

# Cardiovascular Risks in Prediabetes: Preliminary Data on “Vasculopathy Triad”

Ezekiel U Nwose<sup>1,3</sup>, Ross S Richards<sup>1</sup>, Phillip T Bwititi<sup>2</sup>

<sup>1</sup>School of Community Health, Charles Sturt University, Australia, <sup>2</sup>School of Biomedical Sciences, Charles Sturt University, Australia, <sup>3</sup>College of Health Sciences, Novena University, Ogame Nigeria

## Abstract

**Background:** Subclinical cardiovascular disease is inherent in complications of diabetes mellitus. It occurs before the overt manifestation of cardiovascular disease complication in diabetes, and involves vasculopathy triad or three major vascular events comprising stasis, endothelial dysfunction, and atherothrombosis. **Aim:** This study was to examine evidence of vasculopathy triad in prediabetes, biomarkers of stasis, endothelial dysfunction, and atherothrombosis in prediabetes were compared with apparently healthy group. **Materials and Methods:** Eighty-one participants with results for plasma D-dimer, homocysteine, and whole blood viscosity were selected from a research database. The participants consisted of control ( $n = 44$ ) and prediabetes ( $n = 37$ ) based on clinical history and laboratory results. **Results:** Multivariate analysis shows a significantly higher level of vasculopathy in prediabetes than in the control group ( $P > 0.0001$ ). Blood viscosity ( $P < 0.04$ ) and homocysteine ( $P < 0.03$ ) are significantly higher in prediabetes than in controls. Average levels for plasma D-dimer are also higher in prediabetes than in control, but not statistically significant in this particular analysis. **Conclusion:** This study suggests a novel application of known idea, vasculopathy triad that could be used for assessment of subclinical cardiovascular disease in prediabetes.

**Keywords:** Atherothrombosis, blood viscosity, homocysteine, laboratory evaluations, subclinical vascular pathology

**Address for correspondence:** Dr. Uba Nwose, School of Community Health, Charles Sturt University, Orange NSW2800 Australia.  
E-mail: nwoseeu@gmail.com

## Introduction

### Early mechanism of cardiovascular complication in diabetes and prediabetes

The mechanisms for the propensity to develop cardiovascular disease (CVD) among persons with diabetes and prediabetes are varied and associated with several factors, prominent amongst is hyperglycaemia toxicity. Hyperglycemia can mediate its adverse effects through multiple pathways including polyol, hexosamine, protein kinase C, and glycation pathways. The unifying occurrence in these pathways is the overproduction of the oxidant, superoxide ion ( $O_2^{\cdot-}$ )

that increases susceptibility to intracellular oxidative stress.<sup>[1]</sup> In diabetes/hyperglycemia, erythrocytic glycolysis increases and so does production of  $O_2^{\cdot-}$  which results in erythrocyte oxidative stress (EOS). Accumulating reports have consistently demonstrated that EOS in diabetes mellitus (DM) leads to complications such as macrovascular diseases. What requires (but yet) to be equally emphasized is that the hyperglycemia is the same in biochemical context whether it is clinical (diabetes) or subclinical (prediabetes).

The generation of ROS in the erythrocytes coupled with depletion of its defensive natural antioxidants enhance the cells' membrane lipid peroxidation, which lead to decreased membrane fluidity of the red blood cells. The attendant effects are increased aggregation and decreased blood flow rate. Besides, increased glycolysis and pentose phosphate pathway (PPP) activities have the propensity to generate high levels of glyceraldehyde-3-phosphate, which enhance advanced glycation process that in turn exacerbates OS.<sup>[2]</sup>

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10.4103/1947-2714.136913

## Definition of vasculopathy triad and how it is implicated in prediabetes

There is a set of three factors hypothesized to underlie atherothrombogenesis.<sup>[3]</sup> The factors include alterations in blood flow (marked by blood viscosity), injury to the vascular endothelium (marked by endothelial dysfunction), and alterations in coagulation/fibrinolysis balance indicated by plasma D-dimer.<sup>[4]</sup> In our review,<sup>[5]</sup> it was illustrated how these three factors are associated with hyperglycemia-induced erythrocyte oxidative stress and macrovascular complications. Figure 1 is a representation of how the three factors constitute VT. Thus, VT is a diagnostic concept of three markers of vascular pathology.

