ORIGINAL ARTICLE

Risk Factors Associated with Hypercoagulability in Type 2 Diabetes Mellitus Patients at Ndola Central Hospital Zambia

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ABSTRACT

Background: Thrombosis, attributed to atherosclerosis, is the leading cause of morbidity and mortality in patients with diabetes mellitus. Pathogenesis of atherosclerosis in diabetes mellitus is not entirely clear and conventional risk factors such as smoking, obesity, blood pressure and serum lipids fail to explain fully this excess risk. We set out a cross-sectional study to determine the risk factors and patient attributes that may predispose type 2 Diabetes Mellitus (T2DM) patients to be in hypercoagulable state.

Methods: A structured questionnaire was used to capture Age, Sex, duration of diabetes mellitus and knowledge on T2DM of study participants. Body weight and height were also measured and BMI calculated.VWF, Cholesterol, and Glycated haemoglobin were measured in 213 T2DM patients. VWF was used as a proxy marker for hypercoagulability in T2DM patients. Participants with VWF of >2.0 IU/ml plasma concentration were regarded to be in hypercoagulable state.

Results: Chi-square analysis revealed that hypercoagulability in T2DM patients was associated with Age (P=0.001), Sex (P=0.000), glycaemic control (P=0.003), duration of diabetes (P=0.000), BMI (P=0.000) and knowledge on type 2 diabetes mellitus (P=0.001). In multivariate analysis after adjusting for confounders, knowledge on T2DM was not independently associated with hypercoagulability AOR being 1.00(CI 95% 0.80-2.20). However Age, sex, glycaemic control, duration of diabetes and BMI were found to be independent risk factors for hypercoagulability in T2DM patients giving AOR of 1.45(95%CI[1.19-3.16]), 4.42 (95% CI [2.77-10.63]), 6.12 (95% CI 2.27-8.36) 5.28(CI 95% 3.01-8.21) and 1.05(95% CI 0.75-2.86) respectively.

Conclusion: Age, Sex, poor glycaemic control, duration of diabetes and obesity should be taken into account in the management of T2DM patients as these variables are independent risk factors of hypercoagulability in T2DM patients.

INTRODUCTION

The incidence of Type 2 Diabetes Mellitus (T2DM) is rapidly growing in the world. In 1985, an estimated 30 million people suffered from this chronic disease, which, by the end of 2006, had increased to 230 million, representing 6% of the world population. Of this number, 80% is found in the developing world (1). An increase of as high as 146% is predicted to occur in developing countries, while the increase would only be 47% in developed countries. This means that developing countries will contribute 77.6% of the total number of diabetic patients in the world by the year 2030 (2). A population based survey conducted in Zambia found the prevalence of type 2 Diabetes Mellitus to be 2.1 % among males and 3.0% among females (3). This rapidly growing prevalence among developing countries is primarily as a result of demographic and epidemiological transition occurring in these countries as a consequence of urbanization, industrialization and globalization (4).

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Thrombosis is the leading cause of morbidity and mortality in patients with diabetes mellitus. The thrombosis is attributed to atherosclerosis. The pathogenesis of the atherosclerosis in diabetes mellitus is not entirely clear and conventional risk factors such as smoking, obesity, blood pressure and serum lipids fail to explain fully this excess risk. Fibrin deposition is an invariable feature in atherosclerotic lesions. Therefore, disturbances of haemostasis leading to accelerated fibrin formation (hypercoagulability) and delayed fibrin removal (impaired fibrinolysis) may contribute to the development of atherosclerosis.

Several mechanisms contribute to the diabetic prothrombotic state, such as endothelial dysfunction, coagulative activation and platelet hyper-reactivity. In particular, diabetic platelets are characterized by dysregulation of several signaling pathways leading to enhanced adhesion, activation and aggregation (5). These alterations result from the interaction between hyperglycemia, insulin resistance, inflammation and oxidative stress. Many studies have shown a variety of diabetes mellitus-related abnormalities in hemostasis and thrombosis. Venous thrombosis has also been found to occur more frequently in diabetics (6). Hypofibrinolysis is well established in T2DM and characterized by elevated levels of plasminogen activator inhibitor-1 (PAI-1) as well as prolonged clot lysis time. The higher PAI-1 levels can be observed in the more poorly controlled Type 2 Diabetes Mellitus (T2DM) patients (7).

Pandolfi et al., (2007) reported an increase in the number of T2DM patients in hypercoagulable state, indicating that there is an increase in Type 2 Diabetes Mellitus patients at risk of thrombosis (6). The reason for this increase in hypercoagulability among T2DM patients is not known but may be multifactorial. It could be due to poor glycaemic control, aging, obesity, unhealthy diet and sedentary life styles among T2DM patients.

