



Evaluating the effect of antidiabetic treatment on haemostatic and fibrinolytic parameters among type 2 diabetics in Ilorin, Nigeria

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Abstract

Background: Type 2 Diabetes Mellitus is a disease of epidemic proportions and many patients are at a great risk of premature mortality and complication of atherothrombotic disorders affecting coronary, cerebral and peripheral arterial trees. Increased Plasminogen Activator Inhibitor Type 1 inhibits fibrinolysis and predicts cardiovascular risk in those living with Type 2 Diabetes. This study aimed to determine the effect of antidiabetic treatment on haemostatic and fibrinolytic parameters among Type 2 Diabetic subjects in Ilorin.

Methods: This was a comparative cross-sectional study involving 78 Type 2 diabetic patients, (39 treatment naïve, 39 treatment experienced). Full blood count was performed using Sysmex XP 300 while Prothrombin time was determined using one stage test of Owren. Activated partial thromboplastin time was determined by method of Proctor and Rapaport. Fibrinogen and Plasminogen Activator Inhibitor type-1 were assayed using AssayMax Human Fibrinogen ELISA and AssayMax Human PAI-1 ELISA kit. Data Analysis was done using SPSS version 25.0.

Results: Mean PAI-1 levels were significantly higher in treatment naïve diabetics when compared to treatment experienced diabetics (2.44 ± 2.57 vs 2.51 ± 1.47 ng/ml $p=0.002$) as were fibrinogen levels (434.65 ± 366.15 vs 482.24 ± 299.64 mg/dl; $p=0.048$). PAI-1 levels were lowest among diabetics treated with Metformin + DPP4 inhibitors, while fibrinogen levels were lowest among those treated with Metformin + sulfonylurea combination.

Conclusion: Oral hypoglycaemic treatment, combination therapy in particular, improves fibrinolysis in type 2 diabetics thereby reducing the risk of cardiovascular disease in type 2 diabetes mellitus patients.

Keywords: Fibrinolysis, Diabetes, Metformin

Introduction

Diabetes Mellitus is described as an irreversible syndrome of chronic hyperglycaemia due to relative

insulin deficiency, resistance or both.¹ It has grown to epidemic proportions in the past few decades with rapidly rising incidence and prevalence rates.¹⁻³ Global projections estimate 109% rise in prevalence in Africa, and about 41 million Africans are predicted to be living with Diabetes by the year 2035.^{1,4}

Type 2 Diabetes Mellitus is a hypercoagulable state that is accompanied by notable changes to all aspects of the haemostatic mechanism, including

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the endothelium, the platelets, the coagulation factors, natural anticoagulants and fibrinolysis.¹ Studies have however varied in their findings in different geographical locations.⁵⁻⁸ There are racial differences among Diabetics which contribute to these variations and thus make the need for local studies important.⁹⁻¹²

Type 2 Diabetics are at a great risk of premature mortality and complication by atherothrombotic disorders affecting coronary, cerebral and peripheral arterial trees.⁸ Eighty percent of diabetics die from thrombotic events with 75% to 80% of these deaths resulting from cardiovascular events.¹ When compared with non-diabetics, the risk of death by myocardial infarction is 3-5 times higher in patients with Type 2 Diabetes Mellitus.⁸

An endothelial derived antifibrinolytic protein named plasminogen activator inhibitor type 1 (PAI-1) is stimulated by hyperglycemia and has been described to be elevated in patients with Type 2 Diabetes Mellitus.¹³⁻¹⁵ Increased PAI-1 levels in the vessel wall decrease local fibrinolysis and increase thrombus formation and the unfavorable evolution of atherosclerotic plaques, making it an important predictor of cardiovascular risk in those living with Type 2 Diabetes.¹³⁻¹⁵ It is therefore important that efficacy of treatment be evaluated with respect to reducing PAI-1 levels and consequently, the risk of Cardiovascular disease in Type 2 Diabetics.

Hypoglycaemic agents, lipid lowering drugs and antiplatelet agents are used in the treatment of Type 2 Diabetes. The effect of hypoglycaemic agents on coagulation and fibrinolysis has been a source of debate. While metformin is known to reduce plasma PAI-1 levels and increase fibrinolysis, the roles of sulfonylureas are yet unknown.¹⁶ Some thiazolidinediones decrease cardiovascular risk, while others such as rosiglitazones have been found to increase the risk of disease.¹⁷ Gliptins are relatively new agents and studies are ongoing to clarify their role in cardiovascular disease prevention.¹⁶ Insulin treated Type 2 Diabetic subjects are also at a higher risk of cardiovascular events as insulin increases fibrinogen and PAI-1 levels.¹⁸

The aim of this study was to determine the effect of antidiabetic treatment on haemostatic and fibrinolytic parameters of Type 2 Diabetic subjects in Ilorin.

