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Research Article

Relations between Skin Autofluorescence and Hemorheology Assessed by a Microchannel Method in Patients with Traditional Cardiovascular Risk Factors

Abstract

Aim: In recent years, skin autofluorescence (AF), a marker of tissue accumulation of advanced glycation end products, and hemorheology assessed by a microchannel method have been noted for their significance in detecting cardiovascular risk factors. However, there have been no reports regarding the relation between these two biomarkers. The present study attempts to clarify the relation and background factors of these two biomarkers in patients with traditional cardiovascular risk factors.

Methods: A total of 807 outpatients with traditional cardiovascular risk factors (306 males and 501 females; mean age: 64 ± 11 years) and no history of cardiovascular events were enrolled in this study. Skin AF and whole blood passage time (WBPT) as a marker of hemorheology were measured using a commercial device, and the relations among various clinical parameters, including that between skin AF and WBPT, were examined.

Results: There was a significantly positive correlation between skin AF and WBPT (r = 0.41, p < 0.001). Furthermore, multiple regression analysis revealed that these two markers showed a significant correlation (p < 0.01). Furthermore, derivatives of the reactive oxygen metabolites test, an oxidative stress marker, the cardio-ankle vascular index, an arterial function marker, smoking habits, and a number of traditional cardiovascular risk factors in an individual were also selected as independent variables for both skin AF and WBPT.

Conclusion: The results of this study indicated the significant relation between skin AF and hemorheology assessed by a microchannel method in patients with traditional cardiovascular risk factors. In addition, oxidative stress, arterial dysfunction, smoking habits, and a clustering of traditional cardiovascular risk factors were associated with these two biomarkers.

Abbreviations

AGEs: Advanced glycation end products, AF: Autofluorescence, MC-FAN: Micro Channel Array Flow Analyzer, WBPT: Whole blood passage time, eGFR: Estimated glomerular filtration rate, hs-CRP: high-sensitivity C-reactive protein, d-ROMs: Derivatives of reactive oxygen metabolites, CAVI: Cardio-ankle vascular index, PWV: Pulse wave velocity.

Introduction

Cardiovascular risk management in outpatients is mainly based on the treatment of traditional risk factors such as hypertension, dyslipidemia, diabetes mellitus, obesity and smoking habits. In fact, these risk factors are most commonly used to assess cardiovascular risk in clinical practice. Although these risk factors are very important predictors of cardiovascular events, they do not always explain all these events [1]. Therefore, exploring novel biomarker of cardiovascular diseases, in addition to traditional risk factors, is needed for further prevention of cardiovascular events.

Advanced glycation end products (AGEs) play an important role in various diseases, such as diabetic complications, renal dysfunction, or cardiovascular diseases [2,3]. In recent years, simpler methods of detecting AGE accumulation at the volar side of the arm, such as skin autofluorescence (AF), have been explored and used in clinical settings [4]. Skin AF is known...
to be a more reliable marker of tissue AGE levels in vivo than serum AGEs. In fact, some clinical studies have indicated that an increase in skin AF is significantly associated with the development of a range of kidney dysfunctions or vascular complications [5,6].

Impairment of blood rheology has also been reported as an important factor for the incidence of cardiovascular diseases as well as atherosclerosis [7]. Recently, a commercial device that evaluates hemorheology (Micro Channel Array Flow Analyzer (MC–FAN)) using microscopic images has been explored and is being used in clinical settings [8]. MC–FAN is easier to use and is superior to other methods in terms of accuracy of channel dimensions and reproducibility. Furthermore, clinical studies have established the usefulness of whole blood passage time (WBPT), which is measured by MC–FAN, in evaluating cardiovascular disease risk factors [9–12].

Thus, skin AF and WBPT are considered novel risk factors of cardiovascular diseases. However, to the best of our knowledge, there have been no reports regarding the relation between skin AF and WBPT. Therefore, this study aims to clarify this relation in patients with traditional cardiovascular risk factors from the perspective of primary cardiovascular events. This study also examines the background factors of these two markers.

Materials and Methods

Subjects

This cross-sectional study was conducted at the Hitsumoto Medical Clinic in Shimonoseki City from June 2014 to May 2016. The study population comprised outpatients with risk factors of cardiovascular diseases such as hypertension, type 2 diabetes mellitus, dyslipidemia, obesity and smoking habits. None of the patients had a history of cardiovascular events, such as ischemic heart disease, stroke, and perivascular disease. The group of patients with cardiovascular risk factors comprised 306 men and 501 women with a mean age of 64 ± 11 years. All participants provided informed consent, and the study protocol conformed to the ethical guidelines of the Declaration of Helsinki as reflected by the a priori approval of the Local Ethics Committee.