The aim of this study was to determine risk factors and patient attributes associated with hypercoagulability in T2DM patients. These include age, history of diabetes, poor glycaemic control and lack of awareness about Diabetes Mellitus and its complications. It is very cardinal that these factors are investigated so as to have data that will help clinicians to identify type 2 diabetic patients at risk of developing thrombosis and institute early treatment.

PATIENTS AND METHODS

This cross-sectional study was conducted at Ndola Central Hospital (NCH), a third level referral hospital for Copperbelt and the Northern part of Zambia. It is located in Ndola, the provincial headquarters of the Copperbelt Province. The hospital has a bed capacity of 851.

The study included Type 2 Diabetes Mellitus patients attending Ndola Central Hospital Out-patient Department (OPD) Diabetic clinic between November 2012 to May 2013. The total number of type 2 diabetes mellitus patients recruited was 213 and convenience sampling was used to recruit the study participants.

Selection criteria

Inclusion criteria:

The study included male and female Type 2 Diabetes Mellitus patients above the age of 18 years.

Exclusion criteria

Participants who had a history of venous thromboembolism or known inherited coagulation disorders, Cancer and hyperthyroidism were excluded from the study. Others excluded include, those who were Pregnant, Recently undergone surgery, Patients taking standard anticoagulant treatment, less than 18 years and those not willing to consent.

Data collection

A structured questionnaire was used to collect information from the participants. The information collected included Demographic information, Physical measurements (Height and Weight), Levels of awareness about type 2 Diabetes Mellitus risk factors, management, complications and duration of type 2 Diabetes mellitus disease for each participant.

The WHO STEPs surveillance training and practical guide recommends that physical measurements be taken in the following order: Height, Weight and Blood Pressure. Therefore these measurements were taken in that order (3).

Participant' height was measured in meters using the seca Brand 214 Portable stadiometer. It was measured without the participants wearing foot or head gear. and it was recorded in meters. Participants' weight was measured using the Heine Portable Professional Adult Scale 737 and was recorded in kilograms.

Body Mass Index (BMI) was calculated by dividing the participants' weight in kilograms by the square of height in meters.

Assays

Good Laboratory Practice (GLP) principles according to Zambia Ministry of health laboratory quality manual was observed to ensure uniformity, consistency, reliability and reproducibility of all the laboratory test results that were produced in this study. Venous blood collection was done using the evacuated blood collection system. 3 ml of venous blood was collected for each test.

Venous blood for total cholesterol estimation was collected in plain containers. The samples were centrifuged within 4 hours of blood collection and serum was separated from the red cells and frozen at -30°C for subsequent analysis. Total Cholesterol was determined using the Humalyser 2000 semi-automated clinical chemistry analyser. The cholesterol liquicolor reagents manufactured by Human Gesellschaft of Germany were adapted for use on the Humalyser 2000 analyser. The method used was the Cholesterol Oxidase—Phenol-4-aminophenazone (CHO-PAP) method. The principle of this method is based on Flegg and Richmond method (8). Cholesterol levels were classified as normal if less than or equal to 5.2 mmol/l and raised if greater than 5.2 mmol/l.

Three (3) ml of venous blood for vWF was collected from each of the study participants in sodium citrate containers and centrifuged at 1500g for 15 minutes. Plasma was then separated and transferred into siliconized glass tubes and stored at 4C in a fridge until analysis. VWF was determined by the Human ELISA kit manufactured by Abnova of USA. Plasma VWF concentration results were reported in International units/ml (IU/ml).The reference range for plasma concentration of vWF is 0.6 to 2.0 IU/ml. Any result above 2.0 IU/ml was regarded as being in hypercoagulable state.

Venous blood collected in EDTA containers was used for glycated Haemoglobin (HbA1C) estimation. Bio-Quant Glycated Haemoglobin (HbA1C) Enzymatic assay kit produced by BioSupply of United kingdom was used to determine HbA1C in whole blood. This was measured primarily to identify the average plasma glucose concentration over the past 3-4 months and hence indirect indicator of glycaemic control in Type 2 Diabetes Mellitus patients.

Hypercoagulation in Type 2 Diabetes Mellitus patients was determined by estimating vWF and the results obtained were compared with the potential risk factors for hypercoagulation such as age, sex, glycaemic control, duration of type 2 Diabetes Mellitus and awareness of type 2 diabetes Mellitus risk factors and complications. The VWF, which was a continuous variable in SPSS, was recoded so as to categorise the VWF results into two categories; those who had VWF of greater than 2.0 IU/ml were categorized as hypercoagulable and those whose VWF results were $\leq$2.0IU/ml were regarded as normal. Thereafter a chi-square test of independence was done to determine the proportion of hypercoagulability in type 2 diabetes mellitus patients and control subjects.

Age of type 2 Diabetes mellitus patients was categorized and each category's frequency of hypercoagulability (vWF > 2.0 IU/ml) was determined and compared among different categories to determine if the differences were statistically significant.