Materials and methods

This was a hospital based comparative cross-sectional study conducted at endocrinology clinic of departments of Medicine and the General outpatient unit of the Family Medicine Department of University of Ilorin teaching hospital, Ilorin. Approval for this study was sought and obtained from the Ethics and Research Committee of University of Ilorin Teaching Hospital.

A total of 78 Type 2 diabetic patients diagnosed based on WHO criteria for diagnosis of diabetes who met the inclusion criteria for this study were recruited using convenience sampling method.¹⁹ Known Type 1 diabetics who were autoantibody positive were excluded from this study. Subjects on drugs that could affect coagulation, those with impaired hepatic synthetic function, varicose veins, previous history of heart disease, history of bleeding disorders, use of antiplatelet or anticoagulant medications and those that declined consent were excluded from the study. Thirty-nine of these subjects were on antidiabetic treatment, while the other thirty-nine who served as controls were not on any form of antidiabetic treatment.

Written and informed consent was obtained from the patients after thorough explanation of the aim of the study and the procedure involved in a language best understood by them. Structured interviewer administered questionnaires were used to obtain relevant data and history from each participant.

Venous blood (7.5 ml) was taken from each patient between 7am-10am following strict antiseptic procedure. Out of this, 4.5ml was dispensed into plastic tube containing 0.5ml trisodium citrate (3.8%) tightly capped and mixed gently.

The sample was centrifuged at 2000g for 15 minutes, there after the plasma was transferred into a plastic centrifuge tube without disturbing the buffy coat and cell layers. The plasma in the centrifuge tube was spun again at 2000g for another 15 minutes to obtain platelet poor plasma. The platelet poor plasma was separated using a plastic transfer pipette and placed in plastic aliquot tubes. The sample was stored at -800C until assayed for the coagulation tests; PT, APTT.

The remaining 3.0ml of venous blood was dispensed into EDTA bottle and used for analysis of full blood count which was performed using Sysmex XP 300 automated cell counter with strict

adherence to manufacturer's instructions within two hours of sample collection.

Prothrombin time was determined using commercially prepared reagents based on the one stage test of Owren.²⁰ Activated partial thromboplastin time was determined using commercially prepared reagents based on method of Proctor and Rapaport.²¹ Fibrinogen assay was determined using AssayMax Human Fibrinogen ELISA. (Commercially prepared and obtained from Assaypro LLC, 3400 Harry S Truman Blvd, St Charles, MO 63301). Plasminogen Activator Inhibitor type -1 was determined using the AssayMax Human PAI-1 ELISA kit (Product of Saint Charles, Missouri, USA).

Data entry and analysis were done with a micro-computer using Statistical Package for the Social Sciences (SPSS) version 25.0 computer software

packages. After generation of frequency tables and simple proportion, categorical data were compared by Chi square and statistical significance was determined with P value of <0.05, while ANOVA was used to compare the means of more than two independent groups. Correlation between continuous variables was done with Pearson's correlation test.

Results

A total of 78 participants, including 39 type 2 diabetic subjects on treatment and 39 type 2 diabetic subjects not on treatment who served as controls, were recruited for this study.

The mean Platelet count, PT, APTT and INR values were within the normal range and similar in both groups. The mean values of PAI-1 (ng/ml) and Fibrinogen (mg/dl) concentrations were

Table 1: Comparison of haematological and haemostatic parameters in Treatment Naïve and experienced Type 2 Diabetics.

Variables	Treatment Experienced n = 39	Treatment Naïve n = 39	Mann Whitney U test	p –value
Platelet Count (x10⁹)				
Mean (SD)	274.81 (152.80)	259.64 (145.11)	247.00	0.462
Median	228.50	235.00		
PT (Secs)				
Mean (SD)	12.86 (2.15)	12.69 (1.83)	476.50	0.883
Median	12.00	12.00		
INR				
Mean (SD)	1.07 (0.18)	1.06 (0.157)	479.50	0.920
Median	1.00	1.00		
APTT (secs)				
Mean (SD)	30.72 (5.45)	32.92 (6.44)	386.50	0.164
Median	30.00	32.00		
PAI 1 (ng/ml)				
Mean (SD)	2.44 (2.57)	2.51 (1.97)	405.00	0.002
Median	1.33	1.89		
Fibrinogen (mg/dl)				
Mean (SD)	434.65 (366.15)	482.24 (299.64)	625.00	0.048
Median	205.30	136.10		

