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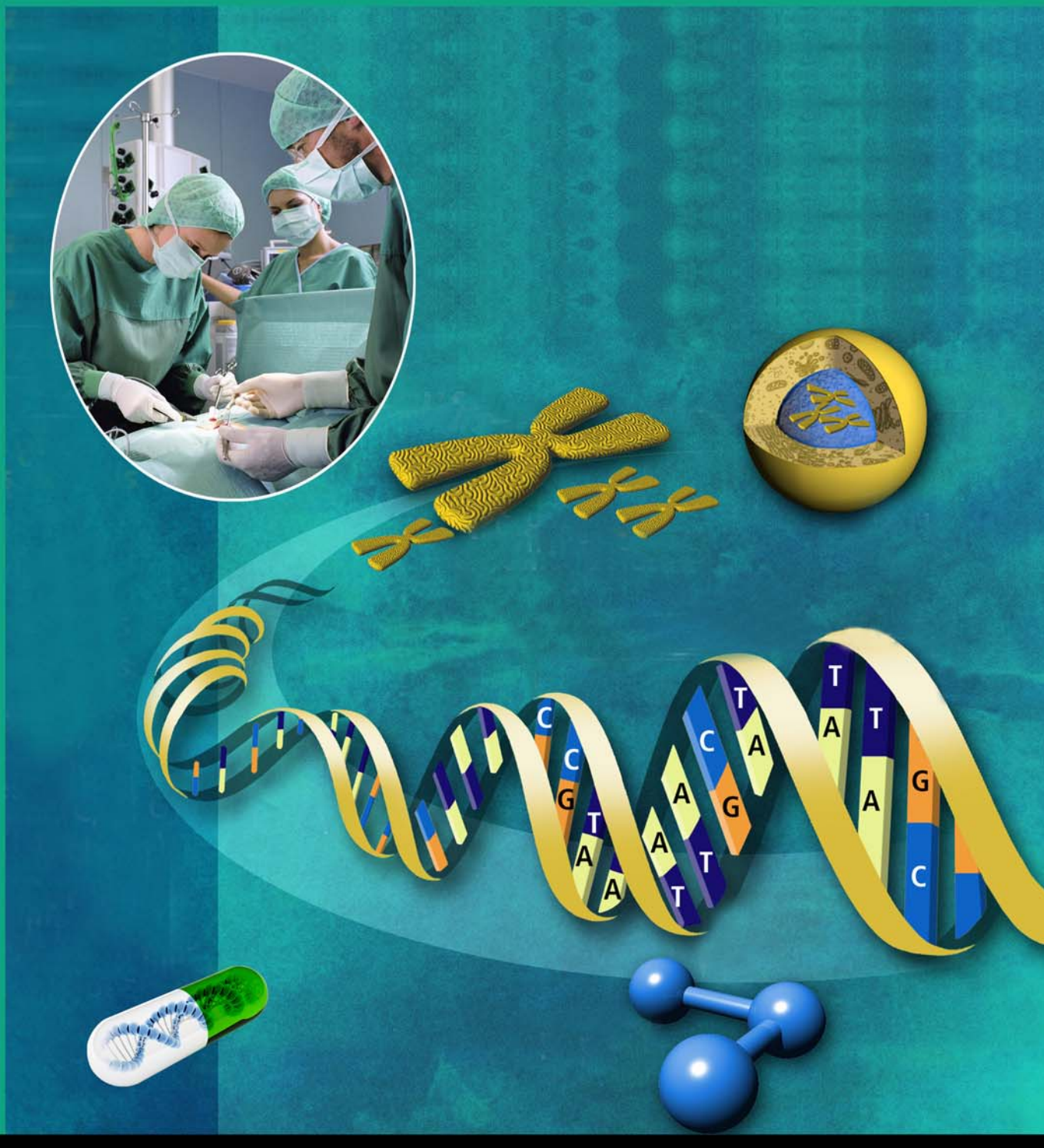