Measurement of skin AF

Skin AF was measured using a commercial instrument (AGE Reader™; DiagnOptics, Groningen, Netherlands), as described previously [13,14]. Briefly, AF was defined as the average light intensity per nanometer between 300 and 420 nm. Levels of skin AF were expressed in arbitrary units (AU). With patients seated, all measurements were taken at the volar side of the lower arm, approximately 10–15 cm below the elbow. The validity and reliability of skin AF levels measured using this method in a Japanese population have been previously established [6,14].

Evaluation of hemorheology using MC–FAN

The evaluation of hemorheology was performed by measuring WBPT using an MC–FAN HR300 rheometer (MC Healthcare Inc., Tokyo) as previously reported [8]. Briefly, the microchannel passage time for 100 μL of physiologic saline was first measured as control. Then, the same measurement was determined for blood obtained from subjects with 100 μL of the heparinization sample. WBPT of the subjects was expressed after correcting for the passage time of physiologic saline. The microchannel formation had a width, length, and depth of 7, 30, and 4.5 μm, respectively. Examination was performed within 60 min of blood sampling. Inter- and intra-assay coefficients of variation for BPT were 8% and 5%, respectively.

Evaluation of cardiovascular risk factors

The degree of obesity was estimated from the body mass index, which was calculated as the weight in kilograms divided by the square of the height in meters; obesity was defined as a Japanese criteria (body mass index ≥ 25 kg/m²). Current smoking was defined as smoking at least one cigarette per day during the previous 28 days. Hypertension was defined as a systolic blood pressure greater than or equal to 140 mmHg, a diastolic blood pressure greater than or equal to 90 mmHg, or the use of anti-hypertensive medication. Diabetes mellitus was defined as a fasting blood glucose level greater than or equal to 126 mg/dL or the use of anti-diabetic medication. Dyslipidemia was defined as a low–density lipoprotein cholesterol level greater than or equal to 140 mg/dL, a high–density lipoprotein cholesterol level less than or equal to 40 mg/dL, a triglyceride level greater than or equal to 150 mg/dL, or the use of anti-dyslipidemic medication. The following blood parameters were measured: blood cell counts, plasma glucose, plasma insulin, serum lipid concentrations, estimated glomerular filtration rate (eGFR), serum high-sensitivity C–reactive protein (hs-CRP) concentrations, and derivatives of reactive oxygen metabolites (d-ROMs). Blood samples were collected from the antecubital vein in the morning after 12 h of fasting. Glucose and insulin concentrations were measured using the glucose oxidase method and an enzyme immunoassay, respectively. To measure insulin resistance, HOMA–IR was calculated as follows [15]: HOMA–IR = fasting glucose concentration (mg/dL) × fasting insulin concentration (μg/mL)/405. Total cholesterol and triglyceride concentrations were measured using standard enzymatic methods. High- and low-density lipoprotein cholesterol concentrations were measured using selective inhibition and Friedewald’s formula, respectively [16]. Subjects with a serum triglyceride concentration ≥ 400 mg/dL were excluded considering the accuracy of this method.

eGFR was calculated using the adjusted Modification of Diet in Renal Disease Study equation, proposed by the working group of the Japanese Chronic Kidney Disease Initiative [17]. The hs-CRP concentration was measured using high–sensitivity latex–enhanced immunonephelometrics, and inter- and intra-assay coefficients of variability has been shown to be less than 2%. The d–ROMs test, which reflects hydroperoxide levels, was measured as an oxidative stress marker in vivo using a commercial device (Dia cron, Grosseto, Italy) [18].

Evaluation of arterial function

The cardio–ankle vascular index (CAVI) was measured as an arterial function marker using a VaSera CAVI instrument (Fukuda Denshi Inc., Tokyo, Japan) according to the previously
described methods [19]. Briefly, the brachial and ankle pulse waves were determined using inflatable cuffs with the pressure maintained between 30 and 50 mmHg to ensure that the cuff pressure had a minimal effect on the systemic hemodynamics. Blood and pulse pressures were simultaneously determined, and the measurements were obtained with the subject in a supine position. CAVI was measured after the subject rested for 10 min in a quiet room. The average coefficient of variation in CAVI has been shown to be less than 5%, which is small enough for clinical use and indicates that CAVI measurement has good reproducibility.