Glycated haemoglobin was used as an indirect measure of glycaemic control in type 2 Diabetes Mellitus patients. Glycated haemoglobin of less than or equal to 7% was regarded as good glycaemic control, while HbA1C of greater than 7% was regarded as poor glycaemic control. Using the chi-square test the frequency of hypercoagulability was compared between those with good glycaemic control (HbA1c $\leq$ 7%) and poor glycaemic control (HbA1c >7.0%) and analyzed for any significance difference in hypercoaugulation. Logistic regression was used to obtain the odds ratios and determine the strength of association between glycaemic control and hypercoagulation.

A general questionnaire was used to investigate the level of awareness about Diabetes Mellitus risk factors, complications and management among type 2 Diabetes Mellitus patients. Participants were asked to mention the risk factors, complications and management of type 2 Diabetes Mellitus. Participants who mentioned 2 or less risk factors/complications and less than 2 ways of managing type diabetes mellitus, were deemed to have inadequate knowledge and those who mentioned 3 or more risk factors/complications and more than 2 ways of managing type 2 diabetes mellitus were deemed to have adequate knowledge about type 2 diabetes mellitus risk factors/complications and management of type 2 diabetes mellitus. The proportion of hypercoagulability between

those with adequate knowledge and inadequate knowledge was analysed statistically using the chi-square and determined any significance difference between these two groups. Logistic regression was used to determine the strength of association between hypercoagulability and level of knowledge about type 2 diabetes mellitus risk factors and complications.

Data for duration of type 2 diabetes Mellitus for each patient was obtained from the questionnaire. The duration was categorized into ≤5 years, 5 to 10 years and > 10 years. Using the chi-square test, the proportion of hypercoagulability was compared among the three categories and determined whether the differences in the proportions were statistically significant. Logistic regression was used to determine the strength of association between duration of type 2 diabetes mellitus and hypercoagulation.

Ethical considerations

This study was performed under a protocol that was reviewed and approved by the University of Zambia-Biomedical Research Ethics Committee (UNZA-BREC). Written permission was obtained from the Permanent Secretary in the Ministry of Health as well as from the Senior Medical Superintendent of Ndola Central Hospital. All the prospective participants in this study were informed about the study, privileges and right to participation. The purpose of the study was thoroughly explained to the participants and those that declined to participate in the study were not forced, but were assured of their protected privileges and rights to treatment. Privacy and confidentiality was maintained. The names of the respondents did not appear anywhere on the forms instead codes were used on the forms. The forms were kept in lockable cabinets and no one apart from the researcher had access to the cabinets. Data on the computer was pass-word protected such that access was limited to only the researcher. The respondents were thus assured of utmost confidentiality. Only qualified medical professionals such as nurses and laboratory staff were involved in the collection of venous blood samples from study participants. Consenting patients and control participants were made to sign the consent form before being enrolled into the study.

Data analysis

The Statistical Package for Social Science (SPSS version 16) was used to analyse the results statistically. Analysis of distribution was made using the Kolmogoroff-Smirnoff test. All the parameters were normally distributed and hence reported as the mean +/- standard deviation. The significance of the differences between patients and controls for normally distributed parameters were determined using the independent samples T-test for continuous variables and Chi-square test for categorical variables. Risk factors and patient attributes associated with hypercoagulability in type 2 Diabetes Mellitus were determined by Binary Logistic regression analysis. Odds ratios and their 95% confidence intervals are reported.

RESULTS

A total of 213 T2DM patients aged 21-83 years participated in the study with the majority of participants [80 (37.6%)] being in the range of 51-60 years. The mean age was 45 years old (SD 4.31). Figure I shows that the proportion of T2DM patients who had good glycaemic control was lower [100(46.9%)] than those who had poor glycaemic control [113(53.1%)]. The proportion of T2DM patients who had adequate knowledge about the risk factors and complications of T2DM was slightly higher [108(50.7%)] than those who had inadequate knowledge [103(49.3%)]. The proportion of participants who were obese [62(29.2%)] was lower than those who had a normal BMI [84(39.4%)]. A higher proportion of participants had T2DM condition for more than 10 years [95(44.6%)] than those who have had the condition for less than 5 years or between 5-10 years [69(32.4%)] and [49(23.0%)] respectively.

Table I and figure II reveals that at the 5% level the proportions of subjects that were hypercoagulable differed significantly among the 21-30, 31-40, 41-50, 51-60 and > 60 age groups in T2DM Patients, P=0.001. The proportion of T2DM Patients who were hypercoagulable was higher in the age range of 51-60 years and above 60 years [60(93.8%)] and [18(94.7 %)] respectively. This shows that there was a significant correlation between age and hypercoagulation.

At 5% level the proportions of male T2DM patients who were in hypercoagulable state [38(40.9%)] was lower than in female T2DM patients [88(73.3%)]. This difference was significant P=0.000 (Table I and Figure III).