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Research Paper

INTRINSIC BLOOD COAGULATION STUDIES IN PATIENTS SUFFERING FROM BOTH DIABETES AND HYPERTENSION

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Thirty (30) citrated blood samples from patients (aged 20-60 years) suffering from both diabetes and hypertension attending diabetic clinics at University of Nigeria Teaching Hospital UNTH Ituku Ozalla in Enugu who have had the disease for at least six months were investigated. Also, investigated was a control group of thirty (30) non-diabetic and non-hypertensive individuals. Their Fasting Blood Sugar (FBS) levels and Blood Pressure (BP) were measured using standard methods. Their Body Mass Indices (BMI) was determined by measuring their weights and heights using standard methods. The results showed that the fasting blood sugar level (249.5±105.66) mg/dL, BP 156+16/9±6.5 mm Hg and Activated Partial Thromboplastin Time (APTT) (27.13±6.4) s were statistically significant (P<0.05). The FBS and BP were significantly higher in the diabetic hypertensive patients than in the control subjects. The APTT was significantly lower in diabetic hypertensive patients than in the control subjects (P<0.05). The pearson correlation coefficient (r) showed an established negative correlation between the age of the diseases and the average APTT values (r=0.804, t=4.8736 and P<0.05). But, there was no correlation established between the BMI and APTT values for these patients (r=0.101, t=0.3660 and P>0.05). It could be inferred from the studies that diabetics with hypertension showed reduced APTT values which occurred in both lean and obese patients.

Keywords: Diabetic, Hypertensive, Patients, BMI, APTT, FBS and BP

INTRODUCTION

Diabetes mellitus represents a group of metabolic disorders in which there is impaired glucose utilization inducing hyperglycemia which is an increase in blood group level beyond normal value (Hazuda, 1991). It has been defined by the World Health Organization (WHO) on the basis of laboratory findings as a fasting venous plasma glucose concentration greater than 7.8 mmol/L (140 mg/L) or greater than 11.1 mmol/L (200 mg/dL) 2 h after oral ingestion of the equivalent of 75 g of glucose (post prandical) even if the fasting

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concentration is normal (Mayne, 1999). These classification include, the primary and secondary diabetes.

The primary diabetes is subdivided into insulin-dependent diabetes (type 1 or Juvenile onset diabetes) and non-insulin dependent diabetes mellitus (type II or adult onset diabetes). Secondary diabetes occurs as a result of chronic pancreatitis, post pancreatomy, adrenal tumors, e.g., Pheochromocytoma and pituitary tumors.

According to WHO, the hypertension is defined as the consistent elevation of the systolic and diastolic pressure within the range of 130/85 mm Hg with regard to the patient's age, when the Blood Pressure (BP) is taken three consecutive days by a skilled worker with a functional sphygmomanometer when a patient attains different posture.

Hypertension is also, classified into primary (essential) and secondary hypertension. Primary hypertension is idiopathic and either benign or malignant. In benign hypertension, the rise of blood pressure is usually slow, progressing over many years and the level is moderately raised (Whaley *et al.,* 1999). Malignant hypertension is characterized by a very high BP by eye changes, which include retinal hemorrhages and exudates, by rapidly progressive renal injury terminating in uraemia, and rare by hypertension encephalopathy. Secondary hypertension has indentifiable causes. The main causes are renal disorders, endocrine causes and medication.

Hypertension and diabetes are interrelated diseases. Alone, each condition is a risk factor for cardiovascular disease and together, they strongly predispose to end-stage renal disease, coronary artery disease, peripheral and cerebrovascular diseases (Bakris *et al.,* 2000). Most diabetic patients die of vascular disease (Mohan, 1992). Complications of hypertension in patients with diabetes include kidney disease, cardiovascular disease like Congestive Cardiac Failure (CCF), coronary artery disease, left ventricular hypertrophy and diabetic retinopathy. Diabetes and hypertension are independently associated with increased prevalence of sexual dysfunction in men and women (Hazuda *et al.,* (1991).

