Correlation of whole blood viscosity and HbA1c with age in diabetes patients: implications for diabetes research and management in low-mid income countries

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Abstract

Background: There is a concept of association between whole blood viscosity (WBV) and glycated haemoglobin (HbA1c), but the odds that both biomarkers increase with age are yet to be exhaustively investigated. The aim of this Health records-based study was to determine the correlation of age with HbA1c as well as age and WBV in diabetes patients.

Methods: The setting of this work was a medical General Practice in a regional Australia. De-identified pathology data of diabetes patients, which included HbA1c, routine biochemistry and haematology results were mined. WBV was derived from haematocrit and serum total protein levels. Statistical analyses included comparison of biomarkers between stratified aged-groups, the correlations with age, and the odds ratio of the variables to increase with age.

Results: Age showed a weak negative correlation with HbA1c ($r = -0.25$), there were odds that HbA1c can increase by 1% in 2-years ($p < 0.04$). There was weak positive correlation of WBV with age in oldest aged-group ($r = 0.27$), but a confounding effect of anaemia causing the odds to decrease 1% in 3-years. HbA1c and WBV were moderately positively correlated in patients with good glycaemic control ($r = 0.39$).

Conclusion: The notion that HbA1c and WBV increase with age requires rethinking, especially for individuals living with diabetes and undergoing treatment. Implications for interpreting the results of these two tests in diabetes research and clinical management is discussed in the context of limitations in low and middle income countries.

Keywords: *Age; blood viscosity; diabetes treatment; glycated haemoglobin; associations*

Résumé

Contexte : Il existe un concept d'association entre la viscosité du sang total (WBV) et l'hémoglobine glyquée (HbA1c), mais les probabilités que les deux biomarqueurs augmentent avec l'âge ne font pas encore l'objet d'études exhaustives. Le but de cette étude basée sur les dossiers de santé est de déterminer la corrélation de l'âge avec l'HbA1c ainsi que de l'âge et WBV chez les patients diabétiques.

Méthodes : Le cadre de ce travail était une pratique médicale générale dans une région de l'Australie. Des données de pathologie non identifiées de patients diabétiques, comprenant l'HbA1c, les résultats de routine en biochimie et en hématologie, ont été extraites. WBV était dérivé de l'hématocrite et des taux de protéines sériques totales. Les analyses statistiques comprenaient la comparaison de biomarqueurs entre les groupes d'âge stratifiés, les corrélations avec l'âge et le rapport de cotes des variables pour augmenter avec l'âge.

Résultats : L'âge montrait une faible corrélation négative avec HbA1c ($r = -0,25$). Il y avait des chances pour que HbA1c augmente de 1% en 2 ans ($p<0,04$). Il y avait une faible corrélation positive entre WBV et l'âge dans le groupe le plus âgé ($r = 0,27$), mais un effet de confusion de l'anémie entraînant une diminution de 1% de la probabilité en 3 ans. HbA1c et WBV étaient modérément corrélées positivement chez les patients ayant un bon contrôle glycémique ($r = 0,39$).

Conclusion : La notion selon laquelle HbA1c et WBV augmentent avec l'âge nécessite de repenser, en particulier pour les personnes atteintes de diabète et en cours de traitement. Les implications pour l'interprétation des résultats de ces deux tests dans la recherche sur le diabète et la gestion clinique sont discutées dans le contexte des limitations dans les pays à revenu faible et intermédiaire.

Mots - clés : *âge ; viscosité du sang ; traitement du diabète ; hémoglobine glyquée ; associations*

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Introduction

There are indications of positive associations between age and some biomarkers of diabetes management including glycaemia (plasma glucose level, glycated haemoglobin (HbA1c), haemorrheological indices (white blood cells, blood viscosity), serum lipid profile and renal function [1, 2]. There is a suggestion of correlation between glycaemia and haemorrheological indices since some treatment regimens in diabetic patients have favourable effects on HbA1c, blood glucose and blood viscosity [3]. The suggestion also corroborates with the speculation that whole blood viscosity (WBV) increases with age [4, 5] and possibly by a mechanism that does not involve the effects of haematocrit and plasma viscosity [5]. Suffice to say that there is abundance of literature supporting the notion that aging impacts on HbA1c [3-6], as well as WBV [7-9]. However, the extent that this notion impacts on interpretation and utilization of HbA1c and WBV test results in diabetes research and clinical management requires elucidation. It must be pointed out that most of the studies reporting on association of aging with HbA1c are on non-diabetes individuals; but how this association may be confounded in diabetic patients is not sufficiently elucidated.

Studies also report that GFR decreases with advancing age and that old age is associated with chronic kidney disease (CKD) [10], which results in proteinuria [11], that leads to hypoproteinaemia [12, 13]. Albuminuria leads to hypoalbuminemia, increases synthesis of apolipoproteins and reduces plasma oncotic pressure, which increases renal sodium reabsorption [14]. The change in serum protein level is affected by renal excretion [13], and besides GFR as well as by factors such as sex, age, race, weight and smoking status [15]. It is reported that albuminuria may be caused by high WBV in non-CKD essential hypertensive patients in view of the fact that increased blood viscosity is associated with decreased renal function [16].