Table I and figure IV shows that the proportion of type 2 diabetes mellitus patients who were hypercoagulable was higher in those with poor glycaemic control [92(81.4%)]

Table I: Proportion of type 2 diabetes mellitus patients with hypercoagulability according to patient attributes

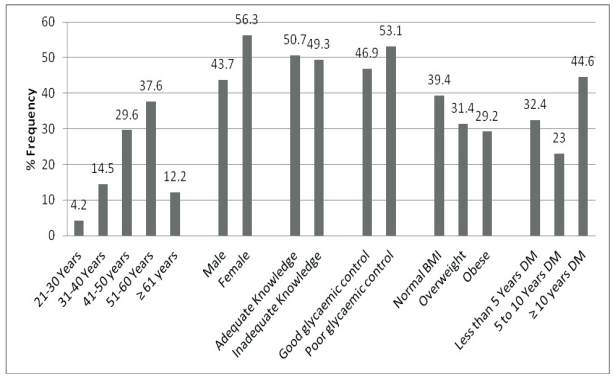
Variable	Total	N	%	P-Value
Age (years)				
21-30	10	0	0	0.001
31-40	40	2	5.0	
41-50	80	46	57.5	
51-60	64	60	93.8	
>61	19	18	94.7	
Gender				
Male	93	38	40.9	0.000
Female	120	88	73.3	
Glycaemic control				
Good	100	34	34.0	0.003
Poor	113	92	81.4	
Duration of type 2 Diabetes mellitus				
Less than 5 years	67	14	20.9	0.000
5 to 10 years	49	22	44.9	
Greater than 10 years	97	90	92.8	
Body Mass Index				
Normal	84	28	33.3	0.000
Overweight	67	42	62.7	
Obese	62	56	90.3	
Knowledge on type 2 Diabetes Mellitus				
Adequate Knowledge	108	48	44.4	0.001
Inadequate knowledge	105	78	74.3	

Table II: Univariate and multivariate regression results: likelihood of hypercoagulability among type 2 diabetes mellitus patients based on patient attributes

	Univariate		Multivariate	
Factor	**OR**	**95% C.I**	**AOR**	**95% C.I**
Sex				
_	Ref		Ref	
Female	**3.98**	2.23-7.10	**4.42**	2.77-10.63
Age (Years)				
21-30®	Ref		Ref	
31-40	1.00	0.15-2.62	**1.16**	0.36-4.77
41-50	**2.12**	0.58-4.23	**1.58**	0.41-6.02
51-60	**2.61**	1.20-4.80	**2.53**	1.21-4.25
_	**2.66**	1.05-4.87	**1.45**	1.19-3.16
Glycaemic Control				
Good®	Ref		Ref	
Poor	**7.50**	4.53-10.95	**6.12**	2.27-8.36
Duration				
<5 years®	Ref		Ref	
5-10 years	**3.08**	1.37-6.97	**2.20**	1.07-4.44
>10 years	**8.67**	5.48-11.85	**5.28**	3.01- 8.21
Body Mass Index				
Normal®	Ref		Ref	
Overweight	**1.52**	1.06-4.32	**1.05**	0.75-2.86
Obese	**5.33**	2.26-11.48	**4.54**	2.88-10.59
Knowledge about Type 2 diabetes Mellitus				
Adequate knowledge®	Ref		Ref	
Inadequate Knowledge	**3.48**	1.96-6.18	1.00	0.80-2.20

OR: odds ratio; AOR: adjusted odds ratio; ®: Reference group

Figure I: Demographic and other attributes of type 2 diabetes mellitus



DM: Diabetes Mellitus

Figure II: Age as a function of hypercoagulability (VWF> 2.0 IU/ml)

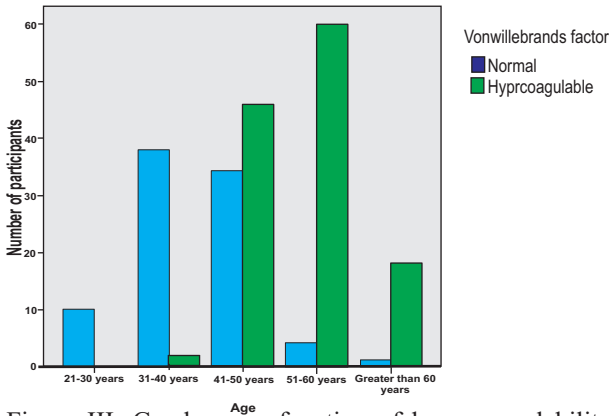


Figure III: Gender as a function of hypercoagulability (VWF 2.0 > IU/ml)