PAI-1: Plasminogen activator Inhibitor Type 1, INR: International normalized ratio, PT: Prothrombin time, APTT: Activated partial Thromboplastin time. PAI -1 range: 5-40ng/ml²²; Fibrinogen range: 150 -400mg/dl²³

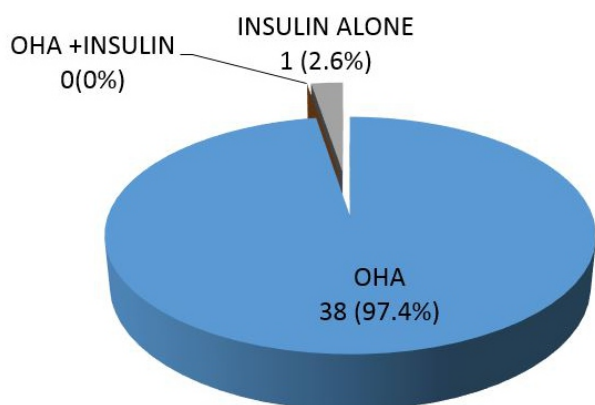


Figure 1a (original): Distribution of Treatment experienced Type 2 diabetics according to Medication.

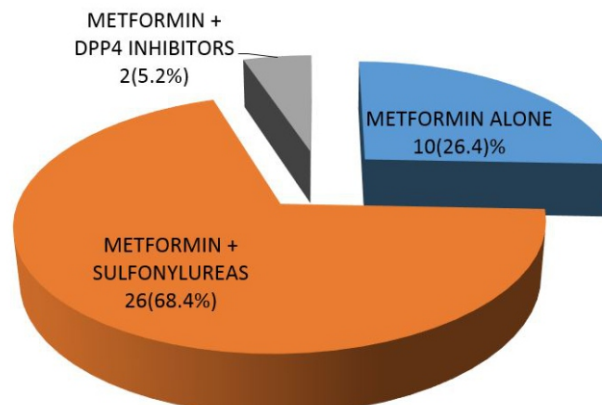


Figure 1b (original): Types of oral hypoglycemics used by Treatment experienced Type 2 diabetics.

Table 2: Comparison between mean plasma PAI 1(ng/ml) and Fibrinogen (mg/dl) levels among treatment experienced Diabetic respondents using different type of oral hypoglycaemics

Type of OHA used	PAI-1 (ng/ml) Mean ± SD	Range	F	p value
Metformin alone	3.40 ± 3.26	1.19 – 10.07	1.784	0.183
Metformin + Sulfonylureas	2.30 ± 2.35	0.50 – 10.79		
Metformin + DPP4 Inhibitors	1.02 ± 0.25	0.76 – 1.43		
Type of OHA used	Fibrinogen(mg/dl) Mean ± SD	Range	F	p value*
Metformin alone	729.14 ± 852.03	34.40 – 2739.12	6.212	0.005
Metformin + Sulfonylureas	140.26 ± 98.38	20.49 – 401.00		
Metformin + DPP4 Inhibitors	239.78 ± 155.31	83.20 – 512.00		

F: ANOVA (Analysis of Variance), PAI-1: Plasminogen activator Inhibitor Type 1

significantly higher among controls than subjects. (p value of 0.002 and 0.048 respectively). (Table 1) There were no statistically significant differences between the Prothrombin time, INR, and APTT between subjects and controls. Table 1. Thirty-eight (97.4%) of the treatment experienced subjects with Type 2 Diabetes were placed only on oral hypoglycaemics, while only 1(2.6%) respondent was on insulin therapy. No patient was on a combination of OHAs and insulin. Figure 1a. As shown in Figure 1b, 26(68.4%) of the 38 subjects

on OHAs were placed on a combination of metformin and sulfonylureas, and while 10(26.4%) of them were treated with metformin alone, only 2(5.2%) were placed on a combination therapy of Metformin and DPP-4 inhibitors. Mean PAI -1 levels were much lower in subjects who were on Metformin + DPP4 inhibitors when compared to those on metformin alone or metformin + sulfonylureas. This was not statistically significant. There was a statistically significant lower mean fibrinogen level among subjects on

metformin + DPP4 inhibitors, and those with metformin alone had the highest mean fibrinogen levels among the three treatment groups. Table 2.