It is important to consider the physiological feedback effect of albuminuria that leads to hypoproteinaemia, which then reduces the WBV (Fig 1) – i.e. that blood viscosity can be influenced by the effect of proteinuria [17]. There is knowledge that:

- Reduced glomerular filtration may proportionally increase blood viscosity [18, 19]
- High blood viscosity induces proteinuria [20-23].
- Proteinuria leads to hypoproteinaemia [24] which feedforwards to low viscosity of blood.

Indeed, blood viscosity may be a target for management in renal complication of diabetes [22], and a useful biomarker in assessment of cardiovascular risk [23]. The implication is the potential for WBV to increase with age, which is complicated or confounded by the impact of renal function on plasma protein as per the negative feedback cycle (Fig 1). Therefore, the question is *what are the odds that WBV increases with age?* Factors such as age and red blood cell (RBC) indices may be responsible for high WBV [16, 25, 26], and indeed, there is opinion that haematocrit (HCT) is an important factor for WBV [16]. HbA1c values and interpretation are affected by haemoglobin

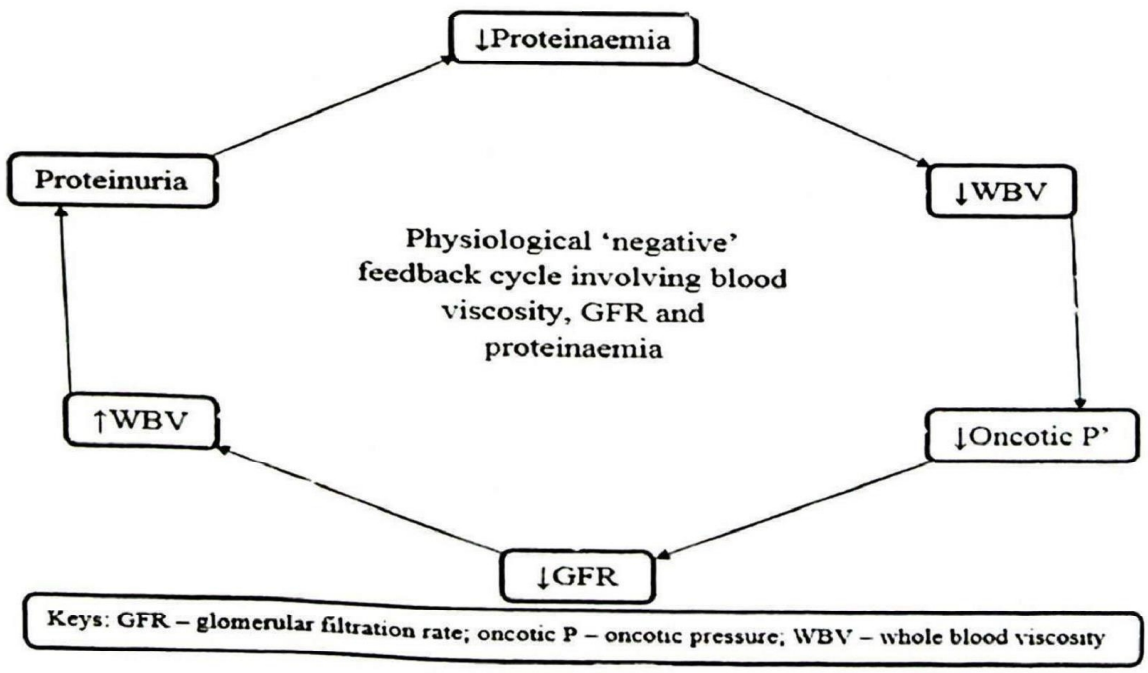

Fig 1. Negative feedback cycle of WBV-GFR-proteinaemia

variants or adducts, as well as conditions that alter the erythrocyte life-span and these include anaemia and renal failure. More so, it is reported that HbA1c goes up by about 0.1% with every 10 years of age [27], and there may be gender differences [28], as well as the impact from platelet functions [29]. Anaemia is a concern in old age with prevalence increasing from 20% to a 63% or more [30]. The idea of aging being associated with both anaemia and HbA1c foretells the question: *Is anaemia (i.e. low haematocrit) inversely or positively related to HbA1c?* This connotes a further conundrum in regards to the association of age and WBV in the sense that:

- It is not clear if anaemia (i.e. low haematocrit) is inversely [31]; or positively related to HbA1c [32]
- WBV is a physiological variable dependent on proteinaemia and haematocrit [25, 33].
- Age is associated with hypoproteinaemia and low haematocrit [12, 13, 30].
- Considering the above points, aging is supposed to be associated with low WBV

