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RESEARCH ARTICLE

Metabolic and Cardiovascular Ageing Indices in Relation to Glycated Haemoglobin in Healthy and Diabetic Subjects

Shruthi Suvarna H.I[1], Shailaja Moodithaya[2,*] and Raghava Sharma[3]

[1]*Department of Physiology, A J Institute of Medical Sciences and Research Centre, Kuntikana, Mangalore, Karnataka – 575004, India;* [2]*Department of Physiology, K.S. Hegde Medical Academy, Nitte University, Mangalore, Karnataka, India;* [3]*Department of General Medicine, K.S. Hegde Medical Academy, Nitte University, Mangalore, Karnataka, India*

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Abstract: ***Background*:** Ageing is a natural phenomenon that has tremendous amount of control over normal physiological functions. Diabetes mellitus and ageing share common symptoms like stiffness and loss of functioning of tissues due to cross-liked proteins and free radicals. Glycated Haemoglobin (HbA1c) is often used as a stable cumulative index of glycemic control and has shown that even in non-diabetic adults, there is a steady increase in HbA1c levels with age.

Aim of the study is to evaluate the strength of association of HbA1c with metabolic and cardiovascular ageing indices in subjects between the age group of 40 to 60 yrs.

***Methods*:** A total of 220 subjects, with (n=110) and without (n=110) diabetes were assessed for the metabolic and cardiovascular ageing indices. BMI, waist hip ratio, fat percentage, Fasting blood sugar and HbA1c were assessed as metabolic ageing indices. The cardiovascular ageing indices measured were resting heart rate, blood pressure and Heart rate Variability.

***Results*:** Ageing indices were compared between subjects with and without diabetes using independent't' test and showed that the T2DM group exhibit significant accelerated ageing as compared to that of the controls. Pearson's and partial correlation coefficient was used to assess the association of HbA1c with the ageing indices without and with controlling for chronological age, indicated that, strength of association of levels of HBA1c with cardiovascular and other metabolic indices of ageing is statistically significant.

***Conclusion*:** The study concludes that the tightness of glycemic control has a significant impact on the biological ageing process.

Keywords: Ageing, type 2 diabetes mellitus, glycosylated haemoglobin, heart rate variability.

INTRODUCTION

Ageing is a complex process that negatively impacts the development and functioning ability of different organ systems due to natural senescence [1]. Lifespan is regulated by genes controlling the activity of metabolism, antioxidant system, DNA repair, cellular senescence and cell death [2]. Ageing results in the natural senescence of multiple organ systems including the kidney, the autonomic nervous system, and the heart [3]. Ageing is associated with physiological changes like atrophy of the heart muscle, loss of elasticity in artery walls, reduced baroreceptor sensitivity and SA node automaticity [4]. Reactive Oxygen Species (ROS) levels increase in damaged or aged mitochondria and their accumulation beyond physiological levels lead to physiological deterioration, genomic instability and accumulation of advanced glycation end products (AGE'S) [5]. These physiological changes associated with advancing age can increase the incidence of a number of pathologies like diabetes, heart diseases, cancer, arthritis, and kidney diseases. These diseases may accelerate the otherwise normal age-related processes, further reducing functional capacity and physiological reserve [6]. Assessing functional status *i.e.* the biological ageing is said to be more rational for categorizing a person's age than chronological age alone. Biological ageing can be determined by various markers called Ageing indices which are said to be the physical properties in a human body which indicates the phenomenon of growing old. Some of the indices that are most frequently used in research on the health of older population are, Cardiovascular indices like resting pulse rate, Systolic Blood Pressure (SBP), Diastolic Blood Pressure (DBP), Pulse Pressure (PP), Mean Blood Pressure (MBP), Heart Rate Variability (HRV), Metabolic indices namely Body Mass Index (BMI), Waist-Hip Ratio (WHR), Body fat %, lipid profile, fasting blood sugar, glycosylated haemoglobin (HbA1c) [7]. There

*Address correspondence to this author at the Department of Physiology, K.S.Hegde Medical Academy, Nitte University, Mangalore, Karnataka, India; Tel: 9845516116; Fax: 0824-2204162;
E-mail: shailaja.moodithaya@nitte.edu.in

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is a linear rise in SBP and PP in middle aged and elderly subjects due to large artery stiffness and an associated risk in wave reflection amplitude. Heart Rate Variability (HRV) changes have been related to modifications in the regulatory mechanisms of Autonomic Nervous System (ANS), suggesting an age-dependent decline in autonomic nervous system activity in elderly subjects [8]. The aging process involves physiological and nutritional changes that are manifested by height and weight loss, muscular mass loss and fat mass increase [9]. It also involves adipose tissue redistribution, with fat accumulation in the trunk and viscera.WHR is a useful index of chronic metabolic dysregulation and adipose tissue deposition [10].