Discussion

This study assessed the coagulation and fibrinolytic parameters of Type 2 diabetics, and evaluated the effect of oral hypoglycaemic treatment on these parameters. The results have shed more light on the optimal pattern of antidiabetic medication that will reduce the prothrombotic risk seen in Type 2 diabetic patients, and consequently reduce the morbidity and mortality associated with the disease. The majority of the respondents with Type 2 Diabetes were between 50 – 65 years of age in both the Treatment Naïve and Experienced groups. This is in contrast to reports by Bullard et al and Xu et al, who found the Type 2 Diabetes was more prevalent in subjects greater than 65 years old.^{22,23} Iloh et al, in Northern Nigeria, however found that Type 2 Diabetes was commonest in patients between 40 – 60 years.²⁴ This disparity may stem from the higher life expectancy and better health care practices among Caucasians, as well as the poorer health seeking attitude among the elderly African population, majorly due to the low socioeconomic status associated with this age group in Africa.

This study compared biochemical and haemostatic parameters between treatment experienced and treatment naïve Type 2 Diabetics. The mean Platelet count, PT, APTT and INR values were within the normal range and similar in both groups. Fibrinogen was also significantly higher among the treatment naïve diabetics. Mean PAI-1 levels were also significantly higher among the treatment naïve diabetics. This is supported by Okuda et al,²⁵ who also reported lower PAI-1 levels in treatment experienced diabetics when comparing values before and after commencement of antidiabetic therapy. This highlights the importance of proper glucose control and its relationship with fibrinolysis, and ultimately the risk of cardiovascular disease.

In this study, all subjects on oral hypoglycaemics had metformin included in their therapy, either alone or as part of a combination regimen. This reliance on metformin based therapy is not surprising, and could stem from its effects on both the fibrinolytic markers and cardiovascular risk

factors. Grant stated that in addition to its hypoglycaemic effect, Metformin reduces PAI-1 levels, thus increasing the rate of fibrinolysis.²⁶ Recent studies also indicate that metformin has direct effects on fibrin structure/function and stabilises platelets.²⁷

In contrast to these studies, Adediran reported poor fibrinolytic activity in patients on oral metformin therapy, and suggested that the complex contributions of other risk factors could explain his findings.²⁸ Metformin has been found to have a differential effect on endothelial activation,²⁹ and since endothelial dysfunction is linked with increase in fibrinolytic markers, it is possible that metformin monotherapy might not optimally reduce fibrinolysis. Metformin monotherapy was also found to have the lowest impact on fibrinogen and PAI-1 levels when compared to a combination of metformin with sulfonylureas or DPP4 inhibitors, and while diabetics on metformin and DPP4 inhibitors were found to have the lowest PAI-1 levels (1.02 ± 0.25), those on metformin + sulfonylureas had the lowest fibrinogen levels. (140.26 ± 98.38).

This theory is supported by Cefalu et al, who found an increased decline in PAI-1 levels with combination of metformin and sulfonylureas, as compared to monotherapy with either agent.³⁰ Gin et al found unchanged fibrinogen levels but reduced PAI-1 antigen levels in patients treated with insulin and metformin when compared to insulin treated patients, and suggested that the reduced PAI-1 levels were as a result of metformins effect on insulin resistance.³¹ Tenecliptin, a DPP4 inhibitor which acts by reducing active incretin concentrations, also reduced PAI-1 levels when used in combination with either metformin or sulfonylureas.³⁰

These reports suggest that while antidiabetic monotherapy may provide effective glycemic control, it might not be enough for optimal improvement in fibrinolytic parameters and combination therapy will provide better results. Also, since it is known that PAI-1 is produced in adipose tissue,^{8,27} and dyslipidemias are also associated with an increase in coagulation factors,⁶ a synergy between antidiabetic medication and lipid lowering drugs is needed for optimal improvement in PAI-1 levels and fibrinolysis.

Conclusions

Treatment naïve Diabetics had higher PAI-1 than treatment experienced subjects, thus fibrinolysis is improved with oral hypoglycaemic treatment. This study has also observed that a combination of antidiabetic drugs had a higher impact on PAI-1 levels than monotherapy. There is a need to educate general practitioners on the advantages of combination therapy over monotherapy with metformin in type 2 diabetic patients. It is also recommended to combine statins with glucose lowering therapy in all diabetic patients at diagnosis, and not just those with complications.

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