Blood coagulation is a process that results in the formation of a fibrin clot. Two pathways lead to the formation of a fibrin clot, the intrinsic and extrinsic pathways. Although, they are initiated by distinct mechanisms, the two converge on a common pathway that leads to clot formation.

Intrinsic blood coagulation pathway is the formation of a clot in response to an abnormal vessel wall in the absence of tissue injury while extrinsic pathway is the formation of fibrin clot in response to tissue injury. Both pathways are complex and involve numerous different proteins termed clotting factors. The intrinsic pathway needs the clotting factors-VIII, IX, XI, XII. Other pathway constituents required are the proteins prekallitkrein, and high molecular weight kininogen, calcium ions and phospholipids secreted from platelets.

Diabetes changes the thrombohaemorrhagic balance, which exists in healthy flowing blood. This change predisposes a diabetic patient to various thromboembolic conditions leading to increased morbidity of these patients (Ghosh, 2002). Increase in certain coagulation factors like XII, XI, VII, Fibrinogen and VWF, increased platelet aggregation, endothelial cell dysfunction coupled with increased blood viscosity are some of the changes that contribute to increased

thromboembolic incidence in the disease (Ghosh, 2002). Vascular disease (hypertension) in diabetes mellitus is a common problem and mortality due to atherosclerotic disorders are high in patients with diabetes mellitus.

The proteins of coagulation which are most often increased in quality in blood of adiabatic patients are prekallitkrein, factor XII, XI, VIII, and fibrinogen molecules (Kanjaksha, 2002). A similar study carried out by (Stegnar, 2000) showed that increased concentration of fibrinogen, VWF and factor VII have predictive value for anthero-sclerosis and can be considered as risk factors for cardiovascular events.

Fibrinogen and factor VIII (Parameters that promote clotting) were elevated in overweight hypertensive compared to normal weight normotensives (Franz, 1993) and activated partial thromboplastin time (APTT) decreased (Meyden, 1993). A person's weight generally has an impact on their cholesterol level. People who are overweight often have high cholesterol and hypertension and their blood is more likely to clot (Banj *et al.,*1989).

Although hypertension occurs more often in patients with type one than type two diabetes mellitus, after adjustment for age, the prevalence of hypertension in type two diabetic patients who have a dual diagnosis of diabetes and hypertension have type two diabetes (Sower *et al*., 2000). Consequently, patients with type two diabetes represents the majority of hypertensive patients.

AIMS AND OBJECTIVES OF THE STUDY

- To investigate the effects of diabetes and hypertension on intrinsic blood coagulation system.
- To investigate whether differences in the age of the disease (diabetic hypertension) affect the value of APTT.
- To find out if there is a significant correlation between the BMI (body mass index) and the APTT.

MATERIALS AND METHODS

Subjects

Two classes of subjects made up of diabetics with hypertension (selected with the help of a physician) and apparently healthy individuals were used for the study. Thirty (30) diabetics with hypertension (aged 20-60 years) who were attending diabetic clinics at University of Nigeria Teaching Hospital (UNTH) Ituku-Ozalla in Enugu and have had the disease for atleast six months were investigated. Also investigated were thirty (30) age and sex matched subjects who served as the control group. They were non diabetic and normontensives. These control subjects, were selected from workers and students of UNTH as well as a few others residing in Enugu.

APTT

Haemoscan (Kaolin/platelets-substitute mixture), $CaCl_2$ 0.025 mol/L, Trisodium Citrate and Plasma.

For Sugar Estimation

Protein precipitation (Sodium tungstate), 4-aminophenazone, plasma (fluoride oxalate bottle)

Preparation of Reagents

1. Sodium Citrate Anticoagulant 3.2 g/dL

Trisodium citrate dehydrate-3.2 g, distilled water-100 mL

2. Protein Precipitant Reagent

Sodium tungstate dehydrate-1 g, disodium hydrogen phosphate-1 g, sodium chloride-0.9 g, phenol-0.1 g, HCl-1 mol/L-12.4 mL and distilled water -100 mL.

