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Original Article

Imbalance between Angiotensin II - Angiotensin (1-7) system is associated with vascular endothelial dysfunction and inflammation in type 2 diabetes with newly diagnosed hypertension

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ABSTRACT

Aim: Diabetes is associated with Renin-angiotensin-aldosterone-system (RAAS) activation. Protective role of Angiotensin (1-7) has been recently identified. The study aims to identify associations between imbalance in RAAS components with vascular endothelial dysfunction and inflammation in diabetics with newly diagnosed hypertension.

Methods: Brachial Flow-mediated-dilation (FMD), Carotid Intima-media-thickness (CIMT), pulse-wave-velocity (PWV), Serum E-selectin, Vascular-Cell-Adhesion-Molecule-1 (VCAM-1), high-sensitivity C-Reactive Protein (hsCRP), Interleukin-10 (IL-10), Renin, AngiotensinII, Angiotensin-Converting-Enzyme 2 (ACE2) and Angiotensin1-7 were measured in 60 diabetic patients with newly diagnosed hypertension. Patients with AngiotensinII/Angiotensin1-7 ratio <1 were classified as Favourable-Axis (FA) group ($n = 22$) and those with ratio >1 were classified as Unfavourable-Axis (UA) group ($n = 38$).

Results: hsCRP was higher [9.52 (4.64–16.19) vs 3.62 (1.77–13.09) (mg/l), $p = 0.04$], IL-10 was lower [2.26 (1.34–12.05) vs 10.98 (4.44–17.78) (pg/ml),$p = 0.006$], %FMD was lower [(5.51 ± 2.97) vs (7.66 ± 3.38) (%), $p = 0.01$] and CIMT was higher in UA compared to FA group [0.7 (0.55–0.79) vs 0.51 (0.49–0.65) (mm), $p = 0.001$]. Renin correlated positively with pressure, PWV, E-selectin and VCAM-1, opposing associations were obtained for Angiotensin1-7 and ACE2.

Conclusion: Imbalance between AngiotensinII – Angiotensin1-7 is associated with increased inflammation and vascular dysfunction in diabetics and can contribute to development of hypertension in these patients.

1. Introduction

Diabetes mellitus is a condition associated with increased risk of morbidity and mortality due to stroke, cardiovascular and renal diseases. This increase in risk has been attributed to concomitant dyslipidemia, obesity and development of hypertension in patients with diabetes.

All of these factors can be linked to an alteration in the vascular function, which can in turn lead to diabetes associated mortality. Multiple mechanisms have been proposed to link diabetic state with endothelial dysfunction which include hyperglycemia, oxidative stress, activation of Protein Kinase C and quenching of Nitric oxide by advanced glycation end products (AGEs) [1]. For example, an increased propensity for oxidative stress can lead to high levels of oxidized LDL, which is known to be pro-atherogenic and can lead to endothelial dysfunction [2]. Additionally, hyperglycemia results in formation of AGEs which increase vascular stiffness by structural alteration of the elastin-collagen ratio leading to stiffening of the extracellular matrix, medial calcification and collagen linkage to advanced-glycation end products [3].

Presence of hypertension in diabetes acts as an additive risk factor for development of cardiovascular diseases [4]. Activation of the renin-angiotensin aldosterone system (RAAS) has been implicated in the development of arterial hypertension in a large population of diabetes [5]. Individual components of RAAS have been

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found to have differential effects on the vasculature, particularly Angiotensin II (Ang II) has a vasoconstrictive effect while Angiotensin 1-7 (Ang 1–7) is a vasodilator [6]. Additionally, Ang II can also lead to endothelial dysfunction and has a proinflammatory role by increasing oxidative stress, adhesion molecules and inflammatory cytokines in the vessel wall [7,8]. Ang II increase has been shown to result in endothelial damage and atherosclerotic changes [9,10]. Ang II is also known to stimulate the atherogenic process by enhancing vascular inflammation and inducing endothelial dysfunction [8]. On the other hand, Ang 1–7 has a protective role and its action on Mas receptors opposes the Ang II mediated AT_1 receptor activation [11].

Diabetes *per se* is known to be a state of inflammation with an increase in the production of inflammatory mediators [12], which may in turn result in vascular dysfunction [13].

Development of hypertension in patients of Diabetes can be a result of vascular derangement mediated by a tilt in the RAAS balance towards a pro-inflammatory profile, further aggravating the pre-existing pro-inflammatory state, finally resulting in hypertension. The disease pathology is complicated by the intricate link between each of these vascular factors, inflammation and RAAS components in the development of diabetes complications.

The current study aims to understand the associations between an imbalance in RAAS components with pro and anti-inflammatory mediators, vascular dysfunction in a diabetic population with recent diagnosed hypertension in an attempt to decipher the possible mechanisms leading to hypertension. Additionally, the study also tries to identify the interrelationships between above mentioned factors in these patients.

2. Methods

Type 2 diabetes individuals with newly diagnosed hypertension without any previous history of intake of antihypertensive medication were recruited in the study ($n = 60$), age 46.1 ± 8.1 years, 49% males. The patients were recruited from outpatient department of Department of Endocrinology & Metabolism, AIIMS, New Delhi.

To study the effect of RAAS imbalance, the patients were divided into 2 groups based on their Ang II to Ang 1–7 ratio (Ang II/Ang 1–7). An Ang II/Ang 1–7 of <1 was considered to be 'Favourable Axis' (FA) while a ratio of >1 was considered to be 'Unfavourable Axis' (UA).