**Statistical analysis**

A commercially available statistical software program (StatView-J 5.0; Hulinks Inc., Tokyo, Japan) was used for all statistical analyses. Continuous variables were expressed as mean ± standard deviation. Between-group comparisons were performed using the Student’s t test or Mann–Whitney’s U test. Simple regression analysis was estimated using Spearman’s rank correlation analysis, while multivariate analysis was performed using multiple regression analysis. A p value of <0.05 was considered significant.

**Results**

Baseline clinical characteristics are shown in table 1, while the histograms of skin AF and WBPT are shown in figure 1. The mean value of skin AF was 2.5 ± 0.5 AU, ranging from 1.2 to 4.1 AU, with a median value of 2.5 AU. On the other hand, the mean value of WBPT was 60.7 ± 16.8 s, ranging from 31.1 to 113.5 s, with a median value of 58.3 s. These two parameters indicated nearly normal distribution. Correlations among skin AF, WBPT, and various clinical parameters are shown in table 2. Age, smoking habits, presence of diabetes mellitus, fasting blood glucose levels, insulin levels, HOMA-IR, a number of traditional cardiovascular risk factors, eGFR, hs-CRP, d-ROMs test, CAVI, and WBPT were significantly correlated with skin AF. On the other hand, sex differences, body mass index, smoking habits, white and red blood cell counts, hematocrit, serum triglyceride concentration, fasting blood glucose levels, insulin levels, HOMA-IR, a number of traditional cardiovascular risk factors, eGFR, hs-CRP, d-ROMs test, CAVI, and skin AF were significantly correlated with WBPT.

Multiple regression analysis for skin AF or WBPT as a subordinate factor was performed with explanatory variables that were significant during univariate analysis. WBPT, d-ROMs test, CAVI, and WBPT were significantly correlated with skin AF. On the other hand, skin AF, a number of traditional cardiovascular risk factors, CAVI, smoking habits, and age were selected as independent variables for skin AF. On the other hand, skin AF, a number of traditional cardiovascular risk factors, CAVI, d-ROMs test, smoking habits, and hematocrit were selected as independent variables for WBPT (Table 3).

**Discussion**

This study aimed to clarify the relation between skin AF, which reflects tissue accumulation of AGEs, and WBPT, a marker of hemorheology assessed by microchannel methods, in patients with traditional cardiovascular risk factors from the viewpoint of primary cardiovascular events. Results of the multivariate analysis showed a significant relation between skin AF and WBPT. In addition, oxidative stress, arterial function, smoking habits, and a number of traditional cardiovascular risk factors in an individual were significantly related with these two biomarkers.

Hemorheology estimated using MC–FAN is an in vitro study that uses artificial blood vessels, with the vessel lumen having a width and depth of 7 and 4.5 μm, respectively. Therefore, the lumen and capillary have similar sizes. On the other hand, levels of pentosidine, which is a major component of AGEs,
were measured at the volar side of the lower arm by skin biopsy and appeared to correlate with skin AF [20]. Capillary vessels are considered to be present in the area of skin biopsy. Therefore, a significant relation between skin AF and WBPT could possibly reflect the accumulation of AGEs in tissues through impairment of blood rheology within the capillaries. On the other hand, some basic studies have reported that AGEs influence hemorheology through mechanisms such as leukocyte–endothelial interaction or platelet aggregation [21,22]. Furthermore, skin AF has been reported to reflect various organ damages or accumulation of AGEs, including the coronary or carotid arteries, kidneys, retinas, neurons, and cardiomyocyte cells [5,6,23,24]. Thus, the results of this and previous studies indicate that AGEs are associated with the impairment of hemorheology not only at the volar side of the lower arm but also in various organs, consequently causing systemic organ damage. In addition, we can predict systemic organ damage by measuring skin AF and WBPT.