3. Color Reagent for Glucose Assay

To make 115 mL: 4 aminophenazone 5 g/L-8 mL, Fermcozyme 952DM-2 mL, phosphate buffer-30 mL and distilled water-75 mL.

Sample Collection

The subjects were made to sit comfortably. Then, the tourniquet was applied above the venepuncture site. Without wasting time, the venepuncture site was cleaned with 70% alcohol and collected from the antecubital vein. 3.8 mL of the sample was delivered into 0.4 mL of 3.25% trisodium citrate containing tube. The remaining 1.2 mL of the sample was delivered into fluoride oxalate bottles for FBS estimation and each sample was mixed by inversion. The sample method of collection was used for the two classes of subject involved. The samples were analyzed within 1 h of collection.

Sugar, Estimation was by Glucose Oxidase Peroxide Method

Principle of the Test

Glucose Oxidase (GOD) catalyses the oxidation of glucose to give hydrogen peroxide and gluconic acid. In the presence of the enzyme peroxidase (POD), the hydrogen peroxide is broken down and the oxygen released reacts with 4-aminophenazone (4-amino antipyrine) and phenol to give a pink color.

The absorbance of the color produced is measured in a colorimeter using a filter (11 ford no. 604).

1. For each patient, four tubes were provided and labeled as follows:

   B- Reagent blank

   S-Standard

   C-Control serum

   T-patients, test

2. 1.5 mL of protein precipitant reagent was pipette into each tube.
3. To each tube, the following were added.

Tube B- 50 µL distilled water

Tube S- 50 µL standard 10 mmol/l

Tube C-50 µL control serum

Tube T-50 µL patients plasma

4. The contents of each tube were well mixed.
5. The control and patients sample (tube C and T) were centrifuged for 5 min at high speed to obtain clear supernatant fluids.
6. 0.5 mL from the blank, standard, control and patients samples were transferred to a second set tubes (labeled as in step 1).
7. 1.5 mL of color reagent (4-aminophenazone) were added to each tube and the content mixed well.
8. The tubes were incubated for 37 °C in water bath for 10 min and shaken occasionally to ensure adequate aeration of the samples.
9. The absorbance of each solution in a colorimeter was read using a green filter (II Ford No 6040). The instrument was zeroed with blank solution in tube B.
10. The concentration of glucose in the control and patients sample were calculated by the formula.

AT/AS* 10 (Conc. of Std).

Calculation of the Body Mass Index (BMI)

BMI is the function of the individuals' weight (kg) and height squared (M^2).

$$\text{Mass index (BMI) Kg/ M}^2 = \frac{\text{Weight (kg)}}{\text{Height (M}^2\text{)}}$$

Measurement of weight was done using a weighing balance. The patients as well as the control subjects were instructed to take off their shoes after the other.

A tape calibrated in inch was used to measure their heights. The results in inches were converted to values in meters by the following formula.

Height (inch)*0.25= Height (M).

APTT (Activated Partial Thromboplastin Time)

The method for APTT described by Cheesbrough (2000) was employed. The activated partial thromboplastin time was mainly used to detect deficiencies in the first stage of coagulation mechanism, principal factors, VIII, IX, XI, XII and prekallitrein (Fletcher factor). It is also sensitive to deficiencies of the rest of factors except factor VII.

Principle of the Test

Kaolin (surface activator) and platelet substitute (phospholipids) are incubated with citrated plasma at 37 °C for the time specified in the test method. Calcium chloride ($CaCl_2$) is added and the time taken for the mixture to clot is measured.

Reagents

Kaolin/platelet substitute mixture, Calcium chloride 0.025 mol/L, Control plasma, which was ran with each batch of tests.

Sample

(a) Anticoagulant

Sodium citrate 32 g/1.36 mL of blood were mixed with 0.4 mL of trisodium citrate.

(b) Sample Processing

Immediately after the extraction, the blood sample was gently mixed with the anticoagulant part of the blood: One part of anticoagulant. A Platelet Poor Plasma (PPP) was obtained by centrifuging the citrated blood for 15 min. After, spinning the plasma was carefully separated and covered to avoid any undesired PH change that might affect the final results. The samples were tested within 2 h after collection.