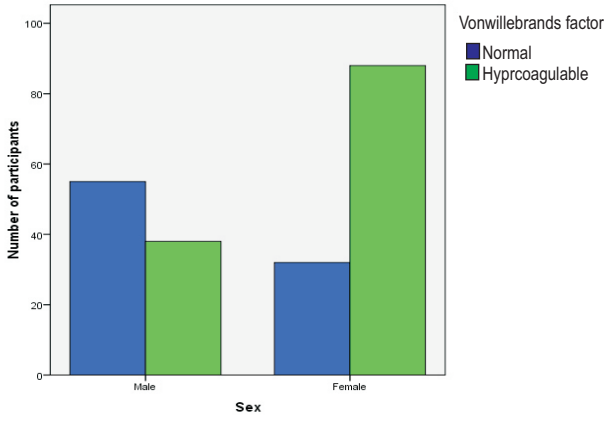


Figure IV: Glycaemic control as a function of hypercoagulability (VWF >2.0 IU/ml)

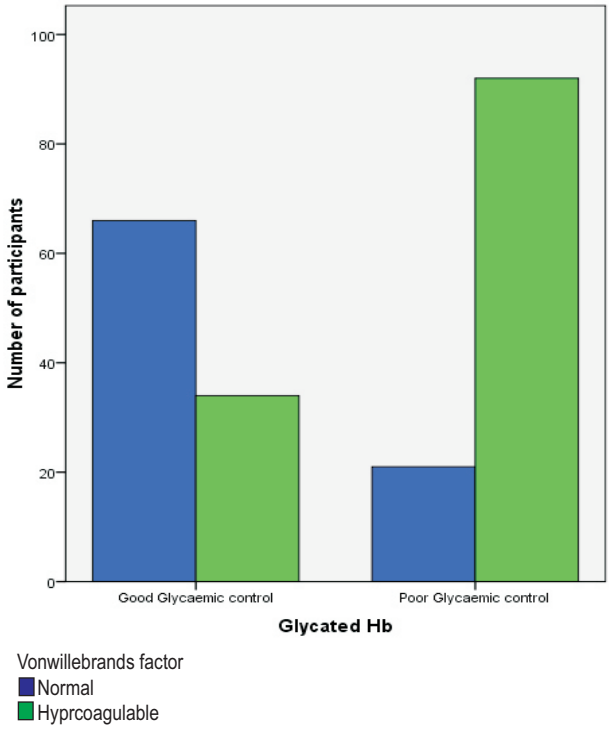


Figure V: Duration of Diabetes Mellitus as a function of hypercoagulabilty (VWF> 2.0 IU/ml)

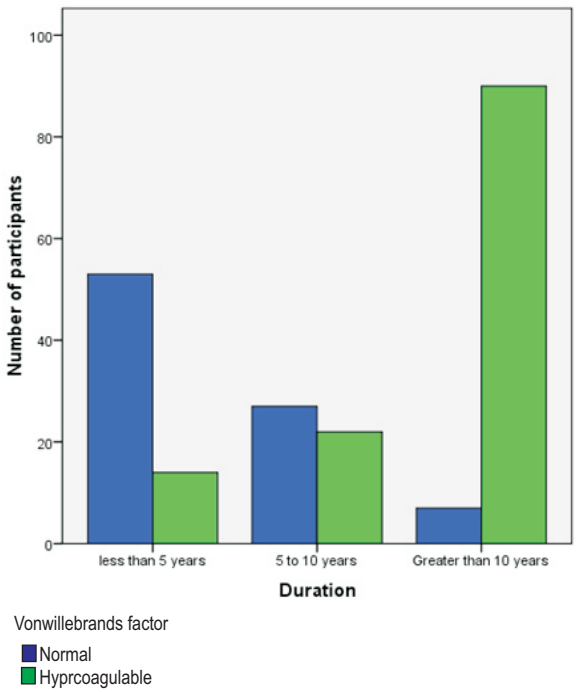


Figure VI: Knowledge about type 2 Diabetes Mellitus risk factors, complications and management as a function of hypercoagulability. (VWF >2.0 IU/ml)