In the series on WBV assessment from serum proteins levels and haematocrit [33], WBV and HbA1c were analysed and WBV was reported to be lower in good glycaemic control relative to poor control [34]. However, HbA1c is known or speculated to increase with age, especially in non-diabetic individuals [6-9], and this may be confounded in diabetes patients who are responsive to treatment, given that glycaemic control indicated by lower HbA1c is the target. It has been reported that association of age with higher HbA1c is unexplained by the features of aging and may reduce diagnostic speciûcity of HbA1c [35]. Thus an addition to the first question expounds to: '*what are the odds that HbA1c and WBV increase with age among diabetes patients?*'

Study aims and hypothesis

Aims: The overreaching objective of study was to evaluate archived clinical data in terms of the three questions posited in the introduction. Specific aims of this study were to determine HbA1c and WBV in diabetes patients in terms of: (1) how the biomarkers compare between stratified aged-groups, (2) the separate correlations of HbA1c and WBV with age; and (3) the odds ratio and probabilities of the variables to increase with age.

Hypotheses

These objectives are based on consideration that given that anaemia is a concern in old age [30], haematocrit is positively associated with WBV [16, 25, 33], and may be inversely related to HbA1c [31]; it is not clear how HbA1c and WBV can both positively correlate with age. It is hypothesized that correlations between age and HbA1c as well as age and WBV may not be consistent or significant in all stratified age groups. This hypothesis is based on the notion that diabetes patients' management has favourable effects on HbA1c and blood viscosity [3]. A secondary hypothesis is that HbA1c is reduced with advancing age of diabetes patients.

Materials and methods

Design and setting

De-identified archived clinical pathology data for the period of 2014 – 2016 were obtained from community-based General Practice (The Wellness House) in Orange, NSW for retrospective review.

Ethics and data

This study was approved by the University's Human Research Ethics Committee (protocol number 2014/158). The most recent of each data case (N = 245) collected were laboratory results of diabetes patients including HbA1c and routine haematology; as well as liver and renal function tests. All data were de-identified and re-coded before delivery to the researcher. WBV values were derived by extrapolation from haematocrit and serum total protein levels based on validated formula [25, 33]. Among the 'N = 245', 17 were excluded from analysis – including 16 with missing eGFR results and one that could not be differentiated by the code.

Statistical analyses:

For the first research objective, age stratification was done on all the included data (n = 228), which was ranked by age and grouped into 1st – 4th quartiles. Descriptive statistics (mean, median and standard deviation values) and MANOVA assessed significant differences between age groups.

On the second objective and main hypothesis that *correlations between age and HbA1c as well as age and WBV may not be consistent or significant in all stratified age groups, Pearson's* correlations of age with HbA1c and WBV were performed three times.

- First, with a presumption to avoid confounding impacts of kidney failure and poor glycaemic control; only the patients with eGFR e" 90 mL/min/1.73m^2 and HbA1c d"6.5% were selected (N = 26) – there were 66 cases with HbA1c d"6.5% out of which 26 had eGFR e" 90 mL/min/1.73m^2.
- Second and third correlations analyses were performed in 1st and 4th quartile age groups,

respectively; to determine the hypothesized inconsistency i.e. possible differences. Interpretation of correlations was based on statistical guidelines [36].

On the secondary hypothesis, two groups of HbA1c levels were generated using 6.5% value as initial dichotomizing cut-off point. The 'low-HbA1c' group comprised all 66 cases with HbA1c < 6.5% values (representing good diabetes control). The 'highest HbA1c group comprised the 66 cases at the other end of the sorted data (representing poor diabetes control). Age and WBV levels were compared between the groups.

For the third objective, the odds of HbA1c and WBV increasing with age were assessed using 'R statistical package version 3.3.2. In this analysis, both HbA1c and WBV were categorical while age remained a continuous variable. Further, the model generated was used to convert to a probability ($P = 1/(1+e^{-Logit\,P})$) – that is, whether HbA1c and/or WBV increase, given a particular age.

Results

Descriptive statistics and multivariate comparison between age groups show directional, but statistically non-significant decreases in HbA1c and WBV from youngest age-group to the oldest (Table 1).

The results show low correlation between biomarkers and age but in varying circumstances. Age appears to show negative correlation with HbA1c, only if glycaemic control and renal functions are normal. The eGFR levels show a weak negative association and WBV weakly positively associated with age; but both only in the oldest age-group (Table 2).

When age and WBV are separately compared between groups of good diabetes controls (n = 66) versus those with highest HbA1c levels; statistical significant differences are observed in age, but not in WBV (Table 3). In particular, the mean age of the group with good glycaemic control is significantly higher ($p < 0.04$). Therefore, there is contrasting observations that may underlie confounding pathophysiology worthy of note.

