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Comparative Assessment of Routine Haemostasis Parameters of Diabetics with or Without Hypertension Attending Sokoto Specialist Hospital, Nigeria

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Abstract

Background: The pathological events during diabetes mellitus (DM) and hypertension (HTN) could induce hyper-vascular injuries and coagulopathies in affected patients. Thus, this prospective comparative study aims to assess the prothrombin time (PT), partial thromboplastin time with kaolin (PTTK) and *international normalized ratio* (INR) as biomarkers of potential coagulopathies in diabetics with hypertension (test group) and those without hypertension (control group) at Specialist Hospital Sokoto, North-western Nigeria. ***Materials and methods:*** Whole blood samples were obtained from 74 diabetic patients with hypertensive and 37 diabetic patients without hypertension and analysed for the PT, PTTK and INR values using commercially available kits. The sociodemographic variables of the enrolled participants were collated and statistically analysed against the coagulometric values using suitable tests. ***Results:*** Participants between 50-59 years (39.2%) were the most frequently enrolled participants, followed by 40-49 years (29.73%). Females comprised 56.8% while males were 43.2% of the participants. The PT, PTTK and INR were not statistically significant between diabetes with hypertension and diabetes without hypertension which were (9.22 ± 1.78, 30.27 ± 18.18 and 0.70 ± 0.15) and (9.51 ± 1.85, 36.57 ± 17.17 and 0.72 ± 0.14) respectively ($p = 0.532$, $p = 0.061$ and $p= 0.575$). The age group of the subjects showed a significant increase in PTTK when compared with PT and INR ($p = 0.392$, $p = 0.002$ and $p = 0.467$). There were increases but statistically non-significant in PTTK when compared with PT and INR based on occupation ($p =0.833$, $p = 0.000$ and $p = 0.779$) respectively. ***Conclusion:*** Findings from this study showed no significant variation in PT, PTTK and INR of diabetics and diabetics with hypertension. However, future studies could be done to focus on platelet factors and other highly sensitive biomarkers of the coagulation system of these populations. Nevertheless, monitoring the PTTK in older diabetic patients with diabetes mellitus is important to prevent hypercoagulation.

Keywords: Diabetic and hypertension comorbidity, Coagulation cascade, Coagulopathy, Sokoto, Nigeria.

INTRODUCTION

Hypertension and diabetes could interrelate as each of the conditions is a risk factor for cardiovascular disease and both can predispose to multi-organ and multisystemic disorders (Petrie *et al*., 2018). Diabetes (both type-1 and type-2) affects millions of individuals worldwide (Sobczak *et al*., 2020). A major cause of death for individuals with diabetes is cardiovascular diseases, in part because both types of diabetes lead to physiological changes that affect haemostasis (Sobczak *et al*., 2020). Those changes include altered concentrations of coagulatory proteins, hyper-activation of platelets, changes in metal ion homeostasis, alterations in lipid metabolism (leading to lipotoxicity in the heart and atherosclerosis), the presence of pro-coagulatory microparticles and endothelial dysfunction. In this review, we explore the different mechanisms by which diabetes leads to an increased risk of developing

coagulatory disorders and how this differs between type-1 and type-2 diabetes.

Despite advances in pharmacology that have led to increasingly effective antihypertensive drug treatments, the precise pathophysiological mechanisms of hypertension and its complications are still poorly understood (Lip and Blann, 2000). Increasing clinical and laboratory evidence suggests that hypertension per se may confer a prothrombotic or hypercoagulable state. This may explain in part why, despite the exposure of the blood vessels to high pressures, the main complications of hypertension (that is, heart attacks and strokes) are paradoxically thrombotic in nature rather than hemorrhagic (Lip and Blann, 2000).