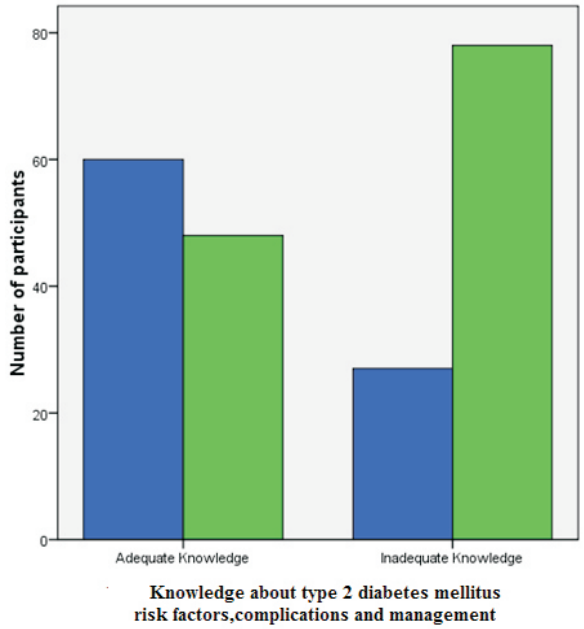
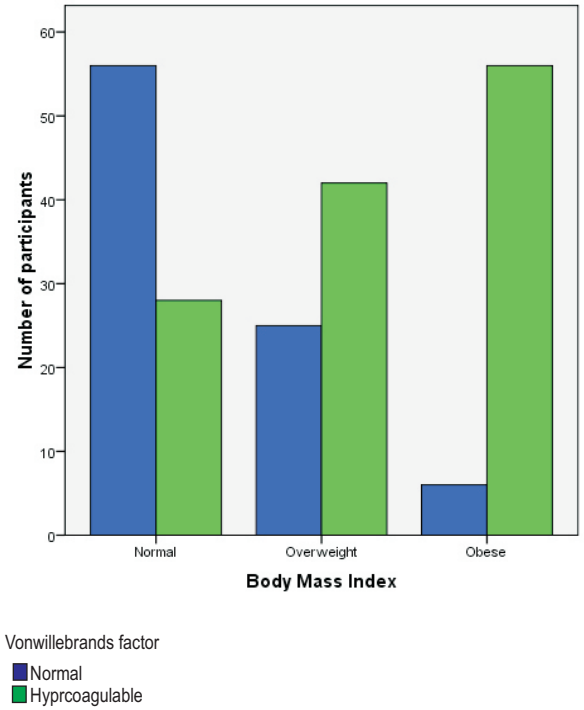


Figure VII: BMI as a function of hypercoagulability (VWF > 2 IU/ml)



as compared to those with good glycaemic control [34(34.0%)]. The difference was significant, P=0.003.

Table I and figure V shows that hypercoagulability in Type 2 Diabetes Mellitus patients was dependent on the duration of the disease. Those who had the disease for over 10 years had a higher proportion of hypercoagulability [90(92.8%)] than those who had it in less than 5 years [22(44.9%)]. P=0.000. Hence there was a significant correlation between duration of T2DM and hypercoagulability.

The proportion of type 2 diabetes mellitus patients who were hypercoagulable was lower in those who had adequate knowledge about the risk factors of T2DM [48(44.4%)] than in patients who had inadequate knowledge [78(74.3%)]. This difference was statistically significant. P=0.000. (Table I and figure VI)

Table I and figure VII shows that hypercoagulability in T2DM patients was dependent on body mass index. The proportion of obese patients who were hypercoagulable was significantly higher 56(90.3%)] than those who had a normal body mass index [28(33.3%)]. P=0.000. Hence there was a significant correlation between body mass index of T2DM and hypercoagulability.

Logistic regression was used to determine the risk factors associated with hypercoagulability in T2DM patients.

Table II reports the results of multivariate regression analysis revealing patient attributes that were also independent risk factors for hypercoagulability in type 2 diabetes mellitus patients. Sex was significantly associated with hypercoagulability. Type 2 diabetes mellitus female patients were 3.98(95% CI [2.23-7.10]) times more likely to be hypercoagulable than the male patients. Even after adjusting for confounders such as obesity, hypercholesterolemia and hypertension, the odds of female T2DM patients being hypercoagulable was still significant AOR being 4.42 (95% CI [2.77-10.63])

In an unadjusted model, Age was significantly associated with hypercoagulability in type 2 Diabetes Mellitus patients. Participants aged 51-60 years and those aged 61 years and above were more likely to be hypercoagulable than those in the age range of 21-30 years OR 2.61(95% CI[1.20-4.80]) and 2.66(95%CI[1.05-4.87) respectively. Even after adjusting for cofounders participants whose age was between 51-60 years and above 61 years were more at risk of hypercoagulability than those in the age range of 21-30 years giving an AOR of 2.53(95% CI[1.21-4.25]) and 1.45(95%CI[1.19-3.16]) respectively.

In an unadjusted model, type 2 diabetes mellitus patients who had poor glycaemic control 53.1% (OR=7.50; 95% CI [4.53-10.95]) were more likely to be hypercoagulable than those who had good glycaemic control. The odds of being hypercoaugulable were still high even after adjusting for age, hypercholesterolemia and obesity, giving an AOR of 6.12 (95% CI 2.27-8.36).

Duration of type 2 diabetes mellitus was significantly associated with hypercoagulability. Participants who had the disease between the duration of 5 – 10 years and those who have had the disease more than 10 years were OR=3.08 (95% CI 1.37-6.97) and OR= 8.67 (95% CI 5.48-11.85) times more likely to be hypercoagulable than those who had the disease for the duration of less than 5 years. The odds of being hypercoaugulable was still high even after adjusting for age, hypercholesterolemia and obesity, giving an AOR of 2.20(95% CI 1.07-4.44) and 5.28(CI 95% 3.01-8.21).