The analyses of odds ratio show that the chance of HbA1c changing with age is 12.99 (RR = 1.02; $p < 0.04$) while that of WBV is 1.01 (RR = 0.99; $p > 0.12$). Going step further in the analysis, the probabilities were derived based on model from the summary using the following formulae:

HbA1c: the model from the summary is 'Logit P = 0.019133 x Age - 0.555026'

- To convert to a probability: $P = 1/(1+e^{-Logit\,P})$, or $P = 1/(1+e^{-(0.01933 \times Age - 0.555026)})$

WBV: The model from the summary is: Logit P = -0.013405 x Age + 0.785269

- To convert to a probability: $P = 1/(1+e^{-Logit\,P})$, or $P = 1/(1+e^{-(-0.013405 \times Age + 0.785269)})$

The derived values were plotted against unit increase in age visualize probability trends over time, using line plot in Microsoft Excel – with age on the X-axis while HbA1c and WBV probabilities are on the Y-axis. The results show that HbA1c probably

Table 1: Descriptive statistics of variables among age groups (N = 228)

		Agee (years)	GFR	HbA1c (%)	HCT (%)	Sr. Protein (g/L)	WBV (208/sec)
1st Quartile	Mean	42.18	88.21	8.28	0.45	71.33	11.83
	Median	45	90	7.6	0.44	71	11.77
	SD	6.86	7.68	2.23	0.03	4.24	0.72
2nd Quartile	Mean	53.26	85.47	8.03	0.44	71.3	11.82
	Median	53	90	7.6	0.44	71	11.77
	SD	3.02	8.85	4.99	0.03	3.97	0.68
3rd Quartile	Mean	63.53	76.11	7.96	0.44	70.88	11.75
	Median	63	80	7.6	0.44	70	11.61
	SD	3.28	16.72	1.91	0.04	4.59	0.78
4th Quartile	Mean	75.82	65.16	7.65	0.42	70.91	11.75
	Median	76	63	7.4	0.43	71	11.77
	SD	6.64	17.89	1.59	0.05	4.07	0.69
P values (MANOVA)		NA	0.00001*	NS	0.001**	NS	NS

*Except between 1st vs. 2nd Quartile groups; **between 1st vs. 4th Quartile groups onlyNA: not applicable, NS: not significant at $p < 0.05$

Table 2: Correlation of age and HbA1c variation with WBV groups

A: Normal eGFR and normal HbA1c (N = 26)	WBV	Sr. protein	HCT	Age	eGFR	HbA1c
WBV (208/Sec)	1.00					
Serum protein (g/L)	1.00	1.00				
HCT (%)	0.22	0.21	1.00			
Age (years)	-0.04	-0.04	0.31	1.00		
eGFR (mL/min/1.73m^2)	0.30	0.30	0.26	0.13	1.00	
HbA1c (%)	0.39	0.39	-0.10	-0.26	-0.11	1.00
B: 1st Quartile age group (N = 57)						
	WBV	Sr. protein	HCT	Age	eGFR	HbA1c
WBV (208/Sec)	1.00					
Serum protein (g/L)	1.00	1.00				
HCT (%)	0.25	0.24	1.00			
Age (years)	-0.13	-0.13	-0.21	1.00		
eGFR (mL/min/1.73m^2)	0.29	0.29	0.23	0.06	1.00	
HbA1c (%)	-0.17	-0.17	0.16	-0.17	0.02	1.00
C: 4th Quartile age group (N = 57)						
	WBV	Sr. protein	HCT	Age	eGFR	HbA1c
WBV (208/Sec)	1.00					
Serum protein (g/L)	1.00	1.00				
HCT (%)	0.19	0.18	1.00			
Age (years)	0.22	0.22	-0.19	1.00		
eGFR (mL/min/1.73m^2)	0.14	0.13	0.53	-0.25	1.00	
HbA1c (%)	0.15	0.15	0.02	-0.15	0.16	1.00

Table 3: Comparison of age and WBV between groups of HbA1c

	HbA1c groups	Mean	Std. Dev'	N
Age (years)	Good glycaemic control	61.42	15.65	66
	Poor glycaemic control	55.3	17.09	66
WBV (208 Sec-1)	Good glycaemic control	11.83	0.71	66
	Poor glycaemic control	11.80	0.87	66

increases by 0.01 (or 1%) in every two to three years, whereas WBV may decrease by the same margin in every three years (Fig 2).