Diabetes is considered to mimic an ageing process since it affects all of the organ systems. Diabetes and ageing share common symptoms like stiffness and loss of functioning of tissues due to cross-linked proteins and free radicals; damage to nerves, eyes, skin, kidney, immune system and other organs.

American Diabetes Association (ADA), the International Diabetes Federation (IDF) and European Association for the Study of Diabetes (EASD) have recommended the evaluation of Glycosylated Haemoglobin (HbA1c), with a cut-off point of $\geq$ 6.5% to diagnose diabetes [11]. HbA_{1c} is often used as a stable cumulative index of glycemia, reflecting the average level of blood glucose (up to 3 months) more reliable than commonly used fasting glucose [12]. Studies have shown that even in non-diabetic adults with normal fasting glucose, there is a steady increase in HbA1c levels with age. This indicates ageing to be a contributing factor for variation in glycemic control [13]. Persistent Hyperglycemia results in increased protein Glycation [14] which undergoes slow and complex rearrangements to form advanced Glycation end-products (AGEs) [15]. AGE accumulation leads to endothelial dysfunction resulting in vascular complications [16]. Elevated levels of HbA1c are regarded an independent risk factor for cardiovascular outcomes and mortality in individuals with or without diabetes [17]. Regardless of the level of hyperglycemia, improvement in glycemic control will lower the risk of CVD and mortality.

With the given background, this study hypothesised that T2DM patients would have accelerated ageing as compared to healthy controls and indices of ageing would be inversely related with the level of glycemic control. Therefore, the current study compared the Metabolic and Cardiovascular ageing indices of T2DM patients and normal controls and also evaluated the association of HbA1c with Metabolic and Cardiovascular ageing indices.

MATERIALS AND METHOD

Subjects

A total of 220 subjects were recruited for this study by convenience sampling method, out of which 110 were T2DM patients and 110 age matched non- diabetic controls who volunteered to participate in this research and were ready to give written consent. Both the control and study groups included 48 males and 62 female subjects. This study was conducted in Department of Physiology and Medicine of K.S. Hegde Medical Academy, Deralakatte, Mangalore, Karnataka, India.

Participants were selected on the inclusion criteria of age between 40-60 years and T2DM patients who were under Oral Hypoglycemic medication or insulin therapy for at least one year. Patients with diabetic complications, T1DM, infections like cold, fever, and allergy and those under anti-ageing therapy were excluded from the study.

Experimental Procedure

Detailed study protocol was explained to the subjects and informed consent was taken from both groups, and the institutional ethics committee approval was taken. History taking included medical history, family history and personal history. Participants were advised to observe 12 hours overnight fasting before the investigations.

For the assessment of metabolic indices, the current study evaluated both anthropometric and biochemical measurements.

All the subjects underwent following anthropometric assessment; height, weight, waist and hip circumference, skin fold thickness of biceps, triceps, and sub scapular and supra iliac sites using standard techniques [15]. The BMI, waist hip ratio, and fat percentage were calculated from these measures using standard equations [16].

For biochemical analysis, 5 ml of fasting blood sample was obtained under aseptic precautions in fluoride and EDTA vial from each study subject with the help of disposable syringe and needle. The sample was analysed for biochemical indices like Fasting Blood Sugar (FBS) by the glucose oxidase-peroxidase method and Glycosylated Haemoglobin levels (HbA1C) estimated by Nephelometric method, in Fasting lipid profile; Total cholesterol was estimated by cholesterol oxidase method, Triglycerides by GPO-POD method, HDL by HDL-C-direct and LDL by Friedwald Equation [17].

For the assessment of cardiovascular ageing, the indices included were resting heart rate, systolic, diastolic, mean blood pressure, pulse pressure and heart rate variability.

Heart rate variability is measured using a data acquisition unit; Power Lab, AD Instruments, Australia, is a computerized 4- channel acquisition system. To quantify the heart rate, ECG signal is recorded using lead II with subject supine, awake and resting for five minutes with the sampling frequency of 1000 Hz. A standard spectral analysis routine is applied to such modified recording and the following parameters evaluated on 5-min time interval: Total Power (TP), High Frequency (HF), Low Frequency (LF) and Very Low Frequency (VLF). The HF power spectrum is evaluated in the range from 0.15 to 0.4 Hz. This band reflects parasympathetic (vagal) tone and fluctuations caused by spontaneous respiration known as respiratory sinus arrhythmia. The LF power spectrum is evaluated in the range from 0.04 to 0.15 Hz. This band can reflect both sympathetic and parasympathetic tones. The VLF power spectrum is evaluated in the range from 0.0033 to 0.04 Hz. The LF/HF Ratio is used to indicate balance between sympathetic and parasympathetic tone [18].