This was a cross sectional observational study. Type 2 diabetes individuals with newly diagnosed hypertension without any previous history of intake of antihypertensive medication were recruited for the study ($n = 60$). The study was approved by the institutes' ethical committee for human research (Ethical clearance no. IESC/T- 138/01.04.2015) and performed in accordance with the ethical standards as laid down in the 1964 Declaration of Helsinki and its later amendments or comparable ethical standards. Written informed consent was taken from all participants. Blood pressure measurement was done for consecutive diabetic individuals reporting to the Department of Endocrinology & Metabolism, AIIMS, New Delhi, India. Hypertension was diagnosed according to the Eighth Joint National Committee (JNC 8) criteria i.e., systolic blood pressure (SBP) of $\geq$140 mmHg and diastolic blood pressure (DBP) $\geq$ 90 mmHg in two measurements 5 min apart. Patients were recruited for the study only if they recorded a consistently high blood pressure on 2 consecutive visits with no reported hypertension on previous visits based on older records and history of the patient to rule out the effect of long-standing hypertension on vascular function. Vascular function was assessed by measuring endothelium-dependent brachial artery Flow Mediated Dilation (FMD), serum levels of E-selectin, assessment of central and peripheral Pulse Wave Velocity (PWV), Augmentation Index (AIx) and Carotid Intima Media Thickness (CIMT). RAAS activity was measured by serum levels of Renin, Angiotensin II (Ang II), Angiotensin Converting Enzyme 2 (ACE 2) and Angiotensin 1-7 (Ang 1–7). Inflammatory state was assessed by serum levels of High-Sensitive C-Reactive Protein (hsCRP), Vascular Cell Adhesion Molecule-1 (VCAM-1) and Interleukin-10 (IL-10).

Endothelial function was assessed by measuring the percentage change in the brachial artery diameter (%FMD) in response to increased shear stress. Right-arm brachial artery diameter was imaged using a vascular ultrasound (Vivid e, GE Healthcare, Germany) by a 12 MHz linear array probe. Lead II ECG was simultaneously recorded to correlate the brachial artery diameter with the phase of cardiac cycle. To create a flow stimulus in the brachial artery by inducing reactive hyperaemia, a sphygmomanometric cuff was first placed around the forearm. Brachial artery was visualised 2–3 cm above the antecubital fossa. Baseline images of the brachial artery were taken after which the cuff was inflated to 50 mm Hg above systolic blood pressure to occlude arterial inflow for 5 min. Longitudinal images of the artery were recorded in the B mode every 10 s for 3 min after release of occlusion. Diameter was measured at end-diastole manually using an offline electronic calliper and percentage FMD was calculated by using the peak diameter after release of occlusion and the baseline brachial artery diameter.

Arterial stiffness was assessed by measuring the carotid-femoral (cf) carotid-radial (cr) PWV, Augmentation index (AIx) using SphgmoCor® CVMS CPVH System (Atcor Medicals, Australia) and Carotid Intima Media Thickness (CIMT) using vascular ultrasound (Vivid e, GE Healthcare, Germany). For measurement of PWV, the tonometer was placed sequentially at the carotid artery and at the femoral artery with simultaneous recording of Lead II ECG to allow for R-wave gating of the pressure signal. The distance between the two arterial sites was estimated from the sternal notch by first measuring up to the best palpable point for the carotid artery pulsation. The distance to the radial artery was measured by keeping the arm abducted and parallel to the floor. Distance to the femoral artery was measured by first measuring the distance from suprasternal notch to the umbilicus and then from umbilicus to the best palpable point of femoral artery pulsation. Pulse wave velocity was calculated automatically by the software using the distance between the two arterial sites and foot-to-foot detection of the pressure waveforms at the two sites. Augmentation Index which is a composite marker of wave reflections and arterial stiffness, was assessed by using the same tonometer probe at the radial artery and using a validated transfer function analysis to derive the central waveforms [14]. Carotid intima media thickness was measured using ultrasound (Vivid e, GE Healthcare, Germany) and the thickness was measured at the level of posterior wall of right common carotid artery. Carotid artery was scanned along its length in longitudinal sections and 4 to 5 images with at least 3–4 s cardiac cycles were captured for offline analysis. Analysis of CIMT was done at the end-diastolic phase of cardiac cycle by using automated software (Vivid e, GE Healthcare, Germany). The average of 3 images was used to calculate CIMT.

Serum levels of E-selectin, hsCRP, IL-10, VCAM-1, Renin, Ang II, ACE 2 and Ang 1–7, were measured by a sandwich enzyme linked immunosorbent assay (ELISA) using commercially available standard kits. The supplier for all the kits was Immunoconcept India Pvt. Ltd. The product numbers of the kits used for the study were E-selectin – [cat no.- EK0501, Booster, USA], hsCRP – [cat no. – K250, XEMA, China], IL-10 – [cat no. EK0416, Booster, USA], Renin – [catalogue (cat) No. E13651551, Sincere™, China], Angiotensin II – [cat no. E13652287, Sincere™, China], Angiotensin converting enzyme 2 [cat no. – EK 0997, Booster, USA] and Angiotensin 1-7 – [cat no. – E13652170, Sincere™, China]; VCAM-1 – [cat no. –

EK0537, Booster, USA]. All the samples were measured in duplicate. The protocol for ELISA of all the biomarkers was followed as per the manufacturer's instructions. The sensitivity or minimum detection range of the ELISA kits were E selectin <4 pg/ml, hsCRP 0.05 pg/ml, VCAM-1 <4 pg/ml, IL-10 < 0.5 pg/ml, Renin 25.5 pg/ml, Ang II 25.4 pg/ml, ACE 2 < 10 pg/ml and Ang 1–7 15.3 pg/ml. The concentrations of all the biomarkers were calculated by using standard curves as a reference. The absorbance of all the biomarkers was read at wavelength of 450 nm by using microplate reader Benchmark plus (BIORAD, USA).