Discussion

The first and preliminary aim of this study was to determine the association of age with HbA1c as well

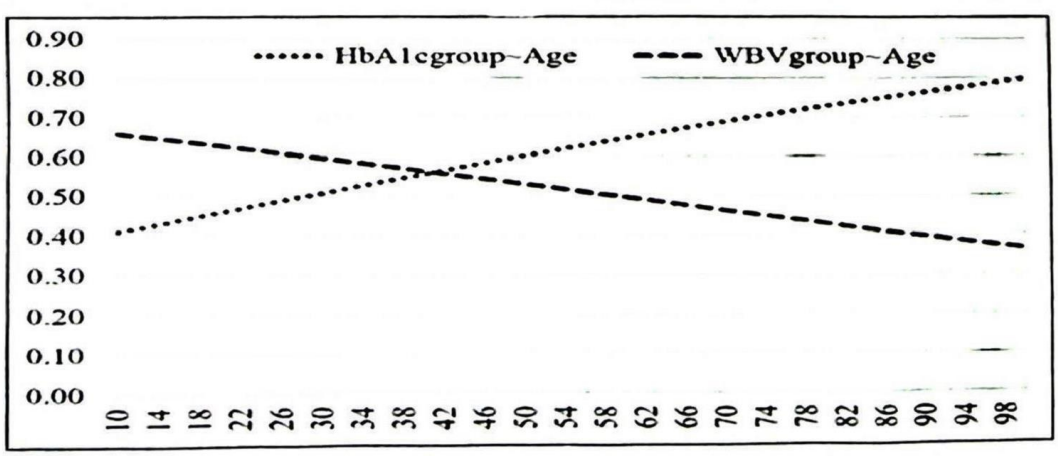

Fig 2. Probability* of HbA1c increasing and WBV decreasing with age*X-axis while HbA1c and WBV probabilities are on the Y-axis

as age and WBV in diabetes patients. Results of the analysis show that neither HbA1c nor blood viscosity achieved statistical significance changes with age, although there was directional decrease in both parameters from youngest aged-group to the oldest (Table 1). Here we report observation of association that implies negative correlation of age with both HbA1c and WBC. This is in contrast to abundance of literature supporting the notion that aging impacts on HbA1c [3-6], as well as WBV [7-9]. However, it is noteworthy that the cohort of this particular study is strictly individuals living with diabetes.

The second, but main research objective was the correlation analysis. The age of patient appears to show negligible to weak negative correlation with HbA1c only among those with good glycaemic control (r 0.15 – 0.26); and a weak positive association with WBV only in the oldest age-group (r = 0.22). This supports our first hypothesis that correlation between age and HbA1c as well as age and WBV may not be consistent or significant in all stratified age groups. HbA1c and WBV are moderately and positively correlated in patients with good glycaemic control and normal renal function (r = 0.39; Table 2). This in part supports the notions in the literature. However, age ($p < 0.04$), but not WBV was statistically significantly different between 'good versus poor' glycaemic control subgroups (Table 3).

Again, similar to the results reported in Table 1, this observation does not appear to support the idea that HbA1c and WBV positively associate with aging [3-9]. Repeat evaluation of age and WBV separately compared between quartile groups of WBV showed no statistical difference, although there was a more linear inverse correlation between age and WBV. Thus, the results further uphold the first hypothesis of this study that HbA1c and blood viscosity may not consistently increase with aging, especially among diabetes patients. However, WBV being correlated with HbA1c in good glycaemic control corroborated our previous report "that WBV is statistically significantly lower ($p < 0.05$) in the group with excellent glycaemic control compared to the group with poor glycaemic control" [34].

The subgroup with good glycaemic control appeared to comprise older people with mean age H" 61 years, compared to the poor control group of mean age H" 55 years (Table 3). This corroborates observations in Table 1 that show directional decrease (8.28 > 8.03 >> 7.96 >>> 7.65) in HbA1c inferring that lower HbA1c can be associated with aging among individuals living with diabetes. A plausible interpretation of the observation is that the patients were obviously younger at the time of diagnosis and when glycaemic control treatment (i.e. HbA1c reduction) commenced. Therefore, age at post-treatment check-up is higher while HbA1c level became lower – where glycaemic control was achieved in the diabetes patients. Further, the contrasting observation reported in regards to Table 3 indicates that good glycaemic control can occur in any age group. In the cohort evaluated, an older people showed lower HbA1c level possibly indicative of adherence to clinical management, relative to a non-adherent younger individual ($P < 0.04$). This indicates that medical treatment may cause negative correlation, which contrasts with expectation of HbA1c increasing with age. This is in agreement with proposition that diabetes patients' management has favourable effects on HbA1c and blood viscosity [3].

The third research objective was about odds ratio; and results show that there is the probability of HbA1c to increase with age ($p < 0.04$), while that of WBV may be insignificant ($p > 0.12$). This observation agrees with the general notion (6-9). It is inferred that the probability of HbA1c to increase with age is significant, and indeed, a critical review of the correlations show that HbA1c is negatively correlated only among the subgroup with good glycaemic control and WBV is only positive in the oldest aged group. In the further analysis where Logit models are converted to probabilities of WBV changing with age, the results show that HbA1c probably increases by 0.01 (or 1%) in every 2 to 3 years, whereas WBV may decrease by the same margin in every 3 years (Fig 2). The observation of the probability that HbA1c increases with age is closely in line, but slightly differs, with the existing reports that indicate about 0.1% increase per 10 years [27, 35].