STATISTICAL ANALYSIS

Statistical analysis was performed with Statistical software IBM SSPS Version 17.0. The study compared the means of metabolic and cardiovascular parameters by unpaired-t test. Association of glycemic control with metabolic and cardiovascular indices of ageing were ascertained by Pearson's correlation coefficient (r) and partial correlation was used for analysis after adjusting for confounding variables. P value < 0.05 was considered statistically significant and p value <0.001 was considered statistically highly significant.

OBSERVATIONS

Of the 240 subjects recruited for this study, 20 were excluded due to disturbance in ECG recording and inability of the software to analyse the HRV and therefore study included 220 subjects for statistical analysis.

Table **1** shows demographic profile and metabolic parameters in control group and T2DM patients. Analysis shows that WHR is significantly higher in T2DM patients of 40-60 years age group when compared to controls. Whereas body fat % as assessed by skin fold thickness is significantly lower in T2DM patients. FBS & HbA1c were highly significant (p-<0.001) in diabetics as expected. Lipid profile parameters like TG, LDL/HDL-C, TC / HDL-C, TG/HDL are significantly higher (p- <0.001) and HDL-C is significantly lower (p- <0.001) in T2DM group as compared to normal.

Table **2** shows highly significant differences in all the cardiovascular parameters of the two groups. T2DM patients are having significantly higher Blood Pressure and Resting Heart rate; further, the autonomic function test results shows significantly reduced heart rate variability in diabetics as compared to their age matched healthy volunteers.

Table **3** represents association of HbA1c with chronological age among healthy volunteers of our study group showing there is significant positive correlation .between HbA1c and chronological age as expected.

Pearson correlation results in Table **4** show very strong positive correlation between glycated haemoglobin and metabolic indices of ageing like waist hip ratio, serum lipid profile parameters like TG, LDL-C/HDL-C TC/HDL-C and TG/HDL. A very strong negative correlation was obtained between glycated haemoglobin and HDL-C.

Very strong positive correlation was observed between glycated haemoglobin and cardiovascular indices of ageing namely Blood Pressure and Resting Heart Rate. Further, the Parameters of Heart Rate Variability (HRV) namely Low frequency power in normalized units (marker of sympathetic activity) and the LF to HF ratio (indicator of sympathovagal balance) showed very strong positive association with glycemic level (Table **5**).

Other parameters of HRV namely High frequency power (marker of vagal activity) and Low frequency power in absolute units showed very strong negative correlation with glycated haemoglobin. Even the total power was negatively correlated but not statistically significant (Table **5**).

Table 1. Comparison of Metabolic indices of ageing in Control group and T2DM patients.

Variables	Control Group (n= 110)	T2DM (n = 110)	p- value
Age (Years)	50.01 + 8.08	51.12 + 6.16	0.22
BMI (kg/m^2)	24.12 + 3.83	24.25 + 3.91	0.99
WHR	0.80 + 0.08	0.86 + 0.05	< 0.001**
Fat %	31.15 + 7.65	28.98 + 7.51	0.03*
FBS (Mmol/L)	5.15 + 0.44	9.61 + 2.72	< 0.001**
HbA1C %	5.09 + 0.61	8.96 + 2.18	< 0.001**
TC (mg/dl)	202.20 + 3.83	202.03 + 4.22	0.97
TG (mg/dl)	129.10 + 4.82	165.99 + 6.93	< 0.001**
LDL-C (mg/dl)	127.92 + 3.62	125.21 + 3.71	0.60
HDL-C (mg/dl)	49.49 + 1.13	43.19 + 1.05	< 0.001*
LDL/HDL-C ratio	2.75 + 0.10	3.07 + 0.11	0.04*
TC / HDL-C ratio	4.26 + 0.12	4.86 + 0.13	< 0.001**
TG / HDL-C Ratio	2.86 + 1.60	4.06 + 2.09	< 0.001**

Data expressed as Mean and SD.

Comparison was done using Unpaired t test.

* Significant.

** Highly significant.

BMI-Body mass index, WHR-Waist to hip ratio, FBS-Fasting Blood Sugar, HbA1c-Glycosylated haemoglobin, TC-total cholesterol, TG- riglycerides, LDL-C-low density lipoprotein, HDL-C-high density lipoprotein.

Table 2. Comparison of Cardiovascular indices of ageing in Control group and T2DM patients.

