton. The levels of this enzyme in the blood depend on factors such as age, sex, or blood type. Blood levels of alkaline phosphatase also increase by two to four times during pregnancy. This is a result of additional alkaline phosphatase produced by the placenta and the liver (Gronowski, 2004). In humans, alkaline phosphatase is present in all tissues throughout the body, but is particularly concentrated in the liver, bile duct, kidney, bone, intestinal mucosa and placenta. In the serum, two types of alkaline phosphatase isozymes predominate: skeletal and liver.

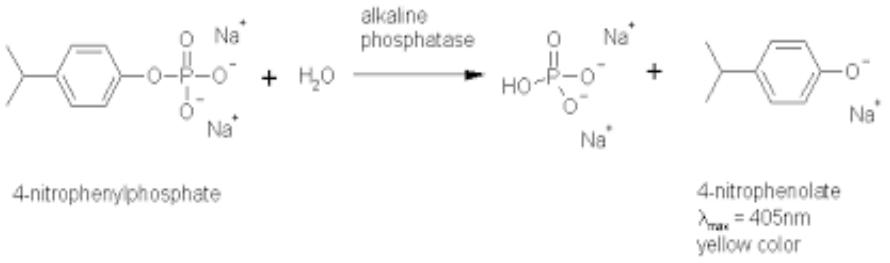

Figure 1.5: Action of Alkaline phosphatase enzyme (Gronowski, 2004).

Since chronic inflammation, which is a known risk factor for hypertension and endothelium dysfunction, stimulates bone marrow activity, ALP could be positively associated

with hypertension. Elevated levels are also associated with diabetes, hypertension, and cardiovascular disease; it was found that elevated levels are associated with elevated serum C-reactive protein (CRP), which could reflect an inflammatory and atherogenic milieu, possibly an alternative cause for elevated serum alkaline phosphatase (Gronowski, 2004).

1.8 AIM & OBJECTIVES

AIM

The aim of this study is to determine the activities of plasma AST (Aspartate Transaminase), ALT (Alkaline Aminotransferase) and ALP (Alkaline Phosphatase) in juvenile hypertensive patients.

OBJECTIVES

The objectives of this study include;

I. To evaluate the plasma activities of plasma AST (Aspartate Transaminase), ALT (Alkaline Aminotransferase) and ALP (Alkaline Phosphatase) in juvenile hypertensive patients and normotensive subjects.
II. To compare the plasma activities of plasma AST (Aspartate Transaminase), ALT (Alkaline Aminotransferase) and ALP (Alkaline Phosphatase) in juvenile hypertensive patients and normotensive subjects.

1.9 SIGNIFICANCE OF STUDY

The significance of this study lies in its potential to contribute to the understanding of the activities of liver enzymes (AST, ALT & ALP) in juvenile hypertensive patients. Given the rising incidence of hypertension among both male and female patients, exploring the activities of liver enzymes could serve as biomarkers for the regulation of blood pressure in juvenile hypertensive patients.

MATERIALS AND METHODS

2.0 Materials

- Anticoagulant bottle
- Sterile Needle and Syringe
- Cotton wool
- Plasma micropipette
- Cuvette
- Methylated spirit.
- Spectrophotometer
- Hematocrit centrifuge
- Statistical Analysis Software

2.1 Method

This study will be conducted following ethical guidelines and with approval from Ekiti State University.

The subjects examined are handled with care and hospitality. Materials and equipment used would also be handled with care during the practical work.

❖ Sample Collection

5mls of various bloods was collected from the patients using a sterile syringe and carefully dispensed into a lithium heparin bottle to avoid blood clotting.

❖ Experimental Design

Forty (40) subjects made of young adults were recruited for this study from Ekiti State University Teaching Hospital (EKSUTH) and were grouped As shown below:

Group 1: Twenty (20) young adults (male and female 18-30 years) with hypertension that is, blood pressure of over 140/90mmHg.

Group 2: Twenty (20) young adults (male and female 18-30 years) without hypertension (normotensive) that is, blood pressure less than 140/90mmHg.

2.2 Anthropometric Measurement

Height (meter) without shoe was measured with a wall-mounted ruler. Weight (Kg) in light clothing was measured with a bathroom scale. Body Mass Index (BMI) was calculated using the formula: weight(kg)/height(m^2).

2.3 Biochemical Analysis

2.3.1 Assay of Plasma Aspartate Aminotransferase (AST) Activity

This was assayed spectrophotometrically using enzymatic method as reported by Reitman and Frankel (1957).