However, it must be recognized that whereas our observation of 1% in 2 – 3 years is based on diabetic patients undergoing treatment, the reports indicating 0.1% were note. Indeed, one of the reports is exclusively based on non-diabetes population. Thus the contribution and implication of this observation is affirmation and elucidation of another existing report [35], that the HbA1c diagnostic speciûcity is confounded by age and medical treatment. That is, on the basis of incremental increase

- Age will confound diagnosis of diabetes with HbA1c, if the same reference is used e.g. for young and old adults
- The incremental increase in HbA1c may also confound response to treatment regimen. Or vice versa, medical treatment is a confounding factor to

the incremental increase if the diabetes is very well under control

The decreasing of WBV with increase in age is evident from descriptive statistics shown in Table 1 and appears to disagree with the notion that blood viscosity increases with age. However, a critical review of the data shows that there is significant decline in haematocrit with increasing age (Table 1). Given the importance of haematocrit in WBV [16, 25, 26], and the knowledge that the effect of age is independent of serum protein level contribution to viscosity [5], it is inferred that the tendency for haematocrit to decrease with age is a confounder in this observation. The significance is that WBV may increase with age [4, 5], and the presence of anaemia is a confounding factor that age-induced anaemia can cause the opposite effect of anaemia-induced hypoviscosity. The addition of this observation to knowledge regarding the speculated WBV increase with age is:

- Firstly, affirmation that it may not be based on haematocrit [5], which tends to decrease with aging, but
- Secondly, age-related ill-health possibly associated with hyperproteinaemia that needs to be further determined.

Implications for diabetes research and management in low-mid income countries

Two themes that are of significance in this report are research and management. This paper discusses two laboratory tests that are integral in diabetes pathophysiology, research and clinical practice.

- HbA1c is well appreciated and pushed for expounded clinical utility, but currently not accessible at most pathology facilities in low-mid income communities and will incur major costs to adopt.
- WBV is useful for therapeutic decisions, but yet to be accepted or acknowledged by clinicians. Yet, it is easily adoptable (i.e. can be extrapolated) at no extra operational cost from haematocrit and serum protein results.

Diabetes research:

Over the years, diabetes research has brought the idea that HbA1c should be delimited to glycaemic control monitoring of patients who are already diagnosed. At least, guidelines recommended blood glucose for diagnosis of diabetes as at 2010. The push to use HbA1c for diabetes screening and diagnosis of new cases, i.e. in addition to the traditional usage in monitoring of patients' management, has garnered momentum and integrated into recommendations within the last decade [37, 38]. However, the need for variations in reference values is still being discussed [38, 39]. This paper contributes to the discourse by reporting the probability of HbA1c increasing with age by 1% in 2-years, which confounds HbA1c diagnostic speciûcity and sensitivity.

Until date, haemorrheology research, specifically WBV testing, has yet to be fully translated from research to bedside. Previous reports have attempt to advance the specificity of the test parameter [40], and "that WBV is a valid clinical laboratory parameter for evidence-based contraindication, indication and monitoring of antiplatelet medication" [41]. Yet, discussion about antiplatelet is without recourse to WBV test for evaluation of stasis [42]; either for initial determination of bleeding risk, or post-intervention to assess antiplatelet efficacy towards reduction in level of stasis. Given the continued interest to elucidate the relative significance of haemorrheological alterations in the pathophysiology of cardiovascular diseases [43], and the basis of ineffectiveness of aspirin [44], our observation that WBV may decrease by 1% with every 3 years constitutes addition to knowledge. This new knowledge calls for validation to support adoption in clinical practice, especially with regards to antiplatelet monitoring.

Diabetes management

Considering that the basis of overlooking the odds ratio of HbA1c has been because 0.1% increase in 10-years "is unlikely to necessitate a change in treatment goals" [27], our observation of 1% in 2 years calls for a re-evaluation of recommended laboratory result interpretation in diabetes monitoring. More so, due to the fact that diabetes perhaps manages to reduce HbA1c level relative to when diagnosis was made is confounding factor to consider. The same applies for screening and diagnosis in terms of reference values. The need to interpret HbA1c results with references to both stratified age-groups and any ongoing medical treatment is warranted.

Diabetes care has involved prophylactic management of patients with antiplatelet, which is meant to maintain blood stasis *vis-à-vis* WBV [41, 45]. Guidelines for the clinical prescription of antiplatelet recommends albeit with limited evidence that risk of bleeding is a contraindication [46]. Yet, current platelet function tests assess only antiplatelet effectiveness or failures, but not stasis *per se*. Blood viscosity (whether plasma or WBV) test that assesses stasis level is neither fully appreciated for this

purpose, nor accessible outside very few referral laboratories. What this report advances is the knowledge that WBV test can be performed at no extra cost or need of resources in a clinical laboratory facility that already has capacity to perform haematocrit and total serum protein.