Prothrombin time (PT) and PTTK are the most commonly used tests to detect coagulation defect and are both considered as being functional tests as they measure enzymatic activities that lead to clot formation. The PT is often used to screen caugulation disorders of the extrinsic and common pathways whereas the PTTK Partial thromboplastin time with kaolin is used to screen for abnormalities of the intrinsic and common clotting systems and to monitor the anticoagulant effect of circulating heparin (William et al., 2017). The pathological events during diabetes mellitus (DM) and hypertension (HTN) could induce hyper-vascular injuries and coagulopathies in affected patients. Thus, the current study aims to assess the prothrombin time (PT), partial thromboplastin time with kaolin (PTTK), and *international normalized ratio* (INR) as biomarkers of potential coagulopathies in diabetics with hypertension and those without hypertension at Specialist Hospital Sokoto, North-western Nigeria.

MATERIALS AND METHODS

Study Area

This study was conducted in Sokoto state, located in extreme north-western Nigeria between longitude $4^{0}8$E and 6054^{0}E and between latitude 12^{0}N and $13^{0}58^{1}$. It shares a boundary with the Niger Republic to the north, Kebbi state to the west and southwest and Zamfara state to the East. The state has a land area of about 28, 232,37 square kilometres and a population of 4,244,399 million people based on the 2006 census made up of two major ethnic groups namely, Hausa and Fulani. The specialist hospital Sokoto is the major secondary refereal hospital that provides healthcare services to the residents of Sokoto metropolis.

Study Population

One hundred and fifty (150) diabetic patients of 50 treated, 50 untreated and 50 apparently healthy non diabetic controls where selected for the research. They were recruited from patients attending diabetes clinic of Usmanu Danfodiyo University Teaching Hospital, Sokoto, North West Nigeria. Institutional ethical clearances were obtained and an informed consent form was explained to each participant before they signed the consent

Study Design: Comparative propectuve study.

Participants Selection: All participants were randomely delected from both groups.

Selection Criteria

Diabetic on warfarin or heparin or any other anticoagulation therapy such as aspirin, and with other complications such as a history of liver diseases, liver dysfunction, hepatotoxic drugs, history of alcohol intake or cigarette smoking, hypertensive and psychic patients were excluded from the study. All participants were seronegative for human immunodeficiency virus, hepatitis B and C and negative for malaria.

Ethical consideration

This study was conducted following the Declaration of Helsinki. Ethical clearance was obtained from the health research ethics committee (HREC) of the Sokoto State Ministry of Health, Nigeria. Written informed consent was obtained from all participants before enrollment.

Data Collection

Questionnaires were used to obtain demographic data such as age, gender, marital status, type of HAART the participant used for treatment, duration of medication, diet, occupation, and educational level among others. All data collated from participants were aggregated and anonymized with coded numbers. A password-protected database was linked to the code and vital information (age, gender, personal/family medical history, smoking history, and medications) was stored appropriately.

Samples collection and processing

Blood samples were collected from diabetic patients. Seventy-four (74) samples were obtained from diabetes mellitus with hypertension while 37 samples were obtained from diabetic patients without hypertension. A total of 111 samples were collected by vein puncture, 4.5 ml of whole blood was dispensed into a container containing 0.5 ml sodium citrate anticoagulant and was centrifuged at 3000 rpm for 15 minutes on a benchtop centrifuge. The platelet-poor plasma was used to determine the PT, PTTK, and INR.

Laboratory analytical protocols

a. PT was determined using Spectrum diagnostic reagent, Egyptian Company for Biotechnology, Cairo, Egypt 2020, with lot number 10104020). Briefly, 100µl of plasma was pipetted into the test tube and was incubated at 37^{o}C for 2 minutes. Then 200µl of PT reagent was added into the test tubes. Timer was started simultaneously, and the clotting time was recorded in seconds

Physiological reference range: 10-15 seconds.

b. PTTK was determined using Spectrum diagnostic reagent, Egyptian Company for Biotechnology, Cairo, Egypt 2020, with lot number 0105020-2). Briefly, 100µl of test plasma was pipetted into test tube and was incubated at 37°C for 2minutes. Then, 100µl of PTTK reagent was pipetted into the same test tube. It was well mixed and was incubated at 37°C for another 1 minute. Then, 100µl of $CaCl_2$ was pipetted into the same test tube. Timer was simultaneously started, and the clotting time was recorded in seconds.