2.1. Statistical analysis

The data are expressed as mean ± SD for normally distributed data and as median with inter quartile range for data which was not normally distributed. Gaussian distribution was tested by using standard normality tests: Kolmogorov - Smirnov, D′ Agostino-Pearson omnibus and Shapiro Wilk normality test. Comparison between two groups was done by using unpaired *t*-test for parametric data and Mann-whitney test for non-parametric data. The correlation between two parameters were analysed by Pearson's correlation coefficient for parametric data or spearman's rank correlation coefficient for nonparametric data. P value of less than 0.05 was consider as statistically significant. Modelling was done by stepwise multiple regression using R (version 3.4.1). All other statistical analysis was done by using GraphPad prism version 5.00 for Windows (GraphPad Software, Inc., USA).

3. Results

3.1. Comparison between FA and UA groups

In the study population, 63.4% patients showed an unfavourable axis shift (AngII/Ang1-7 ratio >1). The demographic profile and baseline characteristics of the participants of FA (n = 22) and UA (n = 38) groups are presented in Table 1 and Table 2. There was no difference in age, gender, BMI and duration of diabetes between the two groups. Both, fasting and postprandial sugar were significantly higher in UA group as compared to FA [FS (mg/dL): (219.1 ± 84.16 vs 167.6 ± 53.66); PPS (mg/dL) (281.6 ± 86.16 vs 228.8 ± 71.1), p = 0.01], respectively, while, %HbA1c was not significantly different between the groups. In UA group, total cholesterol was significant higher compared to FA [212.5 (178.3–229.3) vs 153.5 (108.8–221.5) mg/dl, p = 0.02]. However, there was no significant difference in the levels of triglycerides, LDL and HDL levels between the two groups. ACE 2 levels were significantly higher in FA group as compared to UA group [0.04 (0.02–0.08) vs 0.02 (0.01–0.03) (ng/ml), p < 0.0001].

3.1.1. Effect of RAAS balance on inflammation

Serum levels of inflammatory marker hsCRP was significantly higher [9.52 (4.64–16.19) vs 3.62 (1.77–13.09) (mg/l), p = 0.04] and anti-inflammatory marker IL-10 levels was significantly lower [2.26 (1.34–12.05) vs 10.98 (4.44–17.78) (pg/ml), p = 0.006] in UA group as compared to the FA group (Fig. 1a,b). Similarly, vascular inflammatory molecule VCAM-1 was significantly higher in the UA group [805.3 (626.3–888.5) vs 262.5 (63.86–860.9) (pg/ml), p = 0.02].

3.1.2. Effect of RAAS balance on vascular function

Percent FMD was significantly lower in UA group as compared to FA group [(5.51 ± 2.97) vs (7.66 ± 3.38) (%), p = 0.01] (Fig. 1c.). Additionally, there was a trend of higher E-selectin levels in the UA group [158.4 (33.6–396.1) vs 295.7 (119.9–751.5) (ng/ml), p = 0.05]. Carotid IMT was also significantly higher in UA as compared to FA group [0.7 (0.55–0.79) vs 0.51 (0.49–0.65) (mm), p = 0.001] (Fig. 1d.). However, there was no significant difference in the cf, cr and cd PWVs between the two groups. AIx@75 was also comparable between the two groups.

3.1.3. Effect of RAAS balance on blood pressure

There was no significant difference between the groups in both, peripheral and central systolic, diastolic and pulse pressures.

3.2. Interrelationship between diabetic state, RAAS components, inflammation, vascular function and blood pressure

The significant correlations of diabetic state with RAAS components, inflammation, vascular function and blood pressure are described in Table 3.

3.2.1. Correlation of RAAS activity with inflammation and vascular function

Renin levels showed a significant positive correlation with inflammatory marker VCAM-1 (r = 0.65, p < 0.0001) and negative with IL-10 (r = −0.33, p = 0.01).

Both, ACE 2 and Ang 1–7 showed a significant negative correlation with hsCRP [ACE 2: (r = −0.39, p = 0.002) (Fig. 3a); Ang 1–7:

Table 1
Shows the demographic profile of the participants of Favourable Axis (FA) and Unfavourable Axis (UA).