Conclusion

This study investigated the correlation between age and HbA1c as well as age and blood viscosity. It is observed that both HbA1c and WBV tend to decrease with increasing age among individuals living with diabetes. For HbA1c, this was inferred to be due to post-intervention glycaemic control test – i.e. that age has increased while HbA1c is lower at the time of post-intervention test. For WBV, the negative correlation with age is attributable to anaemia. In the subgroup who indicated good glycaemic control, the biomarkers show positive correlations with age, but only in the oldest aged-group. Thus proving the main hypothesis that *correlations between age and HbA1c as well as age and WBV may not be consistent or significant in all stratified age groups*. This report provides elucidation of a complex pathophysiology in diabetes that need consideration when interpreting pathology results of patients.

Acknowledgements

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References

1. Lippi G, Sanchis-Gomar F and Montagnana M. Biological markers in older people at risk of mobility limitations. Curr Pharm Des 2014, 20(19):3222-3244.
2. Marini MA, Fiorentino TV, Andreozzi F, *et al.* Hemorheological alterations in adults with prediabetes identified by hemoglobin A1c levels. Nutr Metab Cardiovasc Dis 2017, 27(7):601-608.
3. Lee MS, Chen KW, Choi TY and Ernst E. Qigong for type 2 diabetes care: a systematic review. Complement Ther Med 2009, 17(4):236-342.
4. Simmonds MJ, Meiselman HJ, Baskurt OK. Blood rheology and aging. J Geriatr Cardiol 2013, 10(3):291-301.
5. Carallo C, Irace C, De Franceschi MS, *et al.* The effect of aging on blood and plasma viscosity. An 11.6 years follow-up study. Clin Hemorheol Microcirc 2011, 47(1):67-74.
6. Wu L, Lin H, Gao J, *et al.* Effect of age on the diagnostic efficiency of HbA1c for diabetes in a Chinese middle-aged and elderly population: The Shanghai Changfeng Study. PLoS One 2017, 12(9):e0184607.
7. Ma Q, Liu H, Xiang G, Shan W and Xing W. Association between glycated hemoglobin A1c levels with age and gender in Chinese adults with no prior diagnosis of diabetes mellitus. Biomedical reports 2016, 4(6):737-740.
8. Pani LN, Korenda L, Meigs JB, et al. Effect of aging on A1C levels in individuals without diabetes: evidence from the Framingham Offspring Study and the National Health and Nutrition Examination Survey 2001-2004. Diabetes Care 2008, 31(10):1991-1996.
9. Roth J, Muller N, Lehmann T, *et al.* HbA1c and age in non-diabetic subjects: An ignored association? Exp Clin Endocrinol Diabetes 2016, 124(10):637-642.
10. Delanaye P, Glassock RJ, Pottel H and Rule AD. An age-calibrated definition of chronic kidney disease: Rationale and benefits. Clin Biochem Rev 2016, 37(1):17-26.
11. Obi Y, Kimura T, Nagasawa Y, *et al.* Impact of age and overt proteinuria on outcomes of stage 3 to 5 chronic kidney disease in a referred cohort. Clin J Am Soc Nephrol 2010, 5(9):1558-1565.
12. Kodner C. Nephrotic syndrome in adults: diagnosis and management. Am Fam Physician 2009, 80(10):1129-1134.
13. Gitlin D, Janeway CA and Farr LE. Studies on the metabolism of plasma proteins in the nephrotic syndrome. I. Albumin, gamma-globulin and iron-binding globulin. J Clin Invest 1956, 35(1):44-56.
14. Stoycheff N, Stevens LA, Schmid C, *et al.* Nephrotic syndrome in diabetic kidney disease: an evaluation and update of the definition. Am J Kidney Dis 2009, 54(5):840-849.
15. Liu X, Foster MC, Tighiouart H, *et al.* Non-GFR determinants of low-molecular-weight serum protein filtration markers in CKD. Am J Kidney Dis 2016, 68(6):892-900.
16. Sugimori H, Tomoda F, Koike T, *et al.* Increased blood viscosity is associated with reduced renal function and elevated urinary albumin excretion in essential hypertensives without chronic kidney disease. Hypertens Res 2013, 36(3):247-251.