***Physiological reference range*:** 21-38 seconds.

c. The clotting in seconds is converted to the international normalized ratio (INR) from the formular, viz: $INR = (PT\ patient/PT\ Control)^{ISI}$

Where,

INR = International Normalised Ratio
PT Patient = Prothrombin Time of Patient
PT Control = Prothrombin Time of Control
ISI = International Sensitivity Index

Statistical analysis

Data were entered into SPSS version 26 for analysis. Descriptive statistics were used to describe the study groups All continuous variables results were presented as mean ± standard deviation (SD) for each group. Student t-test and One-way ANOVA were used to compare the mean ± standard of PT, PPTK, and INR of the study groups. Tables and graphs were used to present the results. A $P < 0.05$ was considered statistically significant.

RESULTS

Socio-demographic characteristics of diabetes with hypertension

The age distribution analyses of the participants showed that those between 50-59 years (39.2%) were the most frequently enrolled participants, followed by 40-49 years (29.73%). Others included those between 60-69 years (21.62%), 30-39 years (4.05%), 20-29 years (2.7%) and ≥70 years (2.7%). Also, females comprised 56.8% while males were 43.2% of the participants. Based on tribes, Hausas (67.5%) were the most frequently enrolled, then Fulanis (29.7%) and Yorubas (2.70%) (Table 1).

The study showed that the non-educated (59.46%), those with primary (5.41%), secondary (20.2%) and tertiary (14.8%) education levels participated in the study. Also, those that participated from rural and urban areas were (66.2%) and (33.7%) respectively. It was noted that traders (36.4%), civil servants (24.3%), housewives (22.9%) and farmers (16.2%) participated in the study. The study showed that married (56.7%), single (10.8%) and widow (32.4%) were part of the study (Table 1).

Table 1: Socio-demographic characteristics of diabetes with hypertension

Socio-Demographic Factor	Group	Frequency N= 74	Percentage (%)
Age	20-29	2	2.70
	30-39	3	4.05
	40-49	22	29.73
	50-59	29	39.20
	60-69	16	21.62
	≥70	2	2.70
Gender	Male	32	43.24
	Female	42	56.76
Marital Status	Single	8	10.81
	Married	42	56.76
	Widow	24	32.43
Occupation	Civil-servant	18	24.32
	Trader	27	36.49
	House-wife	17	22.97
	Farmer	12	16.22
Tribe	Hausa	50	67.57
	Fulani	22	29.73
	Yoruba	2	2.70
Level of education	Primary	4	5.41
	Secondary	15	20.27
	Tertiary	11	14.86
	None	44	59.46
Place of Residence	Rural	49	66.22
	Urban	25	33.78

Prothrombin Time, Partial Thromboplastin Time with Kaolin and INR of Study participants

There were no significant differences between prothrombin time (PT), partial thromboplastin time with kaolin (PTTK) and international normalised ratio (INR) of the study subjects when compared with the control (p= 0.532, p = 0.061 and p = 0.575) respectively (Table 2).

Table 2: Comparison of PT, PTTK and INR between Diabetes and Diabetes with Hypertension

Parameters	DM Mean ± SD	DM/HTN Mean ± SD	t value	p-value
PT (secs)	9.51 ± 1.85	9.22 ± 1.78	0.631	0.532
PTTK (secs)	36.57 ± 17.17	30.27 ±18.18	1.930	0.061
INR	0.72 ± 0.14	0.70 ± 0.15	0.566	0.575

Key: PT= Prothrombin time, PTTK= Partial thromboplastin time with kaolin, INR = International normalized ratio.