Table 2: Correlation between Skin AF, WBPT and various clinical parameters.

|                               | Skin AF | WBPT |
|-------------------------------|---------|------|
|                               | r       | r    |
| Sex (Female=0, Male=1)        | 0.02    | 0.11** |
|                               | 0.11**  | 0.06 |
| Body mass index               | 0.07    | 0.09*** |
| Obesity                       | 0.06    | 0.10** |
| Smoking (No=0, Yes=1)         | 0.13*   | 0.17* |
| Hypertension (No=0, Yes=1)    | 0.06    | 0.01 |
| Systolic BP                   | 0.07    | 0.04 |
| Diastolic BP                  | 0.05    | 0.01 |
| White blood cell              | 0.04    | 0.14* |
| Red blood cell                | 0.03    | 0.15* |
| Hematocrit                    | 0.01    | 0.20* |
| Platelet                      | 0.04    | 0.03 |
| Diabetes mellitus (No=0, Yes=1)| 0.11** | 0.07 |
| FBG                           | 0.11**  | 0.10** |
| IRI                           | 0.08*** | 0.08*** |
| Log-HOMA-IR                   | 0.10**  | 0.09*** |
| Dyslipidemia (No=0, Yes=1)    | -0.08   | -0.02 |
| Total cholesterol             | 0.07    | 0.06 |
| LDL cholesterol               | 0.05    | 0.05 |
| Triglyceride                  | 0.05    | 0.13** |
| HDL cholesterol               | -0.03   | -0.04 |
| Number of risk factors        | 0.43*   | 0.42* |
| eGFR                          | -0.13*  | -0.12* |
| Log-hs-CRP                    | 0.11**  | 0.18* |
| d-ROMs test                   | 0.35*   | 0.30* |
| CAVI                          | 0.27*   | 0.34* |
| Skin AF                       | -       | 0.41* |
| WBPT                          | 0.41*   | -    |
| RAS inhibitor (No=0, Yes=1)   | -0.06   | -0.05 |
| DPP-4 inhibitor (No=0, Yes=1) | -0.06   | -0.05 |
| Statin (No=0, Yes=1)          | -0.03   | -0.04 |

r expressed correlation coefficient. *p<0.001, **p<0.01, ***p<0.05. Abbreviations as in Table 1.
cardiovascular risk factors.

in daily practice is important for novel cardiovascular risk factors to control each traditional cardiovascular risk factor with these two markers. Thus, the results of this study suggest the usefulness of these drugs in improving AGV levels, receptors of AGVs, or hemorheology [31-33]. The results of this cross-sectional study indicated no significant relation between medications, such as renin–angiotensin system inhibitors, dipeptidyl peptidase-4 inhibitors, or statins, and WBPT or skin AF. However, interventional studies are warranted to examine their effectiveness on novel cardiovascular risk factors, such as d-ROMs test, skin AF, and WBPT. Consequently, we expect to discover new applications for these drugs.

CAVI is known to be a novel marker of arterial stiffness, which is independently associated with blood pressure levels [19]. On the other hand, some clinical studies have indicated the relation between skin AF or impairment of blood rheology and arterial stiffness using pulse wave velocity (PWV). Mac-Way et al. reported that skin AF is significantly related with carotid-femoral PWV [34]. Satoh et al. reported the relation between WBPT and stiffness of the aortic artery using PWV in patients with obesity [9]. Other clinical studies have reported that CAVI is indicative of endothelial dysfunction [35]. In addition, some clinical studies have indicated the significant relation between skin AF or increased WBPT and endothelial dysfunction [12,36]. Thus, both skin AF and WBPT reflect arterial function, such as arterial stiffness or endothelial function, which may consequently predict the incidence of cardiovascular events.

It is well known that smoking habits affect AGV levels or blood rheology. Some clinical studies have indicated the relation between smoking habits and skin AF or WBPT [11,37,38]. The results of the present study also indicated that smoking is an independent factor for determining skin AF or WBPT. Smoking has also been known to affect oxidative stress or CAVI [39]. Thus, smoking cessation is strongly recommended to prevent primary cardiovascular events considering various parameters including skin AF and WBPT.

Patients with just one cardiovascular risk factor will not always have a cardiovascular event. Patients with multiple risk factors are most likely to have a problem [40]. In the present study, clustering of traditional cardiovascular risk factors, such as hypertension, diabetes mellitus, dyslipidemia, smoking, and obesity was significantly correlated with both skin AF and WBPT, even though each parameter was weakly correlated with these two markers. Thus, the results of this study suggest that control of each traditional cardiovascular risk factor in daily practice is important for novel cardiovascular risk factors such as skin AF and WBPT. Furthermore, estimation of WBPT and skin AF are expected to increase the reliability of prediction of cardiovascular disease in patients with traditional cardiovascular risk factors.