Variables	Control Group (n= 110)	T2DM (n = 110)	P value
Systolic (mmHg)	120 + 10.37	134 + 11.84	< 0.001**
Diastolic (mmHg)	77 + 4.73	82 + 5.77	<0.001**
PP(mmHg)	43 + 8.54	51 + 9.88	<0.001**
MBP (mmHg)	91 + 5.88	100 + 6.87	<0.001**
HR	70 + 9.73	76 + 10.49	<0.001**
TP	1340 + 1466.55	588 + 628.95	<0.001**
HF (ab)	451 + 655.35	158 + 273.99	<0.001**
LF (ab)	293 + 329.26	130 + 175.89	<0.001**
HF (nu)	52 + 18.38	45 + 21.41	0.005**
LF (nu)	44 + 17.88	54 + 21.11	<0.001**
LF / HF	1.13 + 1.20	1.90 + 2.06	0.001**

Data expressed as Mean and SD.
Comparison was done using Unpaired t test.
Values of TP, HF (ab), LF (ab) were log transformed and statistically analyzed.
** Highly significant.
MBP-Mean Blood Pressure, HR-Heart Rate, TP-Total Power, LF (ab)-Low Frequency absolute, HF (ab)- High Frequency absolute, LF-Low Frequency normalized units, HF-High Frequency normalized units.

Table 3. Correlation of Glycated Haemoglobin with Chronological age in healthy controls. (n=110).

Variable	Glycated Haemoglobin	
	r	p
AGE (years)	0.400	< 0.001**

Association was done using Pearson correlation Test; "r "indicates strength of correlation; ** Correlation is significant at the 0.01 level (2-tailed).

Table 4. Correlation of Glycated Haemoglobin with Metabolic indices of ageing in both diabetics and normal individuals.

MetabolicIndices	Glycated Haemoglobin	
	r	p
BMI	0.053	0.43
WHR	0.415	< 0.001**
Body Fat %	-0.148	0.02*
Fat Mass	0.158	0.01*
FBS(mmol/L)	0.891	< 0.001**
TC (mg/dl)	0.091	0.177
TG (mg/dl)	0.297	< 0.001**
LDL- C(mg/dl)	0.074	0.271
HDL- C(mg/dl)	-0.288	< 0.001**
LDL-C/HDL-C	0.224	<0.001**
TC/ HDL-C	0.290	< 0.001**
TG/HDL	0.344	< 0.001**

Association was done using Pearson correlation Test.
"r "indicates strength of correlation.
** Correlation is significant at the 0.01 level (2-tailed).
* Correlation is significant at the 0.05 level (2-tailed).
BMI – Body mass index, WHR – Waist to hip ratio, FBS – Fasting Blood Sugar , HbA1c- Glycosylated haemoglobin, TC- total cholesterol, TG- Triglycerides, LDL- C – low density lipoprotein, HDL-C –high density lipoprotein.

Table 5. Correlation of Glycated Haemoglobin with Cardiovascular indices of ageing in both diabetics and normal individuals.

Cardiovascular Indices	Glycated Haemoglobin	
	r	p
Systolic BP (mmHg)	0.368	< 0.001**
Diastolic BP (mmHg)	0.442	< 0.001**
PP (mmHg)	0.223	< 0.001**
MBP	0.459	< 0.000**
RHR (bts/min)	0.377	< 0.001**
TP(ms^2)	- 0.068	0.316
VLF(ms^2)	- 0.232	0.001**
LF(ab) (ms^2)	- 0.380	< 0.001**
HF(ab) (ms^2)	- 0.439	< 0.001**
LF(nu) (ms^2)	0.279	< 0.001**
HF(nu) (ms^2)	- 0.240	< 0.001**
LF/HF	0.242	< 0.001**

Association was done using Pearson correlation Test
"r "indicates strength of correlation.
** Correlation is significant at the 0.01 level (2-tailed).
* Correlation is significant at the 0.05 level (2-tailed).
MBP – Mean Blood Pressure, HR – Heart Rate, TP – Total Power, LF (ab) – Low Frequency absolute, HF (ab) – High Frequency absolute, LF - Low Frequency normalized units, HF – High Frequency normalized units.

Table 6. Correlation of Glycated Haemoglobin with Metabolic indices of ageing in both diabetics and normal individuals after adjusting for chronological age.

Metabolic Indices	Glycated Haemoglobin	
	r	p
BMI	0.035	0.60
WHR	0.412	< 0.001**
Body Fat %	-0.177	0.009*
Fat Mass	0.160	0.01*
FBS(mmol/L)	0.890	< 0.001**
TC (mg/dl)	0.078	0.25
TG (mg/dl)	0.290	< 0.001**
LDL- C(mg/dl)	0.061	0.36
HDL- C(mg/dl)	-0.284	< 0.001**
LDL-C/HDL-C	0.214	<0.001**
TC/ HDL-C	0.283	< 0.001**
TG/HDL	0.352	< 0.001**

Association was done using partial correlation Test
"r "indicates strength of correlation.
** Correlation is significant at the 0.01 level (2-tailed).
* Correlation is significant at the 0.05 level (2-tailed).
BMI – Body mass index ,WHR – Waist to hip ratio, FBS – Fasting Blood Sugar , HbA1c- Glycosylated haemoglobin, TC- total cholesterol, TG- Triglycerides, LDL- C – low density lipoprotein, HDL-C –high density lipoprotein.