It is important to note that in current literature, VT is known as Virchow's triad,<sup>[6-8]</sup> though there is argument about the attribution.<sup>[9]</sup> Further, Lincoff had conceptualized a "triad in cardiovascular medicine".<sup>[10]</sup> While Lincoff's concept is based on treatment view point, VT concept is coming from a laboratory perspective. Nevertheless, Lincoff's triad has diabetes vis-à-vis hyperglycemia as a major factor for cardio-vasculopathy.

## Hypothesis and objective

We reported higher levels of plasma D-dimer in prediabetes relative to control<sup>[11]</sup> and we have also reported that whole blood viscosity (WBV) varies between individuals with different stages of diabetic macrovascular pathogenesis, including prediabetes;<sup>[12]</sup> and that WBV is a laboratory index for antiplatelet as international normalized ratio (INR) is for anticoagulant.<sup>[13]</sup> Given that these factors, which constitute VT, are part of emerging laboratory markers for assessment of CVD,<sup>[14,15]</sup> we hypothesize that concomitant prevalence of at least two of three VT indices would be more prevalent in prediabetes compared to healthy individuals. A positive finding will be invaluable for early identification of subclinical macrovascular complications in prediabetes. The overarching objective is to present the potential of laboratory testing of the VT as a profile.

## Materials and Methods

### Participants selection and baseline characteristics

This work was a repeat analysis of data subset.<sup>[16]</sup> Among 90 participants that constituted the control ( $n = 49$ ) and prediabetes ( $n = 41$ ), 81 comprising control ( $n = 44$ ; 27 females and 17 males) and prediabetes ( $n = 37$ ; 15 females and 22 males) whose results for plasma D-dimer, homocysteine, and whole blood viscosity were selected. The process of participants' classification and selection, which was previously published, were based on questionnaire, clinical history, and laboratory results.<sup>[12,16]</sup> Prediabetes in this study was defined as

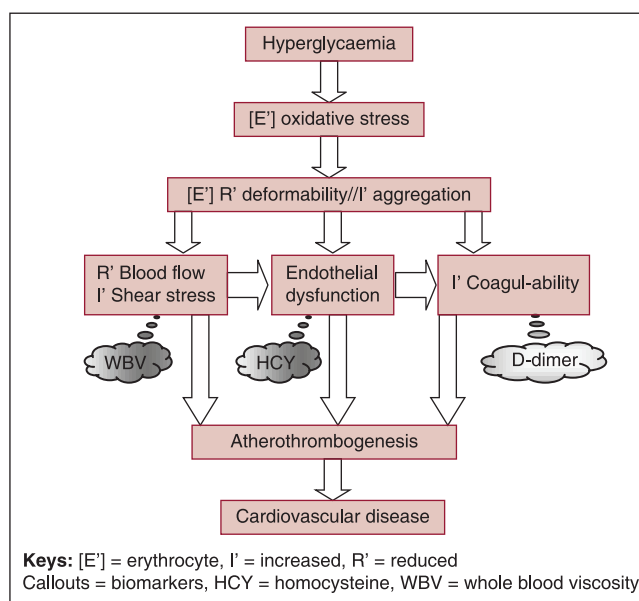
an established prediabetic status identified by general practice, or participants who were otherwise healthy (i.e., undiagnosed) but who presented with elevated blood glucose level.

Information obtained from questionnaire included past results of age and gender as well as blood glucose level, electrocardiography (ECG), history of CVD, diabetes, kidney disease, and any other health conditions. Family history of diabetes and/or heart disease, body mass index (BMI), smoking, alcohol consumption, and ongoing medication were also recorded. All participants included in this study were non-smokers, though there quite a few smokers among the excluded data, which shall be reviewed.