Parameters	Favourable axis (n- 22)	Unfavourable axis (n- 38)	p value
Age (Years)	46.57 ± 9.42	46.03 ± 7.41	0.8
Body Mass Index (kg/m^2)	26.93 ± 5.25	28.2 ± 4.6	0.3
Duration of diabetes (Months)	24 (15.75–54)	50.5 (22.5–87)	0.18
Peripheral Systolic B.P (mmHg)	150 (149–160)	150 (140–161.8)	0.96
Peripheral Diastolic B.P (mmHg)	98.5 (90–102)	92 (90–100)	0.33
Peripheral Pulse Pressure (mmHg)	58 (50–61.5)	60 (50–67)	0.18
Fasting Sugar (mg/dL)	167.6 ± 53.66	219.1 ± 84.16	0.01*
Post-Prandial sugar (mg/dL)	228.8 ± 71.1	281.6 ± 86.16	0.01*
Glycated haemoglobin (HbA1c) (%)	9.49 ± 2.35	9.2 ± 197	0.6
Total Cholesterol (mg/dL)	153.5 (108.8–221.5)	212.5 (178.3–229.3)	0.02*
Triglycerides (mg/dL)	168 (104.8–204.8)	162 (143–196.8)	0.49
Low density lipoproteins (mg/dL)	137.3 (104.3–157.8)	131.5 (103.6–153)	0.77
High density lipoproteins (mg/dL)	40.5 (35.25–48.25)	39 (28.75–48.25)	0.39

Values are represented as Mean ± SD for parametric data and Median with interquartile range for non-parametric data. * representing statistically significant data, P value of less than 0.05 was consider as statistically significant. B.P – Blood Pressure.

Table 2
Shows the baseline characteristics of the participants of Favourable Axis (FA) and Unfavourable Axis (UA).

Parameters		Favourable axis (n-22)	Unfavourable axis (n-38)	p value
Central Blood Pressure (mmHg)	Aortic SP	137.5 ± 14.44	140 ± 14.36	0.5
	Aortic DP	96.32 ± 12.27	96.5 ± 7.8	0.94
	Aortic PP	41.18 ± 12.58	43.53 ± 10.82	0.44
Arterial Stiffness	AI_x@75 (%)	25.23 ± 11.22	26.53 ± 8.94	0.6
	cr-PWV (m/s)	8.5 (7.9–10.03)	8.75 (7.9–10.03)	0.7
	cf-PWV (m/s)	11.1 (9–12.83)	11.5 (10.3–13.25)	0.3
	CIMT (mm)	0.51 (0.49–0.65)	0.7 (0.55–0.79)	0.001*
Endothelial Function	FMD (%)	7.66 ± 3.38	5.51 ± 2.97	0.01*
Vascular Markers (ng/ml)	E-selectin	202.7 (34.56–462.2)	295.7 (119.9–751.5)	0.1
RAAS Markers (ng/ml)	Renin	0.23 (0.12–0.35)	0.21 (0.14–0.41)	0.6
	Angiotensin II	0.1 (0.09–0.15)	0.23 (0.16–0.29)	<0.0001*
	Angiotensin 1-7	0.29 (0.21–0.37)	0.1 (0.08–0.13)	<0.0001*
	ACE 2	0.04 (0.03–0.08)	0.02 (0.01–0.03)	<0.0001*
	Ang II:Ang1-7	0.48 (0.32–0.64)	2 (1.6–2.6)	<0.0001*
Inflammatory Markers	hsCRP (mg/L)	3.62 (1.77–13.09)	9.52 (4.64–16.19)	0.04*
	VCAM-1 (ng/ml)	362.3 (64.2–888.6)	805.3 (626.3–888.5)	0.05
	Interleukin-10 (pg/ml)	10.98 (4.44–17.78)	2.26 (1.34–12.05)	0.006*

Parametric data and nonparametric data are represented as Mean ± SD and Median (interquartile range), respectively. SP: Systolic Pressure; DP: Diastolic Pressure; PP: Pulse Pressure; AI_x@75: Augmentation Index @ Heart Rate 75; cr-PWV: carotid radial Pulse Wave Velocity; cf-PWV: carotid femoral Pulse Wave Velocity; FMD: Flow-Mediated Dilation; Ang II: Angiotensin II; Ang 1–7: Angiotensin 1-7; VCAM-1: Vascular cell adhesion molecule; RAAS: Renin angiotensin aldosterone; ACE 2: Angiotensin Converting enzyme 2; hsCRP: High sensitivity C- reactive protein. * representing statistically significant data, P value of less than 0.05 was consider as statistically significant.