Table **6** and **7** show the association of HBA1c with metabolic and cardiovascular ageing indices using partial correlation test, after adjusting for chorological age as confounding variable. This analysis shows that significant correlation still persists even after adjusting for chronological age.

Table 7. Correlation of Glycated Haemoglobin with Cardiovascular indices of ageing in both diabetics and normal individuals adjusted for chronological age.

Cardiovascular Indices	Glycated Haemoglobin	
	r	p
Systolic BP (mmHg)	0.363	< 0.001**
Diastolic BP (mmHg)	0.437	< 0.001**
PP (mmHg)	0.212	< 0.002**
MBP	0.457	< 0.000**
RHR (bts/min)	0.382	< 0.001**
TP(ms^2)	- 0.272	< 0.001**
VLF(ms^2)	- 0.225	0.001**
LF(ab) (ms^2)	- 0.230	< 0.001**
HF(ab) (ms^2)	- 0.239	< 0.001**
LF(nu) (ms^2)	0.284	< 0.001**
HF(nu) (ms^2)	- 0.243	< 0.001**
LF/HF	0.244	< 0.001**

Association was done using partial correlation Test.
"r "indicates strength of correlation.
** Correlation is significant at the 0.01 level (2-tailed).
* Correlation is significant at the 0.05 level (2-tailed).
MBP – Mean Blood Pressure, HR – Heart Rate, TP – Total Power, LF (ab) – Low Frequency absolute, HF (ab) – High Frequency absolute, LF - Low Frequency normalized units, HF – High Frequency normalized units.

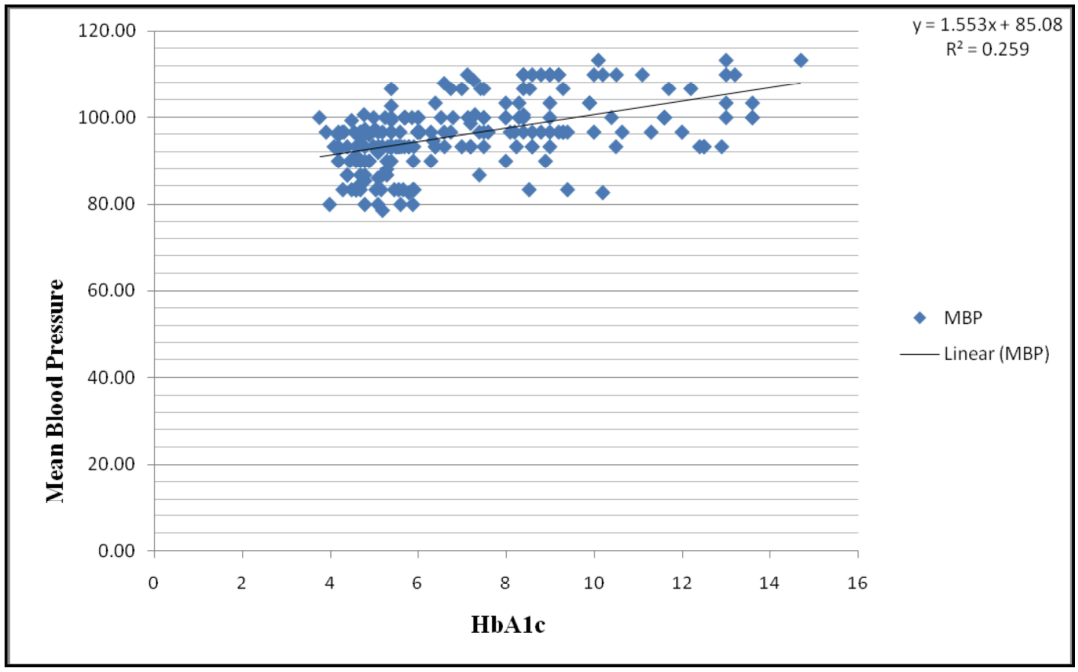

Fig. (1). The figure shows positive correlation between HbA1c and Mean blood Pressure. Statistically significant (r = 0.459**).

Significant linear positive correlation obtained between Mean blood Pressure and Glycated Haemoglobin is shown in Fig. (**1**) and Fig. (**2**) shows negative correlation of high frequency power in absolute units with Glycated Haemoglobin.