This study has several limitations. First, the medical treatments for hypertension, diabetes mellitus, and/or dyslipidemia may have influenced the study results. Second, this was a single-center cross-sectional study with a relatively small population. A prospective study involving a larger number of patients is necessary to confirm the effectiveness of the therapies such as anti-oxidant administration or smoking cessation in improving skin AF levels or blood rheology, as estimated using AGE Reader or MC-FAN. Third, hemorheology estimated using MC-FAN is an in vitro study that uses artificial blood vessels; therefore, the obtained hemorheological data were different from those obtained in vivo because of the influence of vascular factors such as endothelial cells or smooth muscle cells. Furthermore, evaluation of MC-FAN is simple method compared to Coulter-Harkness viscometer. However, the results of this and previous studies indicate that MC-FAN data for WBPT are useful markers for evaluating cardiovascular risk in clinical settings. Finally, an extensive examination of clinical studies will be required in the future to investigate the significance of skin AF and WBPT as novel cardiovascular risk factors.

Conclusions

In conclusion, the present study, which used commercial devices such as AGE Reader and MC-FAN in patients with traditional cardiovascular risk factors, indicated a significant relation between skin AF and WBPT. In addition, oxidative stress, arterial dysfunction, smoking habits, and a clustering of traditional cardiovascular risk factors were associated with these two biomarkers.

References

1. Khot UN, Khot MB, Bajzer CT, Sapp SK, Dhman EM, et al. (2003) Prevalence of conventional risk factors in patients with coronary heart disease. JAMA 290: 898-904. Link: https://goo.gl/85cnMO

2. Kilhovd BK, Juutilainen A, Lehto S, Rönnemaa T, Torjesen PA, et al. (2007) Increased serum levels of advanced glycation endproducts predict total, cardiovascular and coronary mortality in women with type 2 diabetes: a population-based 18 year follow-up study. Diabetologia 50:1409-1417. Link: https://goo.gl/Ljzqgv

3. Yamagishi S, Imaiuzumi T (2005) Diabetic vascular complications: pathophysiology, biochemical basis and potential therapeutic strategy. Curr Pharm Des 11: 2279-2299. Link: https://goo.gl/P7T7pt

4. Meerwaldt R, Hartog JW, Graaff R, Huisman RJ, Links TP, et al. (2005) Skin autofluorescence, a measure of cumulative metabolic stress and advanced glycation end products, predicts mortality in hemodialysis patients. J Am Soc Nephrol 16:3687–3693. Link: https://goo.gl/pWS8hQ

5. Lutgers HL, Graaff R, Links TP, Ubink-Veltmaat LJ, Bilo HJ, et al. (2006) Skin autofluorescence as a noninvasive marker of vascular damage in patients with type 2 diabetes. Diabetes Care 29: 2654-2659. Link: https://goo.gl/RNTaw7

6. Hangai M, Takebe N, Honma H, Sasaki A, Chida A, et al. (2016) Association of Advanced Glycation End Products with coronary Artery Calcification in Japanese Subjects with Type 2 Diabetes as Assessed by Skin Autofluorescence. J Atheroscler Thromb 23: 1178-1187. Link: https://goo.gl/eValMq

7. Tzoulaki I, Murray GD, Lee AJ, Rumley A, Lowe GD, et al. (2007) Relative value of inflammatory, hemostatic, and rheological factors for incident myocardial infarction and stroke: the Edinburgh Artery Study. Circulation 115: 2119-2127. Link: https://goo.gl/8JY2P6
8. Kikuchi Y, Sato K, Mizuguchi Y (1994) Modified cell flow microchannels in a single-crystal silicon substrate and flow behavior of blood cells. Microvac Res 47: 126-139. Link: https://goo.gl/240e0l

9. Satoh N, Kotani K, Wada H, Himeno A, Shimada S, et al. (2009) Unfavorable blood rheology is closely associated with arterial stiffness in obese patients. Endocr J 56: 915-918. Link: https://goo.gl/4CJaXM

10. Matsuo K, Ueda Y, Nishio M, Hirata A, Asai M, et al. (2011) Thrombogenic potential of whole blood is higher in patients with acute coronary syndrome than in patients with stable coronary diseases. Thromb Res 128: 268-273. Link: https://goo.gl/lpQwu7