Procedure

The control plasma and the patient's plasma were tested in duplicate.

1. The $CaCl_2$ 0.025 M solution was pre-incubated at 37 °C.
2. 0.1 mL of plasma was dispensed into a test tube.
3. 0.2 mL of well mixed kaolin/platelet substitute was pipetted into small glass tube.
4. The mixture (plasma-reagent) was incubated for about 2 min at 37 °C.
5. After this period of time, 0.1 mL of the $CaCl_2$ solution was added at 37 °C and the stop watch started.
6. Then, the time for the clot to be formed was recorded.
7. The patients APTT (average of the duplicate tests) was recorded.

The results were expressed in seconds.

RESULTS

Thirty (30) citrated blood samples collected from patients suffering from both hypertension and diabetes were investigated. Also, investigated was a matching control group of thirty (30) non-diabetic normotensive individuals. After measuring their arterial BP , their fasting blood sugar levels were estimated. Their weights and heights were also measured to determine their body mass indices (BMI, kg/m^2).

Then, the Activated Partial Thromboplastin Time test was carried out on each of the patients and the control groups and the results compared.

The mean and standard deviation (XD±SD) for each of the parameters were statistically computed and values obtained from the two sets of samples (Test and control subjects). Further more, student *t*-test was applied to the mean value for test of statistical significance in the different mean results.

Patients were grouped according to the age of their diseases and the average APTT value got for each group. This is to investigate whether the differences in the age of diseases affect APTT values. Also, the patients were grouped according to their body mass indices and the average APTT computed for each group. Here, patients were grouped into underweight, normal, overweight and obese and the correlation calculated between these groups and the APTT.

Finally, the patients's folders were consulted and they were as well asked orally to find out the drugs they were using. Also, information obtained from the patients and their folders showed that 23 (76.7%) out of the 30 patients were diagnosed suffering from diabetes first and then, later, hypertension manifested. In 5 (16.7%) patients, co-existent diabetes and hypertension were dually diagnosed and (6.6%), showed that hypertension can occur first before diabetes.

Fasting Blood Sugar (FBS)

The fasting blood sugar of the patients showed that the mean value of the 249.96±105.66 mg/dL. This showed a deviation from the control group values of 87.7±14.21 mg/dL. The *P*-value was $P<0.001$ which is statistically significant.

Systolic Blood Pressure (SMP)

The systolic BP of the diabetics with hypertension showed a mean value of 156±16 mmHg. This showed a deviation from the control values of 109±10.7 mmHg. The *P*-value was ($P<0.001$) which is statistically significance.

Diastolic BP

The diastolic BP of the patients showed a mean value of 91+6.5 mmHg. This showed a deviation from the control group of 76+7.4 mmHg. The *P*-value was $P<0.001$. There is a statistically significant difference between the patients diastolic BP and that of the control groups.

APTT Reference Range

The *t*-test was used to estimate the normal range of APTT for patients and the control subjects. The normal range got from the Hemoscan kit bought was 27.7-38.0 s.

APTT

The APTT value of the patients showed a mean value of 27.13±6.4 s. The *P*-value is $P<0.01$. There is a statistically significant difference between the patients APTT value and that of control groups.

APTT and the Age of the Diseases

The 30 subjects used were grouped according to the age of their conditions. Then, the average APTT for each group computed and the correlation established using the Pearson correlation coefficient (r). The tables of the two variables are shown below:

Table 1: Age of the Diseases (Year) and Average APTT (s)

Age of the Diseases (Year)	Average APTT (s)
0-2	32.6
3-5	28.3
6-8	28.2
9-11	24.0
12 and above	22.8

The correlation coefficient (r) showed significant correlation (r =0.804 and P<0.05) for the two variables.

Body Mass Index and The Means APTT

The patients were grouped into underweight, normal weight, overweight and obese according to their body mass indices and their corresponding APTT determined. The coefficient of correlation (r) was also computed, and there was no correlation established (t = 0.101, t = 0.3660 and p>0.05) between the BMI of the patients and the average APTT.