DISCUSSION

Chronological age is strongly associated with age-related dysfunction and it varies from person to person within a homogenous age sample. Glycated haemoglobin (HbA1c) is one of the markers to assess age-related physio-

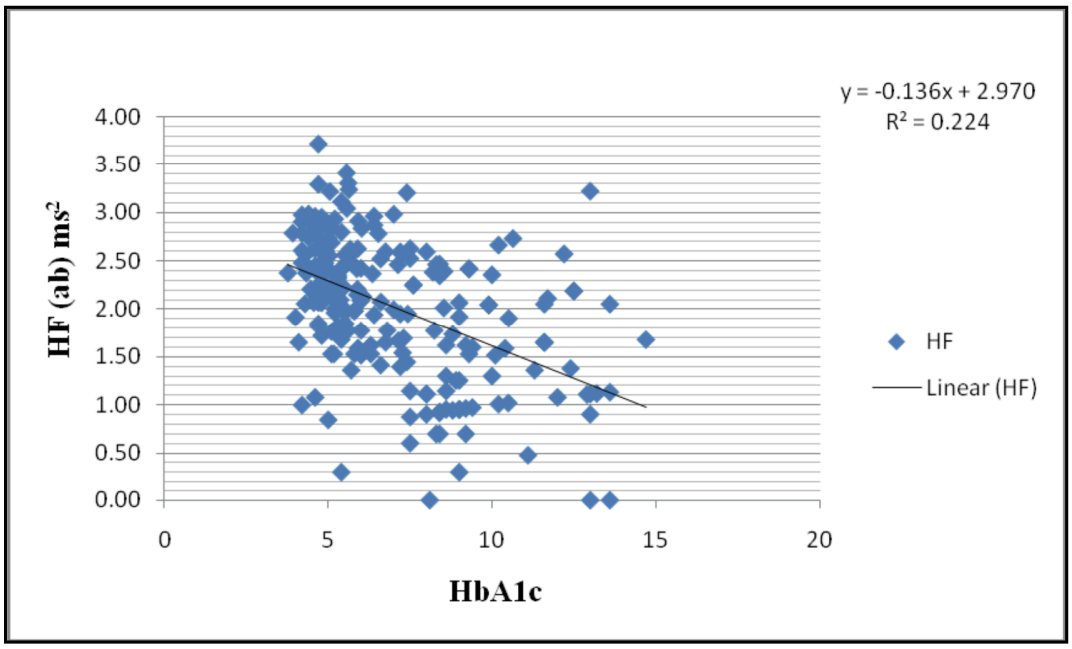

Fig. (2). The figure shows negative correlation between HbA1c and High Frequency power in absolute units. Statistically significant (r = -0.439**).

logical changes [7] and it is also established that endogenous AGEs are generated at higher levels in diabetes. Therefore we postulated that the ageing indices especially the surrogate of metabolic and cardiovascular functions would significantly associate with the level of Glycosylated haemoglobin. We evaluated the ageing indices in diabetic and non- diabetic groups and correlated the association of level of glycemic control with metabolic and cardiovascular ageing indices. The results of the present study showed a significant difference in all the variables of metabolic and cardiovascular ageing indices between T2DM and age matched normals indicating that there is significant acceleration of ageing in patients with T2DM compared to that of normals.

The metabolic indices of this study included anthropometric parameters like BMI, WHR and body fat percentage. The analysis showed a statistically significant increase in WHR of subjects in T2DM group than controls indicating greater proportion of abdominal fat in diabetics. WHR is 'height-independent' measure, which denotes pure central distribution of fat deposition. Studies have shown that increased abdominal fat accumulation is a risk factor for cardiovascular disease and stroke [22]. A study done by Motewar Sapana S *et al.* also showed that T2DM is strongly associated with central obesity [23]. Observations of previous studies suggest that there is a limit up to which subcutaneous fat depots can store energy and as a result, there is an overflow of excess energy into intra-abdominal viscera [24]. However, significantly reduced body fat % in T2DM patients indicates reduced subcutaneous adipose tissue.

The metabolic indices also involved biochemical parameters like Fasting Blood Sugar (FBS), Glycated Haemoglobin (HbA1C) and Fasting Lipid Profile. It was observed that Type 2 diabetics had significantly higher Fasting Blood Sugar and glycated Haemoglobin as expected, compared to that of controls. Significant differences obtained in the lipid parameters like Triglycerides, HDL-C, LDL to HDL ratio, Total cholesterol to HDL ratio, indicated dyslipidemia in T2DM population of our study. These findings were in agreement with findings of Watson *et al.* [25]. Insulin deficiency affects glucose utilization resulting in mobilization of free fatty acids from adipose tissue leading to raised triglycerides (TG) [26]. Hypertriglyceridemia creates TG-rich HDL particles which are hydrolyzed by hepatic lipase and rapidly cleared from circulation, leading to decrease in HDL-C levels in type 2 DM [27]. Hyperlipidemia as reflected by hyper triglycerides is another factor for mortality and morbidity from Coronary artery disease in diabetics. TG/HDL ratio which is considered as a surrogate marker of insulin resistance [28] is significantly higher in T2DM patients of our study. In diabetes, the associated hyperglycemia and insulin changes highly accelerate the progression of atherosclerosis [29].