Aspartate aminotransferase catalyzes the transfer of amino group at pH 7.4, from L-aspartate to α-oxoglutarate to form oxaloacetate and glutamate. The oxaloacetate in turn reacts with 2,4-Dinitrophenylhydrazine in an alkaline medium to form hydrazine derivative whose concentration is measured at 546 nm as the activity of aspartate aminotransferase.

Procedure

Phosphate buffer (100mmol/L at pH 7.4) of 0.5mL was taken with pipette into test- tubes meant for tests and blank. To the sample tubes, plasma (0.1mL) was added and mixed. The reaction mixture was then incubated for 30 min. at 37°C in a water bath. On removal ,2,4-dinitrophenylhydrazine reagent (2mmol/L) of 0.5ml was pipetted into all the tubes. Afterward, plasma (0.1mL) was taken to the tubes of blank (as sample blank), with the content mixed. All the tubes were then allowed to stand for exactly 20 min. at room temperature. Sodium hydroxide (0.4N) of 5mL was added to each tube of test and blank with the content mixed carefully. The absorbance of test at 546 nm were read and recorded against the sample blank after 5 min. incubation at room temperature. The absorbance was compared with the calibrated chart of the reagent as the activity of aspartate aminotransferase in the plasma.

2.3.2 Assay of Plasma Alanine Aminotransferase (ALT) Activity

This was assayed spectrophotometrically using enzymatic method as reported by Reitman and Frankel (1957).

Alanine aminotransferase catalyzes the transfer of amino group at pH 7.4, from L-alanine to α-oxoglutarate to form pyruvate and glutamate. The pyruvate in turn reacts with 2, 4-dinitrophenylhydrazine in an alkaline medium to form hydrazine derivative whose concentration is measured at 546 nm as activity of alanine aminotransferase.

Procedure

Phosphate buffer (100mmol/L at pH 7.4) of 0.5mL was taken with pipette into test- tubes meant for tests and blank. To the sample tubes, 0.1ml of plasma was added and throughly mixed.

The reaction mixture was then incubated for 30 min. at 37˚C in a water bath. On removal,2,4-dinitrophenylhydrazine reagent (2mmol/L) of 0.5mL was pipetted into all the tubes. Afterward, 0.1ml plasma was taken to the tubes of blank (as sample blank), with the content thoroughly mixed. All the tubes were then allowed to stand for exactly 20 min. at room temperature. Sodium hydroxide (0.4N) of 5ml was added to each tube of test and blank with the content mixed carefully. The absorbance of test at 546 nm were read and recorded against the sample blank after 5 min. incubation at room temperature.The absorbances werecompared with the calibrated chart of the reagent as the activity of alanine aminotransferase in the plasma.

2.3.3 Determination of Plasma Alkaline Phosphatase (ALP) Activity

Alkaline phosphatase activity was assayed spectrophotometrically according to enzymatic method of Henry (1964). Alkaline phosphatase catalyzes para-nitrophenylphosphate at pH 9.8, thereby forming a product of para-nitrophenol and phosphate whose reading at 405 nm gives the activity of the alkaline phosphatase.

Procedure:

Plasma (0.02mL) was pipetted into appropriately labelled cuvette and 1mL of alkaline phosphatase reagent [containing magnesium chloride (0.5mmol/L), diethanolamine buffer (1 mol/L at pH 9.8) and para-nitrophenyl phosphate (10mmol/L)] was added respectively to each cuvette. The content of each cuvettes was thoroughly mixed. Timer was started and absorbance of plasma recorded kinetically after 1,2 and 3 min. at 405 nm.

Calculation:

Plasma Alkaline phosphatase = Mean ALP absorbance change per min. X 2760I U/L

Activity (IU/L)

2.4 Statistical Data Analysis

The data collected was analyzed using one way Analysis of Variance (ANOVA) and Ducan Multiple Range Test to compare to compare the data obtained from the equipment and those of the control.

2.5 Budget

COST ITEMS	AMOUNT (N)
40 patients samples	100000
Bathroom scale	20000
EDTA Bottles	30000
Syringes and Oral Gavage Tools	20000
Personal Protective Equipment	25000
Statistical Analysis Software	30000
Miscellaneous	70000
TOTAL COST	295000

2.6 Expected Outcome

It is expected that there are alterations in the activities of AST, ALT & ALP in the experimental group compared to the control group (normotensive). The outcome may show the potential of AST, ALT & ALP as biomarkers in the regulation of juvenile hypertension. Possible increase in BP is due to complex and varied components, which are not only due to aging factors but also to unique environment and lifestyle factors. Increase in the activities of plasma ALT in patients, could be used as a biomarker for the diagnosis and management of hypertension in young adults.

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