Comparison of PT, PTTK and INR of diabetes with hypertension by age groups of participants

The result showed no significant difference between prothrombin time and international normalised ratio (p > 0.05) of study subject when compared based on age group but there was significant increase in partial thromboplastin time with kaolin when compared between age group (p= 0.392, p=0.002 and p= 0.467) respectively (Table 3).

Table 3: Comparison of PT, PTTK and INR of diabetes with hypertension by age groups of participants

Parameter	20-29 n= 2	30-39 n= 3	40-49 n= 22	50-59 n= 29	60-69 n= 16	≥70 n= 2	f value	p-value
PT	8.50±0.71	9.33±2.31	9.77±2.16	9.69±1.83	9.50±1.32	12.50±4.95	1.057	0.392
PTTK	21.50±3.54	26.33±9.81	35.73±18.11	32.17±13.00	49.88±11.90	27.00±18.38	4.149	0.002*
INR	0.65 ± 0.07	0.70 ± 0.17	0.74 ± 0.18	0.73 ± 0.15	0.73 ± 0.11	0.95 ± 0.35	0.930	0.467

Key: PT= Prothrombin time, PTTK= Partial thromboplastin time with kaolin, INR = International normalized ratio.

Comparison of PT, PTTK and INR of diabetes with hypertension by sex of participants

The PTTK of males was relatively higher than that of the femlaes. However, there was no signifant difference between the two groups. Also, there were no significant difference in Prothrombin time, and International normalised ratio of male subjects compared to female subjects studied (p>0.05) (Table 4).

Table 4: Comparison of PT, PTTK and INR of diabetes with hypertension by sex of participants

Parameter	Male N= 32	Female N= 42	f value	p-value
PT	9.72 ± 1.85	9.69 ± 2.02	2.109	0.151
PTTK	50.66 ± 8.52	25.52 ± 10.95	1.206	0.275
INR	0.74 ± 0.14	0.73 ± 0.16	1.255	0.266

Key: PT= Prothrombin time, PTTK= Partial thromboplastin time with kaolin, INR = International normalized ratio.

Comparison of PT, PTTK and INR of diabetes with hypertension base on level of education

The result showed no significant difference in PT, PTTK and 1NR of none educated subjects when compared to primary school, secondary school and tertiary institution (p>0.05) of study subject (Table 5).

Table 5: Comparison of PT, PTTK and INR of diabetes with hypertension base on level of education

Parameter	Primary N = 4	Secondary N= 15	Tertiary N= 11	None N= 44	f value	p-value
PT	9.50 ± 1.29	9.73 ± 2.55	9.27 ± 1.27	9.82 ± 1.92	0.241	0.867
PTTK	43.75±15.37	34.13±14.83	42.73 ± 20.10	34.91 ± 15.24	1.089	0.360
INR	0.73 ± 0.10	0.73 ± 0.20	0.71 ± 0.10	0.75 ± 0.15	0.195	0.900

Key: PT= Prothrombin time, PTTK= Partial thromboplastin time with kaolin, INR = International normalized ratio.

Comparison of PT, PTTK and INR of diabetes with hypertension base on occupation

The result showed no significant difference between Prothrombin time and 1nternational normalised ratio of study subject when compared with occupation but there was significant increase when compared with Partial thromboplastin time with kaolin when compared with occupation (p= 0.833, p= 0.000* and p= 0.779) respectively (Table 6).

Table 6: Comparison of PT, PTTK and INR of diabetes with hypertension base on occupation

Parameter	Civil-servant N= 18	Trader N= 27	House-wife N= 17	Farmer N = 12	f value	p-value
PT	9.39 ± 1.61	9.93 ± 2.15	9.76 ± 2.39	9.58 ± 1.16	0.290	0.833
PTTK	41.28±17.43	32.85±15.46	26.82±11.20	50.58±6.84	7.958	0.000*
INR	0.71 ± 0.13	0.76 ± 0.17	0.73 ± 0.20	0.74 ± 0.10	0.364	0.779

Key: PT= Prothrombin time, PTTK= Partial thromboplastin time with kaolin, INR = International normalized ratio.