Participants were included if their BMI was within the range 20-30 Kg/m, daily consumption of standard alcoholic drinks was <2 for females and <4 for males, and no known disease or ongoing medication was reported or identified. Participants identified known family history of diabetes, diagnosed with diabetes or CVD (including high blood pressure and dyslipidemia) were classified into separate groups, and therefore excluded in this study. Thus, baseline characteristics common to both groups included normal blood pressure and normal cholesterol profile test results.<sup>[16]</sup>

## Statistical study

Mean blood glucose level was first determined to be significantly higher in the control group compared to the prediabetes group ( $P < 0.000001$ ). Plasma D-dimer and homocysteine were, determined by the MiniQuant



**Figure 1:** Illustration of links between hyperglycemia, erythrocyte oxidative stress, and related vascular events

(Biopool) and AxSYM (Abbott Laboratories) methods, respectively. Blood viscosity was measured using a Silenus viscometer. Cholesterol values were assayed using point of care machine (Cholestech LDX®).<sup>[16]</sup>

S-Plus was used to perform multivariate (MANOVA) and univariate analyses to determine and reaffirm previously reported observations of statistically significant differences in levels of the indices between prediabetes and control. Based on the descriptive statistics of data [Table 1], normal levels for the research method and study population were worked out using the formula:

Normal range = Mean ± 2SD; where SD is standard deviation

Thereafter, data were sorted to determine prevalence of abnormality of D-dimer, homocysteine and viscosity, as well as concordant abnormality of ≥2 of the three markers in any participant.

### Results

The descriptive statistics show that average levels for all indices of the vasculopathy triad are higher in prediabetes than in control [Table 1]. In the cohort evaluated overall, MANOVA shows a significant difference between groups ( $P < 0.001$ ). It is also observed that only WBV ( $P < 0.04$ ) and homocysteine ( $P < 0.03$ ) are significantly higher in prediabetes than in controls.

In evaluation of prevalence of abnormality of the three parameters, it is observed that abnormal results of all VT indices are more prevalent in prediabetes than in control group. Abnormal D-dimer is 43% in control compared to 51% in prediabetes. Abnormal homocysteine is 5% in control compared to 8% in prediabetes. Abnormal WBV is 7% in control compared to 11% in prediabetes.

The outcome of evaluation of concordant abnormality of ≥2 of the three markers in any participant is presented in the vein diagrams [Figure 2]. It is observed that in the

control group that half of the abnormal homocysteine and all abnormal WBV results were concordant with abnormally high D-dimer results [Figure 2a]. Also, it is observed that in the prediabetes group that two-third of the abnormal homocysteine and three-quarters of abnormal WBV results were concordant with abnormally high D-dimer results [Figure 2b]. In both the control and prediabetes groups, no individual presented