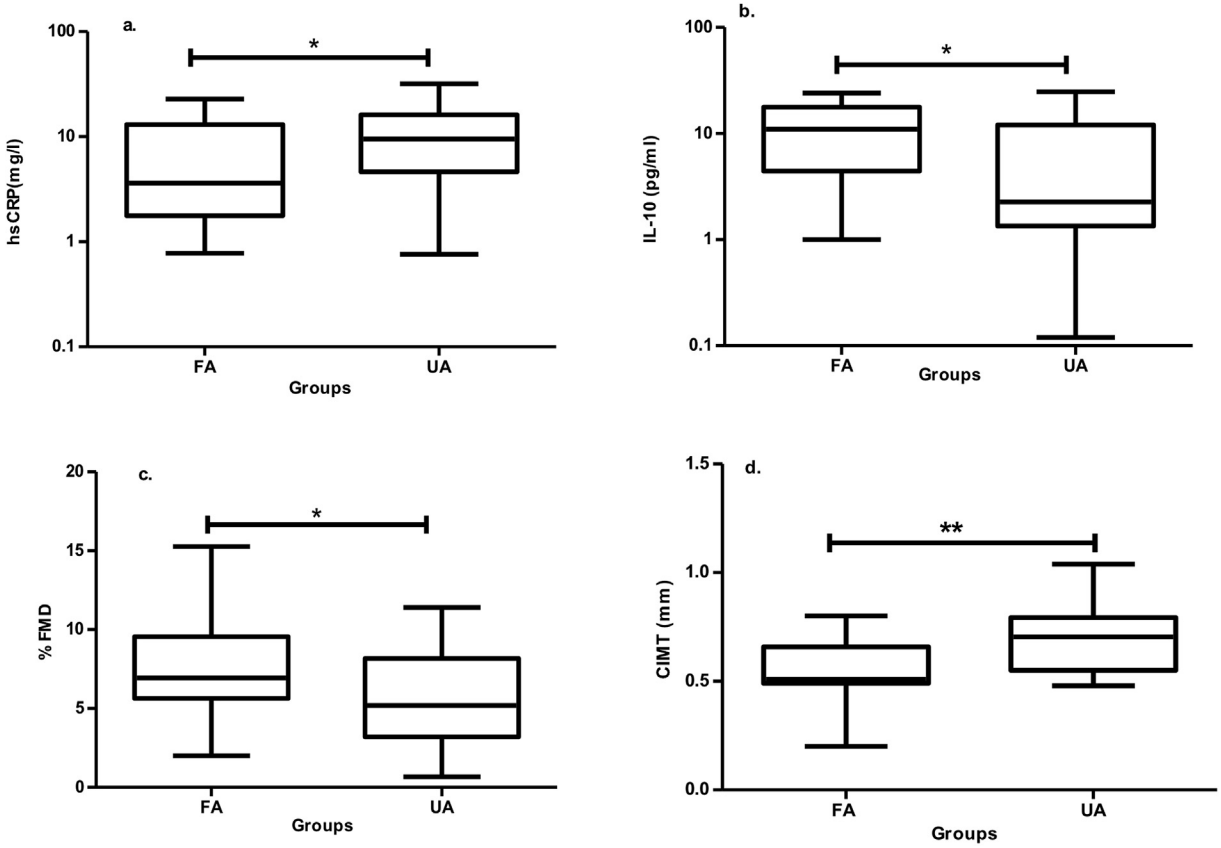

Fig. 1. Figure shows the Comparison between Favourable axis (FA) and Unfavourable axis (UA) groups: a.) High Sensitive C- Reactive Protein (hsCRP), milligram/litre (mg/l) b.) Interleukin-10 (IL-10) picogram/millilitre (pg/ml), c.) Percentage Flow Mediated Dilation (%FMD) d.) Carotid Intima Media Thickness (CIMT) millimetre (mm) comparison between favourable and unfavourable axis. p value of less than 0.05 was consider as statistically significant. * represents $p < 0.05$, ** represents $p < 0.01$.

($r = -0.44$, $p < 0.0001$)] (Fig. 2a) and positive correlation with IL-10 [ACE 2: ($r = 0.54$, $p < 0.0001$) (Fig. 3b); Ang 1–7 ($r = 0.5$, $p < 0.0001$)] (Fig. 2b). ACE 2 also showed a significant negative correlation with VCAM-1 ($r = -0.31$, $p = 0.01$).

A significant positive correlation was seen between renin levels and levels of E– selectin ($r = 0.55$, $p < 0.0001$). Renin levels showed a significant positive correlation with cr and cf PWV ($r = 0.47$, $p = 0.0001$ and $r = 0.45$, $p = 0.0003$ respectively). Renin also showed a significant positive correlation with AIX@75 ($r = 0.3$, $p = 0.01$).

Serum levels of ACE 2 showed a significant negative correlation with central as well as peripheral indices of arterial stiffness i.e. cf-PWV and cr-PWV [($r = -0.26$, $p = 0.04$) and ($r = -0.3$, $p = 0.02$),

Table 3
Shows Inter-relationship between diabetic state, RAAS components, inflammation, anti-inflammation, vascular function and blood pressure.

Parameters			R	p value
Diabetic State & Blood Pressures	Fasting Sugar	Systolic Blood Pressure	0.28	0.03
		Systolic Blood Pressure	0.29	0.02
	Post-Prandial Sugar	Pulse pressure	0.25	0.04
		Aortic Diastolic Blood Pressure	0.3	0.02
		Aortic Pulse Pressure	0.35	0.005
		Diastolic Blood Pressure	−0.31	0.01
		Pulse Pressure	0.3	0.01
	Duration of diabetes			
Diabetic state & Vascular Function	Fasting sugar	E-selectin	0.37	0.003
	Post-Prandial Sugar	carotid-radial-PWV	0.27	0.02
	HbA1c	carotid-femoral PWV	0.25	0.04
	Duration of diabetes	Carotid Intima Media Thickness	0.27	0.03
		E-selectin	0.32	0.01
		carotid-radial-PWV	0.26	0.04
		E-selectin	0.26	0.04
		carotid-femoral PWV	0.31	0.01
		carotid-femoral PWV	0.29	0.02
Diabetic state & RAAS	Fasting Sugar	Angiotensin 1-7	−0.31	0.01
	Post-Prandial Sugar	Angiotensin Converting Enzyme 2	−0.28	0.03
		Angiotensin 1-7	−0.3	0.01
		Angiotensin Converting Enzyme 2	−0.4	0.001
Diabetic state & Inflammation	Fasting Sugar	Interleukin −10	−0.35	0.004
	Post-Prandial sugar	Interleukin −10	−0.32	0.01

The correlation between two parameters were analysed by Pearson's correlation coefficient for parametric data or spearman's rank correlation coefficient for nonparametric data. P value of less than 0.05 was consider as statistically significant.