Body Mass Index was significantly associated with hypercoagulability in type 2 diabetes mellitus patients. Participants who were overweight were 1.52(95% CI 1.06-4.32) more likely to be hypercoagulable than those whose BMI was normal. The odds of being hypercoagulable in participants who were obese were 5.33(95% CI 2.26-11.48). After adjusting for cofounders the odds of being hypercoagulable in the obese group was 4.54(95% CI 2.88-10.59). In the overweight group the odds of hypercoagulability became 1.05(95% CI 0.75-2.86). The null value is 1, and because this confidence interval does include 1 in adjusted model, the result indicates a statistically insignificant difference in the odds of overweight and normal individuals in terms of hypercoagulability.

In univariate analysis type 2 diabetes mellitus patients who had inadequate knowledge on the risk factors, complications and management of type 2 diabetes mellitus were 3.48 more likely to be hypercoagulable than those with adequate knowledge OR 3.48(CI 95% 1.96-6.18). However after adjusting for glycaemic control, age, hypercholesterolemia and obesity, the odds of hypercoagulability was the same in those with adequate knowledge about the risk factors and complications and those who had inadequate knowledge, AOR being 1.00(CI 95% 0.80-2.20).

DISCUSSION

This research revealed that patients aged 51 years and above were at risk of hypercoagulability than those who were below 51 years old. These results are consistent with the findings of past studies. Soltani and colleagues reported a significant correlation between age and hypercoagulability in T2DM patients (9). These results accords that of Zhaolan et al.,(2010), who reported a significant association between age and hypercoagulation in type 2 diabetes mellitus patients (10). The results are further supported by Khattaba et al.,(2010) who reported that older type 2 diabetes mellitus patients were more likely to be hypercoagulable than the younger patients (11). The risk of hypercoagulability with increasing age could be attributed to the changes that occur to the vascular system sclerosis as a result of aging thus tilting the scale to hypercoagulability in older patients.

In the current study, the proportion of female T2DM patients who were in hypercoagulable state was higher than the male T2DM patients. Logic regression analysis showed that women were four times at risk of hypercoagulability than the male patients (Fig IV, Table I and II). This findings was consistent with that reported in Egypt by Soliman G., (2005), who reported that female T2DM patients were more hypercoagulable than male patients by finding significantly higher fibrinogen levels in female T2DM patients than male patients (12). higher plasma fibrinogen level in diabetic females than diabetic males (13). On Similar results were reported by Soedama et al., (2008), The results are also in concomitant with that of Screiber et al., (2005) reported that Women are more hypercoagulable than men early after injury in a study conducted to determine the course of coagulation after injury and to determine whether there is a gender difference (13). The results of this study further accords that of Michael W et al., (2002), who found significantly higher FVII: C, Vonwillebrands and PAI-1 levels in women than in men with T2DM(14). The finding of higher PAI-1 and vWF levels in diabetic women therefore may indicate an important sex-specific interference in the haemostatic system by diabetes that could increase vascular risk (14). A mechanism independent of insulin resistance by which female sex could be associated with higher vWF, FVIII: C and PAI-1 levels in T2DM is not clear. Differences caused by sex hormone levels do not explain the findings. In women higher estrogen levels are associated with lower PAI-1 levels while in healthy postmenopausal women not receiving estrogen replacement, PAI-1 levels are no higher than in men of the same age (15) . In the present study no assessment was made of sex hormone levels. Investigation of the relationship among female hormonal status, haemostatic variables, and the features of insulin resistance in premenopausal and postmenopausal T2DM women may help in the evaluation of the importance of estrogens and vWF and PAI-1 in thrombotic development in women with T2DM.

In this study poor glycaemic control was associated with hypercoagulability in T2DM.These results are consistent with Chantal et al., (2010), who also found significant association between glycaemic control and hypercoagulability (16). The results also accords that of Osende et al., (2008), who reported a correlation between improved glycaemic control and blood thrombogenicity (17). Poor glycaemic control may lead to hypercoagulability because of the effects of glucose on the endothelium. Long term high glucose levels damage the endothelium by accelerating glycosylation of proteins and lipids to generate advanced glycation end products (AGEs) (18). AGEs accumulate in the vessel wall, where they may directly disturb cell structure and function. Furthermore, the receptor for AGEs (RAGE) activation on endothelial cells inhibits nitric oxide (NO) biosynthesis by endothelial NO synthase (eNOS) down regulation, with increased generation of ROS.ROS as a negative effect on NO by forming the highly oxidant peroxynitrite ion, which in turn uncouples eNOS to produce superoxide anion and asymmetric dimethylarginine (ADMA) , an endogenous inhibitor of eNOS. NO is important in haemostasis because it inhibits platelet aggregation therefore a reduction of NO may lead to unregulated platelet aggregation (19).