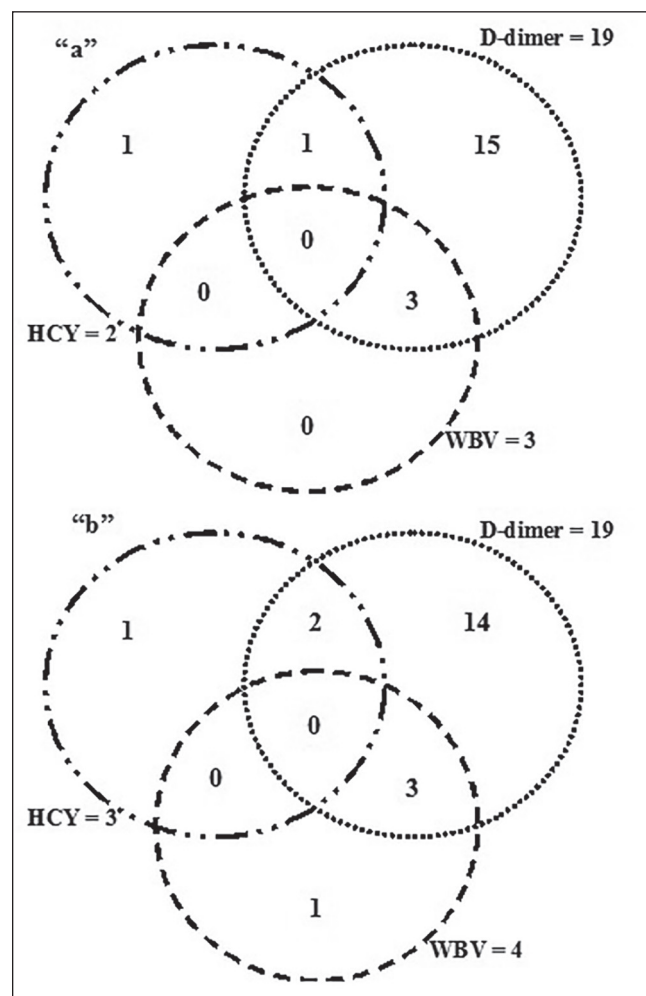


Figure 2: Concordance and prevalence of vasculopathy indices in control and prediabetes groups

Table 1: Descriptive statistics

	Age (Years)		BGL <sup>†</sup> (mmol/L)		D-dimer* (µg/L)		HCY <sup>‡</sup> (µmol/L)		WBV <sup>††</sup> (mPas)	
	C	PD	C	PD	C	PD	C	PD	C	PD
Mean	55.45	58.30	4.46	5.68	152	185	8.58	9.94	80.72	97.54
Median	56.50	57.00	4.65	5.70	140	167	8.10	9.65	73.83	91.45
SD	12.59	11.45	0.70	0.90	2.31	3.06	2.52	2.61	33.04	37.29
Kurt	-0.34	-0.57	-0.38	-0.09	1.5	3.0	0.55	0.33	0.33	0.37
Min	27	37	2.8	3.9	23	23	4.59	6.02	29.1	5.44
Max	84	82	5.4	7.8	1176	4083	15.46	17	168.15	177.45

<sup>†</sup>Blood glucose level ( $P < 0.00001$ ), <sup>‡</sup>Homocysteine ( $P < 0.03$ ), <sup>††</sup>Whole blood viscosity ( $P < 0.04$ ), \*Log normalized values – otherwise, raw data were very widely distributed; C = Control; PD = Prediabetes

abnormal result for all three parameters. However, it is observed that participants with one abnormal VT index presented levels of other two indices that were higher than population average.

## Discussion

Oxidative stress activates nuclear redox-sensitive transcription factor, which results in enhancement of events at the gene level such as production of pro-coagulant tissue factors and pro-inflammatory mediators that lead to endothelial dysfunction and CVD. Although erythrocyte has no nucleus, stimulated red blood cells are associated with redox-sensitive transcription factor NF-kappaB in mononuclear cells.<sup>[17]</sup> Hence, erythrocyte oxidative stress (EOS) may affect certain macrovascular events including hypercoagulation, endothelial dysfunction, and blood flow. It is noteworthy that these three vascular events constitute Virchow's triad and in order to avoid the debate on attribution,<sup>[18]</sup> it is hereby conceptualized and renamed, vasculopathy triad (VT). The issue is that hyperglycemia that is observable in prediabetes undoubtedly causes both EOS and macrovascular complications.<sup>[19-22]</sup> Indeed, hyperglycaemia-induced EOS reduces erythrocyte membrane fluidity that in turn increases red blood cell aggregation and blood viscosity, and both are predictors of arterial thrombosis. Hyperglycemia is also associated with hypercoagulation and endothelial dysfunction.<sup>[16]</sup> However, the biomarkers of these three vascular events have yet to be adequately considered as a laboratory test profile of surrogate makers of cardiovascular events, especially in prediabetes management. This forms the basis of investigating these three factors.

The results of preliminary report indicate that, on the average, high blood viscosity as well as D-dimer and homocysteine are more prevalent in prediabetes than in apparently healthy person. The concordance results further indicate that while no individual may present abnormal level in all three vascular events, any person with one abnormal VT index may present either concomitant abnormality of another vascular event or the levels of other indices being higher than population average.