11. Hitsumoto T (2012) Factors affecting impairment of blood rheology in obese subjects. J Cardiol 60: 401-406. Link: https://goo.gl/PNT5I1

12. Yagi H, Sumino H, Aoki T, Tsunekawa K, Araki O, et al. (2011) Impaired blood rheology is associated with endothelial dysfunction in patients with coronary risk factors. Clin Hemorheol Microcirc 62: 139-150. Link: https://goo.gl/Rb5Jht

13. Meerwaldt R, Hartog JW, Graaff R, Huisman RJ, Links TP, et al. (2005) Increased accumulation of skin advanced glycation end-products precedes and correlates with clinical manifestation of diabetic neuropathy. J Am Soc Nephrol 16: 3567-3663. Link: https://goo.gl/yGqG6

14. Nomoto K, Yagi M, Arita S, Hamada U, Yonei Y (2012) A survey of fluorescence derived from advanced glycation end products in the skin of Japanese: differences with age and measurement location. Anti-Aging Medicine 9: 119-124. Link: https://goo.gl/y3yrf

15. Matthews DR, Hosker JP, Rudenski AS, Naylor BA, Treacher DF, et al. (1985) Homeostasis model assessment: insulin resistance and beta-cell function from fasting plasma glucose and insulin concentrations in man. Diabetologia 28: 412-419. Link: https://goo.gl/xfyJY

16. Fridewald WT, Levy RI, Fredrickson DS (1972) Estimation of the concentration of low density lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge. Clin Chem 18: 499-502. Link: https://goo.gl/pnFlb0

17. Imai E, Horio M, Nitta K, Yamagata K, Iseki K, et al. (2007) Estimation of glomerular filtration rate by the MDRD study equation modified for Japanese patients with chronic kidney disease. Clin Exp Nephrol 11: 41-50. Link: https://goo.gl/LbgFjC

18. Cesarone MR, Belcaro G, Carratelli M, Cornellì I, De Sanctis MT, et al. (1999) A simple test to monitor oxidative stress. Int Angiol 18: 127-30 Link: https://goo.gl/B0g5J

19. Shirai K, Utino J, Otsuka K, Takata M (2006) A novel blood pressure-independent arterial wall stiffness parameter; cardio-ankle vascular index (CAVI). J Atheroscler Thromb 13: 101-107. Link: https://goo.gl/M6LyLd

20. Meerwaldt R, Graaff R, Oomen PH, Links TP, Jager JJ, et al. (2004) Simple non-invasive assessment of advanced glycation end product accumulation. Diabetologia 47: 1324-1330. Link: https://goo.gl/gwhkFFe

21. Morigi M, Angioletti S, Imberti B, Donadelli R, Micheletti G, et al. (1998) Leukocyte-endothelial interaction is augmented by high glucose concentrations and hyperglycemia in a NF-kB-dependent fashion. J Clin Invest 101: 1905-1915. Link: https://goo.gl/2hFqY3

22. Hasegawa Y, Suehiro A, Higasa S, Namba M, Kakishita E (2002) Enhancing effect of advanced glycation end products on serotonin-induced platelet aggregation in patients with diabetes mellitus. Thromb Res 107: 319-322. Link: https://goo.gl/ZN6WGR

23. Araszkiewicz A, Naskret D, Zozulinska-Ziolkiewicz D, Pilacinis C, Uruka A, et al. (2015) Skin autofluorescence is associated with carotid intima-media thickness, diabetic microangiopathy, and long-lasting metabolic control in type 1 diabetic patients. Results from Poznan Prospective Study. Microvac Res 98: 62-67. Link: https://goo.gl/hhc9aU

24. Hofmann B, Jacobs K, Navarrete Santos A, Winkel A, et al. (2015) Relationship between cardiac tissue glycation and skin autofluorescence in patients with coronary artery disease. Diabetes Metab 41: 410-415. Link: https://goo.gl/wC07ag

25. Tzoulakis I, Murray GD, Lee AJ, Runley A, Lowe GD, et al. (2007) Inflammatory, haemostatic, and rheological markers for incident peripheral arterial disease: Edinburgh Artery Study. Eur Heart J 28: 354-362. Link: https://goo.gl/q8aAbm