17. Gordge MP, Patel A, Faint RW, Rylance PB and Neild GH. Blood hyperviscosity and its relationship to progressive renal failure in patients with diabetic nephropathy. Diabet Med 1990, 7(10):880-886.
18. Reubi FC. Glomerular filtration rate, renal blood flow and blood viscosity during and after diabetic coma. Circ Res 1953, 1(5):410-413.
19. Goldschmidt H, Lannert H, Bommer J and Ho AD. Multiple myeloma and renal failure. Nephrol Dial Transplant 2000, 15(3):301-304.
20. Simpson LO. Blood viscosity induced proteinuria. Nephron 1984, 36(4):280-281.
21. Simpson LO. A hypothesis proposing increased blood viscosity as a cause of proteinuria and increased vascular permeability. Nephron 1982, 31(1):89-93.
22. Simpson LO, Shand BI, Olds RJ. A reappraisal of the influence of blood rheology on glomerular filtration and its role in the pathogenesis of diabetic nephropathy. J Diabet Complications 1987, 1(4):137-144.
23. Ercan M, Konukoglu D and Onen S. Plasma viscosity as a cardiovascular risk marker in patients with proteinuria. Clin Hemorheol Microcirc 2003, 29(2):111-116.
24. Takashima T, Onozawa K, Rikitake S, *et al.* Two cases of minor glomerular abnormalities with proteinuria disproportionate to the degree of hypoproteinemia. CEN Case Rep 2014, 3(2):172-177.
25. Tamariz LJ, Young JH, Pankow JS, *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(10):1153-1160.
26. von Tempelhoff GF, Schelkunov O, Demirhan A, *et al.* Correlation between blood rheological properties and red blood cell indices(MCH, MCV, MCHC) in healthy women. Clin Hemorheol Microcirc 2016, 62(1):45-54.
27. Little RR, Rohlfing CL and Sacks DB. Status of hemoglobin A1c measurement and goals for improvement: from chaos to order for improving diabetes care. Clin Chem 2011, 57(2):205-214.
28. Deconinck B, Mathieu C and Benhalima K. Characteristics and cardiovascular complications of a large cohort of adults diagnosed with type 2 diabetes <45 years. Diabetol Metab Syndr 2017, 9:28. https://doi.org/10.1186/s13098-017-0227-z
29. Demirtunc R, Duman D, Basar M, *et al.* . The relationship between glycemic control and platelet activity in type 2 diabetes mellitus. J Diabetes Complications 2009, 23(2):89-94.
30. Mahlknecht U and Kaiser S. Age-related changes in peripheral blood counts in humans. Exp Ther Med 2010, 1(6):1019-1025.
31. Silva JF, Pimentel AL and Camargo JL. Effect of iron deficiency anaemia on HbA1c levels is dependent on the degree of anaemia. Clinical Biochemistry 2016, 49(1):117-120.
32. Abass AE, Musa IR, Rayis DA, Adam I and Gasim IG. Glycated hemoglobin and red blood cell indices in non-diabetic pregnant women. Clin Pract 2017, 7(4):999. https://doi.org/10.4081/cp.2017.999
33. Nwose EU. Whole blood viscosity assessment issues I: Extrapolation chart and reference values. North Am J Med Sci 2010, 2(4):165-159.
34. Nwose EU, Butkowski E and Cann NG. Whole blood viscosity determination in diabetes management: perspective in practice. North Am J Med Sci 2009, 1(3):110-113.
35. Dubowitz N, Xue W, Long Q, *et al.* Aging is associated with increased HbA1c levels, independently of glucose levels and insulin resistance, and also with decreased HbA1c diagnostic speciûcity. Diabet Med 2014, 31(8):927-935.
36. Mukaka MM. Statistics corner. A guide to appropriate use of correlation coefficient in medical research. Malawi Med J 2012, 24(3):69-71.
37. Farmer A. Use of HbA1c in the diagnosis of diabetes. BMJ 2012, 345:e7293 https://doi.org/7210.1136/bmj.e7293
38. Kowall B and Rathmann W. HbA1c for diagnosis of type 2 diabetes. Is there an optimal cut point to assess high risk of diabetes complications, and how well does the 6.5% cutoff perform? Diabetes Metab Syndr Obes 2013, 6:477-491.
39. Florkowski C. HbA1c as a Diagnostic Test for Diabetes Mellitus - Reviewing the Evidence. Clin Biochem Rev 2013, 34(2):75-83.
40. Nwose EU. Whole blood viscosity assessment issues IV: Prevalence in acute phase inflammation. North Am J Med Sci 2010, 2(8):353-358.
41. Nwose EU and Cann NG. Whole blood viscosity issues VI: Association with blood salicylate level and gastrointestinal bleeding. North Am J Med Sci 2010, 2(10):457-460.
42. Metharom P, Berndt MC, Baker RI and Andrews RK. Current state and novel approaches of antiplatelet therapy. Arterioscler Thromb Vasc Biol 2015, 35(6):1327-1338.
43. Raddino R, Ferrari R, Scarabelli TM, *et al.* Clinical implications of rheology in cardiovascular diseases. Recenti Prog Med 2002, 93(3):186-99.

44. Feher G, Koltai K, Kesmarky G and Toth K. Hemorheological background of acetylsalicylic acid resistance. Clin Hemorheol Microcirc 2008, 38(3):143-152.
45. von Tempelhoff GF, Tsikouras P, Rath W, Velten E and Csorba R. Rheological, hemostaseological changes during immunetherapy for prevention of HELLP-syndrome in a patient with elevated phospholipid antibodies. Clin Hemorheol Microcirc 2015, 60(1):123-131.
46. Bertrand ME. When and how to discontinue antiplatelet therapy. European Heart Journal Supplements 2008, 10(suppl A):A35-A41.