We previously reported that up to 76% of oxidative stress is associated with high WBV, reaching 95% prevalence in prediabetes.<sup>[12]</sup> In this report, we present higher prevalence of homocysteine and WBV in the prediabetes group, but relatively equal concomitant abnormality of D-dimer in control [Figure 2]. Plasma D-dimer alone may be used to exclude "no indication" of deep vein thrombosis (DVT) or pulmonary embolism. However, additional tests such as homocysteine or WBV could

provide additive value on the positive D-dimer result, especially in assessing vasculopathy to allow clinical decision on the risk of thrombosis to be made.

In terms of epidemiological evidence of significance, literature on Virchow's triad has been profound.<sup>[6-9,18,23]</sup> It has been known that some candidate biomarkers, including plasma D-dimer and homocysteine, are potential clinical tools to improve risk prediction.<sup>[14]</sup> A cursory look at the biomarkers for cardiovascular events show that WBV is not listed. In other words, while thrombosis is reported to be reaching epidemic proportion partly due to DM, and hyperviscosity is considered as an independent risk predictor;<sup>[24]</sup> and while stasis is managed to prevent development of atherothrombosis, the relevant laboratory marker has yet to be fully acknowledged.

Ridker and his group further suggested that for a proposed biomarker to be widely used, it should (i) provide independent information on risk or prognosis beyond that available from global assessment algorithms such as the Framingham Risk Score; (ii) be easy to measure in a cost-effective manner in outpatient settings; (iii) be measured by an inexpensive and standardized assay with low variability that does not require specialized plasma collection or assay techniques; and (iv) reduction of the biomarker should lead to decreased vascular risk.<sup>[14]</sup>

While we acknowledge that WBV has yet to satisfy the suggestion of providing independent information for diabetic macrovascular complications in our previous study,<sup>[25]</sup> it is noteworthy that the test has a cost-effective user-friendly "chart" option, which has been developed,<sup>[26]</sup> based on a standardized formula.<sup>[27]</sup> The other noteworthy fact is that aspirin is used to reduce vascular risk and we have reported that acetylsalicylate reduces WBV.<sup>[28]</sup> Thus, WBV holds promise, as a laboratory marker, in the clinical management of CVD in diabetes and prediabetes.

## Conclusion

In current clinical practice, the three component indices of VT are being assessed individually, but yet to be recognized together as a surrogate test profile of cardiovascular events. We report a relatively higher prevalence and concomitances of at least two of the VT indices in prediabetes individuals compared to healthy individuals; and suggest that measuring homocysteine or WBV could provide additive value to augment positive D-dimer result during clinical decision on the risk of thrombosis. This will be valuable for the improvement of early identification of subclinical macrovascular complications in prediabetes.



## Acknowledgement

This work is a review of an already published data subset. All authors of the original publication (reference 16) are hereby acknowledged.