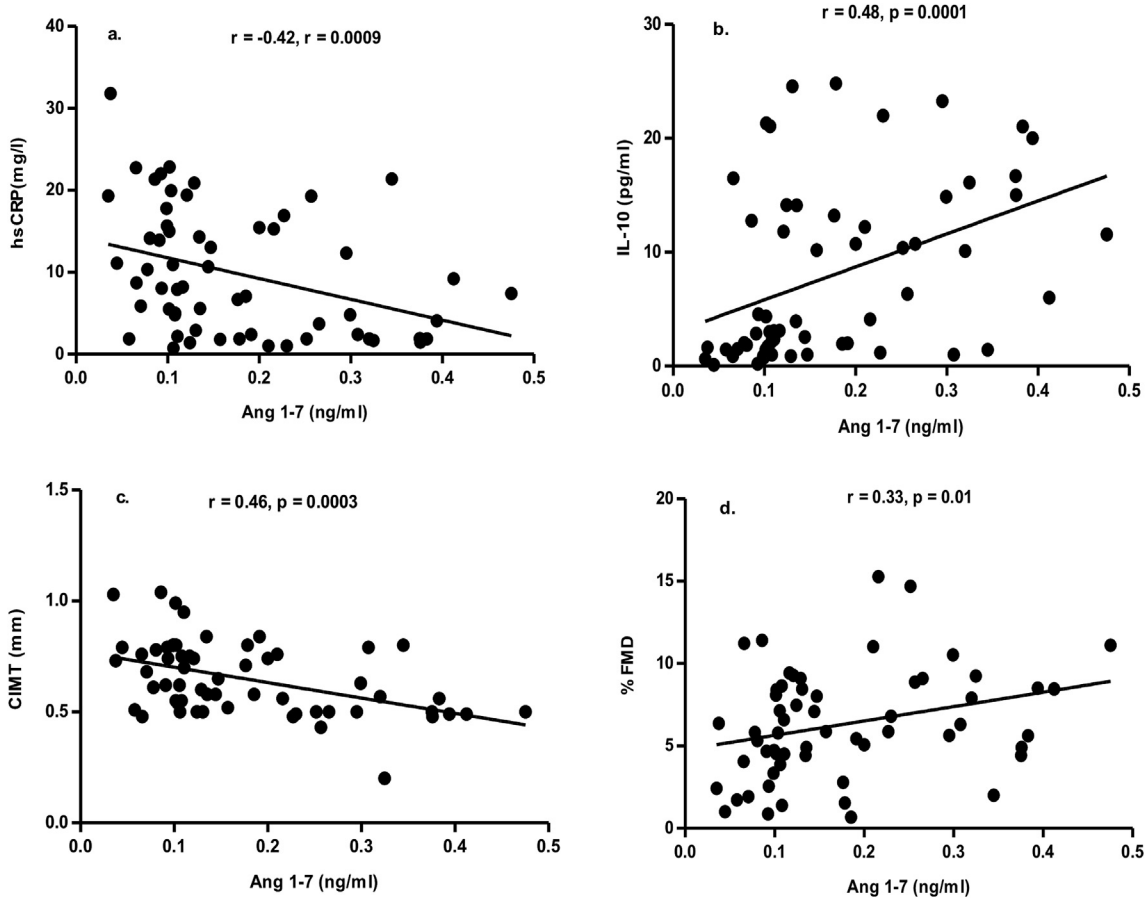

Fig. 2. Figure shows a significant correlation of Angiotensin Converting Enzyme 2 (ACE 2) a.) negative correlation with High Sensitive C- Reactive Protein (hsCRP), b.) positive correlation with Interleukin-10 (IL-10) c.) negative correlation with Carotid Intima Media Thickness (CIMT) d.) negative correlation with carotid-radial pulse wave velocity (cr-PWV). p value of less than 0.05 was consider as statistically significant.