In this study a good statistically significant correlation was found between the prevalence of hypercoagulability and the duration of diabetes mellitus that was consistent with findings of other studies. Participants who had the disease for a period of 10 years and above were more likely to be hypercoagulative than those who had it for duration of less than 5 years. Infact the odds of being hypercoagulable in T2DM patients increased with the increase in the duration of diabetes. Chantal et al., (2010)

and Barbic et al., (2010) reported a significant correlation between hypercoagulability and the duration of diabetes (16,20). The reason for this correlation may be due to the effects of hyperglyceamia on the endothelium over a prolonged period of time. Damage to the endothelium tend to increase with the increase in the duration of T2DM.

In chi-square test of independence the proportion of individuals who were hypercoagulable was significantly higher in individuals who had inadequate knowledge about the risk factors and complications of T2DM. In the univariate analysis the odds of hypercoagulability in participants who had inadequate knowledge about T2DM risk factors and complications was higher than in those who had adequate knowledge. In the multivariate analysis after adjusting for glycaemic control the adjusted odds ratio was 1 indicating that the risk of hypercoagulability in those who had adequate knowledge and inadequate knowledge about the risk factors and complications of T2DM was the same. This is inconsistent with the results obtained by Blankenfeld et al., (2006), who reported that the existence of diabetes related complications was a significant predictor of poor knowledge in T2DM patients (21). However in this study Blankenfeld and colleagues did not adjust for glycaemic control. This may be the reason why there was such a discrepancy. Blankenfeld et al., (2006) further reported that patients who have inadequate knowledge about the risk factors and complications of the disease are more likely not to adhere to treatment or may be inconsistent in going to the Hospital for review so as to have the glucose levels checked (21). The results are also in contrast with Ulvi et al., (2009) who reported a positive correlation between hypercoagulability and levels of knowledge about the risk factors and complications of T2DM patients among the rural community in Pakistan. T2DM patients with inadequate knowledge about the risk factors, complications and management of diabetes are less likely to understand why glucose levels should be monitored and hence usually tend to have poor glycaemic control and consequently increased hypercoagulability. Patients with adequate knowledge on the other hand may be very careful with their lifestyles because they know the complications that may occur if they don't adhere to proper treatment and risk free life style and hence able to have better glycaemic control (22).

In this study obesity was significantly associated with hypercoagulability in T2DM patients . T2DM patients who were obese were more likely to be hypercoagulable than the non-obese T2DM patients. These results are consistent with most of the past studies. Muthu et al., (2009), Soltani et al., (2011) and Kozek et al., (2004) found increased hypercoagulability with increased levels of cholesterol (9, 23, 24). Obesity was first proposed to be a risk factor for the development of atherosclerosis and T2DM over 40 years ago (24). Metabolic alterations accompanying the visceral distribution of fat lead to arterial hypertension, dyslipidemia, insulin resistance and subsequently to T2DM. This phenomenon is associated not only with classical atherosclerotic risk factors but also with coagulation and fibrinolysis abnormalities (25). Hypercoagulation in obesity is thought to be caused primarily by the synthesis of factors activating coagulation and inhibiting fibrinolysis (for example factor VII activator and the fibrinolytic inhibitor PAI-1) in adipose tissue(24). Hemostatic abnormalities may also result from the synthesis in adipose tissue of cytokines that are mediators of inflammation and insulin resistance, such as interleukin 6 and TNF-alpha. In addition to this direct effect, the metabolic and lipid alterations that accompany obesity and T2DM are likely to indirectly influence coagulation properties in these patients. Hypercholesterolemia, obesity and hypertension are interrelated. Both obesity and hypercholesterolemia have been implicated in the development of T2DM and both are also associated with hypercoagulation. Studies have shown that raised plasma insulin levels with insulin resistance appears to be an atherogenic factor. Insulin stimulates cholesterol synthesis in smooth muscle cells and macrophages of the arterial walls and also stimulates the proliferation and migration of smooth muscle cells. Prospective data is needed to clarify whether these factors preceded T2DM or they are a consequence of the disease (26).

CONCLUSION

The main aim of this study was to determine the risk factors associated with hypercoagulability in T2DM patients at Ndola Central Hospital. The variables that were found to be independent risk factors of hypercoagulability among T2DM patients were Age, sex, duration of T2DM, obesity, and poor glycaemic control.

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ABREVIATIONS USED

ADMA:Asymmetric dimethylarginine; **AGEs**:Advanced Glycation End Products; **AOR**:Adjusted Odds Ratio;
BMI: Body Mass Index;
GLP: Good Laboratory Practice;
HBAIC: Glycated Haemoglobin;
NCH: Ndola Central Hospital;
NO: Nitric Oxide;
OPD: Outpatient Department;
OR: Odds Ratio;
PAI: Plasminogen Activator Inhibitor;
ROs: Reactive Oxygen Species;
T2DM: Type 2 Diabetes Mellitus;
UNZA-BREC: University of Zambia Biomedical Research Committee;
VWF: Vonwillebrands' factor;
WHO: World Health Organisation.

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