Table 2: BMI (Kg/m^2) and Average APTT (s)

BMI RANGE (Kg/m^2)	Average APTT (s)
Underweight (<18.5)	22.0
Normal weight (18.5-25.0)	27.15
Overweight (25.0-29.9)	27.2
Obese (30 and above)	23.0

DISCUSSION

The FBS and BP of the patients showed mean values that are statistically significantly higher in the diabetics with hypertension than in the control subject (p < 0.001). These conditions could be due to multiple inherited disordered and/or other predisposing factors such as diet, obesity, stress or some drugs like steroids.

APTT

The mean values of APTT of the patients (27.12 ±6.4 s) deviated from the control values (31.83 ±8.1 s) and thus, there is a statistically significant

Table 3: The Summary of the Result of the Students

Parameters Studied	Mean ± SD of		*T*-Value	*T*-Value	*P*-Value
	Patients	Control			
FBS (mg/dl)	249.96 ± 105.6	87.71 ± 14.21			*P*<0.001
SYSTOLIC BP (mmHg)	156 ± 16	109 ± 10.7			*P*<0.001
DIASTOLIC BP (mmHg)	91 ± 6.5	76 ± 7.4			*P*<0.01
APTT (s)	27.13 ± 6.4	31.83 ± 8.1			*P*<0.001
APTT and Age of diseases			4.8736	0.804	*P*<0.05
BMI and the mean APTT			0.3660	0.101	*P*<0.05

different (p<0.01) between the patients APTT values and that of the control subjects. The APTT values of these patients were found to be reduced when compared with those of the control group. This deviation (low APTT) value of patient could be as a result of elevated blood levels of one or more of the clotting factors – VII, fibrinogen, IX, XII, fibrin monomers – occurring in these patients. This agrees with the findings of Vander (1993) ''Blood clotting parameters-fibrinogen, factor VIII, and fibrin monomer-that promoted clotting were elevated in overweight hypertensive and type 2 diabetic patients''.

APTT and the Age of the Diseases

There was a statistically significant difference between the age of the diseases (diabetes and hypertension) and the average APTT (r=0.804, t=4.8736 and p<0.05). These age-related variation in APTT seemed to be more obvious when the APTT values of the patients showed negative correlation with the age of diseases. These agreed with the findings of the Avelone (1994), ''An attempt to study the effect of the short term hyperglycaemia on factor VIII concentration in NIDDM showed that the high level of factor VIII seen in this diseases are not directly related to hyperglycaemia, similar study by the same author showed that acute changes in the blood sugar concentration are not directly responsible for hypercoaguble state in this diseases but other metabolic changes in the long-term bring about this changes''.

BMI and APTT

There was no significant correlation established between the BMI of the patients and their APTT. This was showed by the value of correlation coefficient (r) computed for APTT and the corresponding BMI (r=0.0101, t=03660 and p<0.05). This agrees with the findings of Aso (2002) whose work showed that blood coagulation system is enhanced in both lean and obese type 2 diabetes compared with healthy subject and the degree of activation of coagulation was similar between lean and obese diabetics patients. The absence of correlation among these group of patients (lean, normal weight, over weight and obese) could be due to the variation in the degree of metabolic changes associated with the diseases.

CONCLUSION

The result of this study showed that, the fasting blood sugar (FBS) and blood pressure of diabetic hypertensive patients were significantly (p<0.05) higher than those in the control subjects. The result also indicated that Activated Partial Thromboplastin Time (APTT) of diabetic hypertensive patients were significantly lower than that of the control subjects. Furthermore, the pearson correlation coefficient (r) showed negative correlation between the age of diseases and average Activated Partial Thromboplastin Time (APTT) values, while there was no correlation established between body mass index (BMI) and Activated Partial Thromboplastin Time (APTT) values for these patients. Result of this study therefore suggest that diabetic hypertensive condition could be associated with decrease in the Activated Partial Thromboplastin Time (APTT).

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