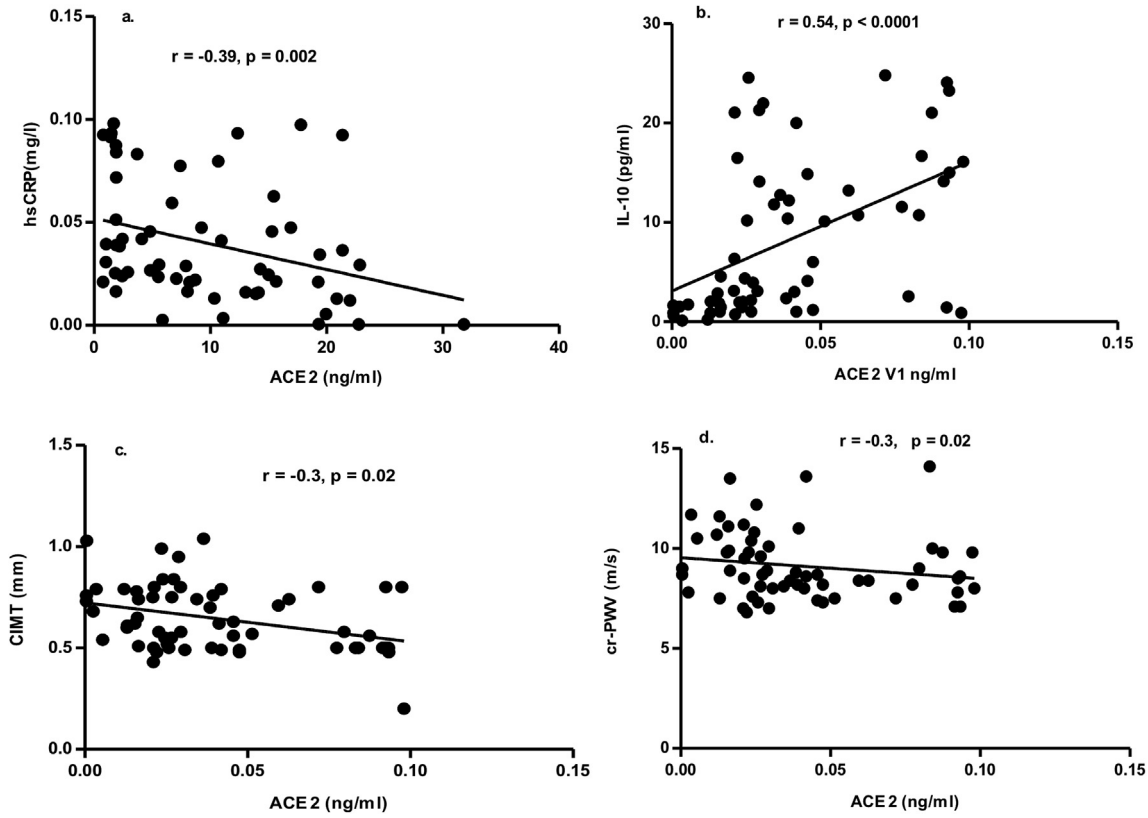

Fig. 3. Figure shows a significant correlation of Angiotensin 1-7 (Ang 1–7) a.) negative correlation with High Sensitive C- Reactive Protein (hsCRP), b.) positive correlation with Interleukin-10 (IL-10) c.) negative correlation Carotid Intima Media Thickness (CIMT) d.) positive correlation with Percentage Flow Mediated Dilation (%FMD). P value of less than 0.05 was consider as statistically significant.

respectively] (Fig. 3d). ACE 2 also correlated negatively with CIMT ($r = -0.3$, $p = 0.02$), and E-selectin ($r = -0.37$, $p = 0.003$) (Fig. 3c). Likewise, Ang 1–7 levels showed a significant negative correlation with cf-PWV ($r = -0.33$, $p = 0.009$), CIMT ($r = -0.47$, $p = <0.0001$) and with E-selectin ($r = -0.33$, $p = 0.009$) (Fig. 2c). Ang 1–7 also showed a significant positive correlation with %FMD ($r = 0.33$, $p = 0.01$) (Fig. 2d).

3.2.2. Correlations of inflammation with vascular function

A significant positive correlation was observed between hsCRP and carotid intima media thickness ($r = 0.4$, $p = 0.001$). VCAM-1 also correlated positively with E-selectin levels, cf-PWV and AIX@75 [($r = 0.69$, $p < 0.0001$), ($r = 0.34$, $p = 0.007$) and ($r = 0.35$, $p = 0.006$), respectively).

IL-10 showed a significant negative correlation with E-selectin, cf-PWV, cr-PWV and CIMT [($r = -0.32$, $p = 0.01$); ($r = -0.37$, $p = 0.003$); ($r = -0.38$, $p = 0.003$) and ($r = -0.48$, $p = 0.0001$), respectively] and significant positive correlation with %FMD ($r = 0.35$, $p = 0.005$).

3.2.3. Correlation of RAAS activity, inflammation and vascular function with blood pressure

Renin levels correlated positively with peripheral and central PP [($r = 0.34$, $p = 0.006$) and ($r = 0.33$, $p = 0.01$), respectively]. On the other hand, Ang 1–7 showed a negative correlation with both peripheral pressures; Systolic and Pulse pressure [(SBP $r = -0.27$, $p = 0.03$) and (PP $r = -0.3$, $p = 0.01$)] and central pressures; Aortic systolic and Aortic Diastolic Pressure [($r = -0.28$, $p = 0.03$) and ($r = -0.29$, $p = 0.02$), respectively]. A significant negative correlation was also found between ACE 2 and peripheral pulse pressure and central aortic systolic pressure [($r = -0.3$, $p = 0.02$) and ($r = -0.26$, $p = 0.02$), respectively].

IL-10 showed a significant negative correlation with peripheral and central systolic pressure [SBP ($r = -0.3$, $p = 0.02$); ASP ($r = -0.29$, $p = 0.02$)] and peripheral pulse pressure ($r = -0.36$, $p = 0.004$).

A significant positive correlation was observed between E-selectin and pulse pressure ($r = 0.31$, $p = 0.01$). A significant positive correlation was observed between AIx@75 and peripheral DBP [($r = 0.35$, $p = 0.005$) and between AIx@75 and central Systolic and Pulse Pressure [Aortic SBP $r = 0.33$, $p = 0.008$; Aortic PP $r = 0.33$, $p = 0.009$]. Similarly, cf-PWV also correlated positively with peripheral and aortic pulse pressure (PP $r = 0.35$, $p = 0.004$; Aortic PP $r = 0.27$, $p = 0.03$).