26. Podrez EA, Byzova TV, Febbraio M, Salomon RG, Ma Y, et al. (2007) Platelet CD36 links hyperlipidemia, oxidant stress and a prothrombotic phenotype. Nat Med 13: 1086-1095. Link: https://goo.gl/bkY6sg

27. Yamagishi S, Nakamura N, Suematsu M, Kaseda K, Matsu T (2015) Advanced Glycation End Products: A Molecular Target for Vascular Complications in Diabetes. Mol Med 21: S32-40. Link: https://goo.gl/oup4NF

28. Lodovici M, Bigagli E, Tarantini F, Di Serio C, Raimondi L (2015) Losartan reduces oxidative damage to renal DNA and conserves plasma antioxidant capacity in diabetic rats. Exp Biol Med (Maywood) 240: 1500-1504. Link: https://goo.gl/pXkhBf

29. Alam MA, Chowdhury MR, Jain P, Sagar MA, Reza HM (2015) DPP-4 inhibitor sitagliptin prevents inflammation and oxidative stress of heart and kidney in two kidney and one clip (2K/1C) rats. Diabetol Metab Syndr 7: 107. Link: https://goo.gl/GKizzz

30. Endo K, Miyashita Y, Sasaki H, Ebisuno M, Ohira M, et al. (2006) Propranolol and atorvastatin decrease urinary 8-hydroxy-2′-deoxyguanosine in patients with diabetes and hypercholesterolemia. J Atheroscler Thromb 13: 68-75. Link: https://goo.gl/TOLcWN

31. Ott C, Raff U, Schmidt S, Kistner I, Friedrich S, et al. (2014) Effects of saxagliptin on early microvascular changes in patients with type 2 diabetes. Cardiovasc Diabetol 13: 19. Link: https://goo.gl/qzHFf6

32. Yamada K, Hirayama T, Hasegawa Y (2007) Antiplatelet effect of losartan and telmisartan in patients with ischemic stroke. J Stroke Cerebrovasc Dis 16: 225-231. Link: https://goo.gl/QLgN0a

33. Shimomura M, Oyama J, Takeuchi M, Shibata Y, Yamamoto Y, et al. (2016) Acute effects of statin on reduction of angiopeptin-like 2 and glyceroldehyde-derived advanced glycation end-products levels in patients with acute myocardial infarction: a message from SAMIT (Statin for Acute Myocardial Infarction Trial). Heart vessels 31: 1583-1589. Link: https://goo.gl/JzY9fT

34. Mac-Way F, Couture V, Utescu MS, Ignace S, De Serres SA, et al. (2014) Advanced glycation end products, aortic stiffness, and wave reflection in peritoneal dialysis as compared to hemodialysis. Int Urol Nephrol 46: 817-824. Link: https://goo.gl/fL0rRb

35. Endo K, Sakai A, Ohira M, Miyashita Y, Shirai K (2011) Cardio-ankle vascular index may reflect endothelial function in type 2 diabetes. Int J Clin Pract 65: 1200-1201. Link: https://goo.gl/0VIAfc

36. Skhova J Jr, Soupal J, Loni Ekali G, Prázný M, Kalousová M, et al. (2013) Skin autofluorescence relates to soluble receptor for advanced glycation end-products and albuminuria in diabetes mellitus. Int J Clin Pract. Diabetes Technol Ther 12: 399-403. Link: https://goo.gl/SNFDZD

37. Koetsier M, Lutters HL, de Jonge C, Links TP, Smit AJ, et al. (2010) Reference values of skin autofluorescence. Int J Clin Pract. Diabetes Technol Ther 12: 399-403. Link: https://goo.gl/SNFDZD

38. Shimada S, Hasegawa K, Wada H, Terashima S, Satoh-Asahara N, et al. (2011) High blood viscosity is closely associated with cigarette smoking and markedly reduced by smoking cessation. Circ J 75: 185-189. Link: https://goo.gl/SnakE9

39. Noike H, Nakamura K, Sugiyama Y, Iizuka T, Shimizu K, et al. (2010) Changes in cardio-ankle vascular index in smoking cessation. J Atheroscler Thromb 17: 517-525. Link: https://goo.gl/UlkExr

40. Nakamura Y, Yamamoto T, Okamura T, Kadowaki T, Hayakawa T, et al. (2006) Combined cardiovascular risk factors and outcome: NIPPON DATA80, 1980–1994. Circ J 70: 960–964. Link: https://goo.gl/H4AEvl