In this study, the Total cholesterol and LDL-C levels are slightly higher in controls compared to diabetics but not statistically significant. This may be due to the fact that controls with dyslipidemia were not excluded from the study. Biomarkers related to aging in human populations listed by author Eileen Crimmins have proposed Resting heart rate, Blood Pressure, Heart Rate Variability, Total Homocysteine as the biomarkers of Cardiovascular system [7]. In this study we have assessed Resting heart rate, Blood pressure, Heart rate variability and the analysis showed that Resting Heart rate and Blood Pressure parameters (SBP, DBP, PP, and MBP) were significantly higher in T2DM group when compared to normals. This finding is in par with results of the study conducted by Sowers J, *et al.* who showed in their study that HTN in T2DM clusters with CVD risk factors contributes to early mortality [30]. Raised Blood Pressure is more common in diabetics than the general population. People with type 2 diabetes have a greater incidence of cardio-

vascular disease, cerebrovascular disease and renal disease than the general population [31].

Cardiac autonomic indices were assessed by frequency domain analysis of short-term HRV at supine resting condition. In the present study, it was observed that the parameters of Heart rate variability are significantly reduced and parameters of HRV depicting the parasympathetic modulation of heart are more reduced when compared to those depicting the sympathetic modulation in diabetics compared to normal subjects. Significant reduction in overall fluctuations in autonomic input to the heart was indicated by a significant decline in Total Power (T.P) parameter of frequency domain analysis. HF power which is an indicator of parasympathetic activity was significantly reduced and LF power reflecting both sympathetic and vagal activity was also significantly reduced in diabetic group. LF when expressed in normalized units is a qualitative marker of sympathetic modulations and this value was significantly higher indicating sympathetic dominance in diabetics. LF to HF ratio which is a marker of sympathovagal balance was also found to be significantly raised in diabetics suggesting sympathovagal imbalance in patients with T2DM compared to normal subjects. Study conducted by Kudat H, *et al.*, Pagani M, *et al.* and Chessa M, *et al.* using HRV in diabetics had also reported an increase in Heart rate and overall reduction in HRV parameters in diabetics with an increase in LF/HF in T2DM patients indicating more parasympathetic damage than sympathetic [32-34]. Parasympathetic modulation is mediated via vagus nerve, which is a long nerve and it is a known fact that long nerves are prone for neuropathy in diabetes mellitus. Results of Framingham study and Hoorn study have shown HRV to be reduced in diabetic individuals [35, 36].

Study conducted by Vera K Jandackova, *et al.* demonstrated that normative aging is accompanied by overall reduction in autonomic control of heart, diminished vagal tone and predominant sympathetic tone [37]. Mechanisms underlying changes in parasympathetic modulation with aging may be related to changes in cholinergic and muscarinic pathways through which vagal signals are transmitted. This may include disturbed cardiac acetylcholine release response to stimulation [38], http://www.ncbi.nlm.nih.gov/pmc/articles/PMC4802439/ - jah31301-bib-0039decrease in muscarinic receptor activity [39], http://www.ncbi.nlm.nih.gov/pmc/articles/PMC4802439/ - jah31301-bib-0040and reductions in M2 muscarinic receptor density with aging [40].

Thus the significant differences observed for various ageing indices of metabolic and cardiovascular system among our control and study groups prompted us to find the association of HbA1c with the above markers. Our study, for the first time has reported the association of various ageing indices with glycated Hb.

The analysis of association of HBA1c with chronological age among healthy controls of current study, showed highly significant positive correlation indicating our study as supporting HBA1c as a sensitive biomarker in healthy ageing as suggested by Lara J, *et al.* and Wagner K H, *et al.* [41, 42]. The analysis of association of metabolic and cardiovascular ageing indices with HbA1c using Pearson's correlation revealed a significant correlation between Glycemic index (HbA1c) and metabolic, cardiovascular indices of ageing. Pearson correlation was performed for entire study population which included both diabetics and controls. Further, outcome of the analysis of the association of HBA1c with metabolic and cardiovascular ageing indices adjusting for chronological age, using partial correlation test, showed that significant correlation still persists, indicating that in our study population, the association of metabolic and cardiovascular ageing indices with HBA1c is attributed to poor glycemic control independent of the chronological age.

WHR showed a very strong linear association with HbA1c. However, our study observed a lower body fat % in T2DM and the inability to demonstrate positive correlation with Glycosylated hemoglobin which is another index of ageing. Therefore, finding of this study indicates that body % fat is not a sensitive index of ageing in T2DM patients.