Comparison of PT, PTTK and INR of diabetes with hypertension base on marital status

The result showed no significant difference in PT, PTTK and INR of single when compared to married and widow of study subject (p>0.05) (Table 7).

Table 7: Comparison of PT, PTTK and INR of diabetes with hypertension based on marital status

Parameter	Single N= 8	Married N= 42	Widow N= 24	f value	p-value
PT	9.75 ± 1.98	9.76 ± 2.13	9.58 ± 1.61	0.066	0.936
PTTK	25.00 ± 5.83	38.31± 16.60	36.83±16.02	2.436	0.095
INR	0.75 ± 0.16	0.74 ± 0.17	0.73 ± 0.13	0.127	0.881

Key: PT= Prothrombin time, PTTK= Partial thromboplastin time with kaolin, INR = International normalized ratio.

Comparison of PT, PTTK and INR of diabetes with hypertension based on place of residence

Table 8 shows the mean comparison of PT, PTTK and INR of diabetes with hypertension based on place of residence. The result showed no significant difference in PT, PTTK and 1NR of rural residents when compared to urban residents (p>0.05) (Table 8).

Table 8: Mean comparison of PT, PTTK and INR of diabetes with hypertension base on place of residence

Parameter	Rural N= 49	Urban N= 25	t value	p-value
PT	9.73 ± 1.83	9.64 ± 2.16	0.207	0.650
PTTK	37.65 ± 15.01	33.92 ± 17.79	1.317	0.255
INR	0.74 ± 0.15	0.73 ± 0.17	0.258	0.613

Key: PT= Prothrombin time, PTTK= Partial thromboplastin time with kaolin, INR = International normalized ratio.

DISCUSSION

Findings from this study showed no statistically significant difference in PT, PTTK and INR of participants with diabetic mellitus and hyperfiction comorbidity and those with only diabetic mellitus. This does not corroborate the report of Abdulrahman and Dallatu (2012) in the same study area. The differences could be due to the study design which compared DM with normal subjects by Abdulrahman and Dallatu (2012) in contrast to the current study which enrolled diabetics without hypertension as the control group.

It is worth mentioning that there was no significant difference between all the sociodemographic variables (except age) of the test group with PT, PTK and INR analysed. Technically, it could be due to the relatively small sample size of participants of the performance characteristic (sensitivity and specificity) of the commercial reagents used for the investigations. Conversely and biologically, this might be due to the effect of a non-insulin hypoglycemic drug on glucose levels which in turn prevents the glycation process in treated DM patients (Ambelu *et al*., 2018), especially as most DM patients were on antihyperglycaemic therapy. Concerning the significant difference in the PTTK of the test group with their age, the highest value was obtained in those between 60 to 69 years. This could reflect the potential and combined effect of ageing, hypertension and diabetic mellitus on the blood vessels and sustained releases of proinflammatory cytokines which could exaggerate the PTTK levels (Rad *et al*., 2021). This supports the findings of Akputuzor *et al*., (2011) and Mirsaiedi *et al*., (2012). Prolonged PTTK commonly reported in elderly test subjects could speculate that this phenomenon is due to diminished prostacyclin synthesis and/or release by the endothelial cells during old age (Nnenna *et al*., 2014).

CONCLUSION

Findings from this study showed no significant variation in PT, PTTK and INR of diabetics and diabetics with hypertension. However, future studies could be done to focus on platelet factors and other highly sensitive biomarkers of the coagulation system of these populations. Nevertheless, monitoring the PTTK in older diabetic patients with diabetes mellitus is important to prevent hypercoagulation.

Conflict of interest: None declared by authors.

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