## References

- Feldman EL. Oxidative stress and diabetic neuropathy: A new understanding of an old problem. *J Clin Invest* 2003;111:431-3.
- Stitt AW. Advanced glycation: An important pathological event in diabetic and age related ocular disease. *Br J Ophthalmol* 2001;85:746-53.
- Previtali E, Bucciarelli P, Passamonti SM, Martinelli I. Risk factors for venous and arterial thrombosis. *Blood Transfus* 2011;9:120-38.
- Higgins C. Recurrence of venous thromboembolism. *Biomed Sci* 2006;50:865-7.
- Nwose EU, Jelinek HF, Richards RS, Kerr PG. Erythrocyte oxidative stress in clinical management of diabetes and its cardiovascular complication. *Br J Biomed Sci* 2007;64:35-43.
- Makin A, Silverman SH, Lip GY. Peripheral vascular disease and Virchow's triad for thrombogenesis. *QJM* 2002;95:199-210.
- Lowe GD. Virchow's triad revisited: Abnormal flow. *Pathophysiol Haemost Thromb* 2003;33:455-7.
- Malone PC, Agutter PS. Is 'Virchow's triad' useful? *Br J Haematol* 2009;145:839.
- Bagot CN, Arya R. Virchow's triad: A question of attribution. *Br J Haematol* 2008;143:180-90.
- Lincoff AM. Important triad in cardiovascular medicine: Diabetes, coronary intervention, and platelet glycoprotein IIb/IIIa receptor blockade. *Circulation* 2003;107:1556-9.
- Nwose EU, Richards RS, Jelinek HF, Kerr PG. D-dimer identifies stages in the progression of diabetes mellitus from family history of diabetes to cardiovascular complications. *Pathology* 2007;39:252-7.
- Richards RS, Nwose EU. Blood viscosity at different stages of diabetes pathogenesis. *Br J Biomed Sci* 2010;67:67-70.
- Nwose EU, Cann NG, Butkowski E. Whole blood viscosity assessment issues III: Association with international normalized ratio and thrombocytopenia. *N Am J Med Sci* 2010;2:301-5.
- Ridker PM, Brown NJ, Vaughan DE, Harrison DG, Mehta JL. Established and emerging plasma biomarkers in the prediction of first atherothrombotic events. *Circulation* 2004;109:IV6-19.
- Lowe GD, Lee AJ, Rumley A, Price JF, Fowkes FG. Blood viscosity and risk of cardiovascular events: The Edinburgh Artery Study. *Br J Haematol* 1997;96:168-73.
- Nwose EU, Richards RS, Kerr PG, Tinley R, Jelinek HF. Oxidative damage indices for the assessment of subclinical diabetic macrovascular complications. *Br J Biomed Sci* 2008;65:136-41.
- Andrassy M, Bierhaus A, Hong M, Sis J, Schiekofer S, Humper PM, *et al.* Erythropoietin-mediated decrease of the redox-sensitive transcription factor NF-kappaB is inversely correlated with the hemoglobin level. *Clin Nephrol* 2002;58:179-89.
- Dickson BC. Virchow's triad. *Br J Haematol* 2009;145:433.
- Brownlee M. The pathobiology of diabetic complications: A unifying mechanism. *Diabetes* 2005;54:1615-25.
- Dincer Y, Akcay T, Alademir Z, Ilkova H. Effect of oxidative stress on glutathione pathway in red blood cells from patients with insulin-dependent diabetes mellitus. *Metabolism* 2002;51:1360-2.
- Dumaswala UJ, Zhuo L, Mahajan S, Nair PN, Shertzer HG, Dibello P, *et al.* Glutathione protects chemokine-scavenging and antioxidative defense functions in human RBC. *Am J Physiol Cell Physiol* 2001;280:C867-73.
- Taniyama Y, Griendling KK. Reactive oxygen species in the vasculature: Molecular and cellular mechanisms. *Hypertension* 2003;42:1075-81.
- Lip GY, Blann AD. Does hypertension confer a prothrombotic state? Virchow's triad revisited. *Circulation* 2000;101:218-20.
- Lowe GD. Measurement of thrombosis and its prevention. *Br J Clin Pharmacol* 2002;54:96-100.
- Nwose EU, Richards RS, McDonald S, Jelinek HF, Kerr PG, Tinley P. Assessment of diabetic macrovascular complications: A prediabetes model. *Br J Biomed Sci* 2010;67:59-66.
- Nwose EU. Cardiovascular risk assessment and support techniques: Whole blood viscosity assessment issues I: Extrapolation chart and reference values. *N Am J Med Sci* 2010;2:165-9.
- Tamariz LJ, Young JH, Pankow JS, Yeh HC, Schmidt MI, Astor B, *et al.* Blood viscosity and hematocrit as risk factors for type 2 diabetes mellitus: The atherosclerosis risk in communities (ARIC) study. *Am J Epidemiol* 2008;168:1153-60.
- Nwose EU, Cann NG. Whole blood viscosity issues VI: Association with blood salicylate level and gastrointestinal bleeding. *N Am J Med Sci* 2010;2:457-60.

**How to cite this article:** Nwose EU, Richards RS, Bwititi PT. Cardiovascular risks in prediabetes: Preliminary data on "vasculopathy triad". *North Am J Med Sci* 2014;6:328-32.

**Source of Support:** Nil. **Conflict of Interest:** None declared.