Further serum lipid parameters like TG, LDL-C to HDL-C ratio and Total Cholesterol to HDL-C ratio, which were assessed as metabolic indices showed linear positive association and HDL-C showed significant inverse correlation with HbA1c.These findings are in agreement with the observations of Prashant Tayde, *et al.* and Khan, *et al.* who concluded that greater abdominal adiposity and deranged lipids are significant factors for cardiac outcomes [43, 44]. A positive correlation between glycated haemoglobin and dyslipidemia observed in this study suggests that controlling the glycemic levels may significantly reduce the risk of cardiovascular events due to dyslipidemia. Khaw, *et al.* has reported that reducing HbA1c level by 0.2% could lower the mortality by 10% [45].

Similar trend was observed in the correlation results of cardiovascular indices with glycated haemoglobin. Blood pressure parameters like SBP, DBP, PP and MBP showed significant positive correlation with HbA1c. This observation is in par with the results of Buhler, *et al.* who reported that increased HbA1c levels increases blood viscosity by reducing the flexibility of RBC's and increasing their aggregability which in turn increases peripheral resistance and blood pressure [46]. Earlier, the study conducted by Julie K, *et al.* demonstrated the association of hyperglycemia with vascular stiffness and risk of CVD [47].

Further frequency domain parameters of Heart Rate Variability showed a significant negative correlation with glycated Haemoglobin indicating reduced heart rate variability in hyperglycemia. A significant positive correlation of LF to HF ratio with glycated haemoglobin shows that sympathetic dominance is significantly associated with tightness of glycemic control. This is consistent with the study conducted by Faulkner M S, *et al.* [48]. Similarly cardio-vagal function as measured by HF power exhibited a significant negative correlation with glycated Hb. The above findings suggest that chronic hyperglycemia leads to progressive autonomic dysfunction. The vagus nerve, the longest autonomic nerve which mediates maximum parasympathetic activity is the first nerve to be denervated in diabetes and as a compensatory mechanism cardiac sympathetic tone is increased [49]. Study conducted by Mika P Tarvainen, *et al.* has also suggested that elevated glycemic values have an unfavourable effect on cardiac autonomic function [50]. Some of the studies on HRV have suggested that autonomic modulations of

heart can be affected not only by hyperglycemia but also by other factors like age and accumulation of body fat [51].

Limitations

T2DM patients included in this study were on oral hypoglycaemic or insulin therapy. Novelty of the study would have been better if this study had recruited T2DM patients prior to the medication though it is practically not feasible to enroll a sample size of 220 T2DM patients prior to medication. However, our primary aim is to evaluate the association of poor glycemic control with ageing indices and hence we recruited the diabetic group irrespective of medications. Further, analysis of sensitive ageing biomarker like telomere length as marker for ageing would have provided better understanding of accelerated ageing in T2DM.

Clinical Implications

The outcome of the study suggests that individuals with T2DM age differently from healthy counterparts. This finding would facilitate to build the knowledge in T2DM patients about how the poor glycemic control will have risk for age-related decline in functionality in addition to the complication of hyperglycemia. The outcome of this study will also aid to create awareness in T2DM patients to plan the dietary diaries and structured physical activities to slow down the age-related decline in health status and promote healthy ageing.

CONCLUSION

The study concludes that T2DM patients exhibit accelerated ageing as compared to non-diabetic individuals as assessed by metabolic and cardiovascular ageing indices. Further, study also demonstrates that indices of ageing are inversely related with the level of glycemic control. Findings of our study suggest that tightness of glycemic control in terms of glycated haemoglobin should be achieved to decrease the risk of cardiovascular morbidity and mortality. This would help in promoting healthy ageing which is likely to have a significant impact on the quality of life.

LIST OF ABBREVIATIONS

AGE'S	=	Advanced Glycation End products
BMI	=	Body Mass Index
CVD	=	Cardio Vascular Disorder
DBP	=	Diastolic Blood Pressure
FBS	=	Fasting Blood Sugar
GPO-POD	=	Glycerol Phosphate Dehydrogenase - Peroxidase
HbA1c	=	Glycosylated Haemoglobin
HDL	=	High Density Lipoprotein
HF	=	High Frequency
HRV	=	Heart Rate Variability
HTN	=	Hypertension
LDL	=	Low Density Lipoprotein
LDL/ HDL	=	Low Density Lipoprotein To High Density Lipoprotein Ratio
LF	=	Low Frequency
LF/HF	=	Low Frequency to High Frequency Ratio
MBP	=	Mean Blood Pressure
PP	=	Pulse Pressure
RHR	=	Resting Heart Rate
ROS	=	Reactive Oxygen Species
SBP	=	Systolic Blood Pressure
T1DM	=	Type 1 Diabetes Mellitus
T2DM	=	Type 2 Diabetes Mellitus
TC	=	Total Cholesterol
TC / HDL	=	Total Cholesterol to High Density Lipoprotein Ratio
TG	=	Triglycerides
TP	=	Total Power
VLF	=	Very Low Frequency
WHR	=	Waist Hip Ratio

CONFLICT OF INTEREST

The authors confirm that this article content has no conflict of interest.

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