To identify factors influencing blood pressure, stepwise-multiple regression modelling was performed for aortic mean and pulse pressures. For aortic mean pressure, 7 key variables emerged in the model, which are – DOD, Ang II, Ang II/Ang 1–7 ratio, IL10/hsCRP ratio, fasting and post prandial blood sugar levels. Of these, Ang II emerged as an independent predictor of aortic mean blood pressure ($p = 0.04$) while Ang II/Ang 1–7 ratio appeared to be an important variable in the model ($p = 0.09$). The model explained 21.5% of the total variability in aortic MBP.

The model for aortic PP includes Age, BMI, Ang 1–7, Ang II/Ang 1–7, IL 10, HbA1c and FMD. Of these, Age, Ang II/Ang 1–7 ratio and Triglyceride levels emerged as independent predictors of aortic PP [($p = 0.02$), ($p = 0.03$) and ($p = 0.03$), respectively]. The model explained 27.4% of the total variability in aortic PP.

4. Discussion

The effect of activation of RAAS in the pathogenesis of hypertension has been well established [15]. In light of recent animal studies on ACE 2 and Ang 1–7, an opposing cardio-protective role of RAAS [16] is also being considered. It therefore appears that a balance between the two arms of the RAAS would be crucial in the development and progression of cardiovascular diseases (CVD). In the current study, we examined the effect of an imbalance in RAAS components on vascular dysfunction and inflammation which are the critical factors underlying the development of CVD.

Group-wise comparison revealed that patients with an unfavourable axis had higher levels of fasting and post-prandial sugar levels. Fasting and postprandial blood sugar levels also correlated with Ang 1–7 and ACE 2 levels. In a study on rats, glucose was shown to increase Ang II production by mesangial cells in a concentration-dependent manner [17]. Additionally, RAS blockade by ACE inhibitors have shown to ameliorate glycaemic control in diabetic patients. Evidence is also emerging to suggest that ACE2 – Ang 1–7 axis mediated activation of Mas receptors may have an important role in regulating insulin sensitivity. $Mas^{-/-}$ mice have shown to exhibit elevated levels of leptin and insulin in addition to causing impaired insulin sensitivity and glucose tolerance [18]. These mice also display elevated serum and muscle triglycerides and blood cholesterol levels. In the current study, patients in the unfavourable axis group had higher levels of total cholesterol as compared to those with favourable axis. Ang II has been shown to upregulate mRNA for HMG-CoA reductase, which is blocked by Fluvastatin [19]. Indirect evidence through studies on statins have shown that statin therapy alone can also result in lowering of blood pressure [20]. While the exact mechanisms have not been deciphered, this effect has been postulated to be mediated by inhibition of the RAAS by statins. This suggests that an imbalance between Ang II and Ang 1–7 can exacerbate the disease state by potentiating hyperglycaemia and dyslipidaemia which are key regulators of vascular function and inflammation.

In the current study, hsCRP and VCAM-1 levels were higher in patients with an AngII/Ang 1–7 ratio >1 while IL-10 levels were lower than in those with a ratio <1. Additionally, both ACE 2 and Ang 1–7 showed a negative correlation with pro-inflammatory and a positive correlation with anti-inflammatory markers. Ang 1–7 has been shown to reduce Ang II mediated expression of VCAM-1 in cultured endothelial cells [21]. The anti-inflammatory effects of Ang 1–7 have been established over the past few years in multiple studies which have elucidated the pathways activated by ACE2/Ang 1–7/Mas axis and the regulation of cytokine levels by the axis. Disease models have also been used to establish the role of ACE 2/Ang 1–7 activation in reduction of inflammation in obesity, chronic renal failure etc [22]. However, to the best of our knowledge, this is the first study to provide evidence of a direct association of ACE 2 and Ang 1–7 with inflammatory markers and the effect of Ang II/Ang 1–7 imbalance on the inflammatory state.

An imbalance in Ang II/Ang 1–7 levels also showed a higher propensity for vascular dysfunction and arterial stiffness as determined by lower %FMD and higher E-selectin levels and carotid IMT in patients with an unfavourable axis as compared to those with a favourable axis. In a study on spontaneously hypertensive stroke-prone rats (SHSPR), presence of transgenic ACE 2 was found to result in lower blood pressure and better endothelial function by an increase in production of Ang 1–7 in comparison to rats without SHSPR [23]. In the current study, Ang 1–7 levels were found to correlate positively with %FMD and ACE 2 showed a negative correlation with E-selectin levels. To the best of our knowledge, this is the first study in humans to report a lower endothelial function in humans with an imbalance in the AngII/Ang 1–7 ratio. Ex-vivo treatment in renal arteries of diabetic patients with Ang 1–7 improved endothelium dependent relaxation and ACE2/Ang 1–7 activation was found to lower reactive oxygen species in diabetic mice which suggests that ACE2/Ang 1–7 activation preserves endothelial function in diabetic mice by increasing NO bioavailability and decreasing reactive oxygen species [24].

Impairment of endothelial function could be possibly mediated by the inflammation induced by RAAS imbalance. A significant positive correlation was observed between VCAM-1 and E-selectin. While IL-10 showed a negative correlation with E-selectin and a positive correlation with FMD. In a recent study, ACE 2 gene transfer

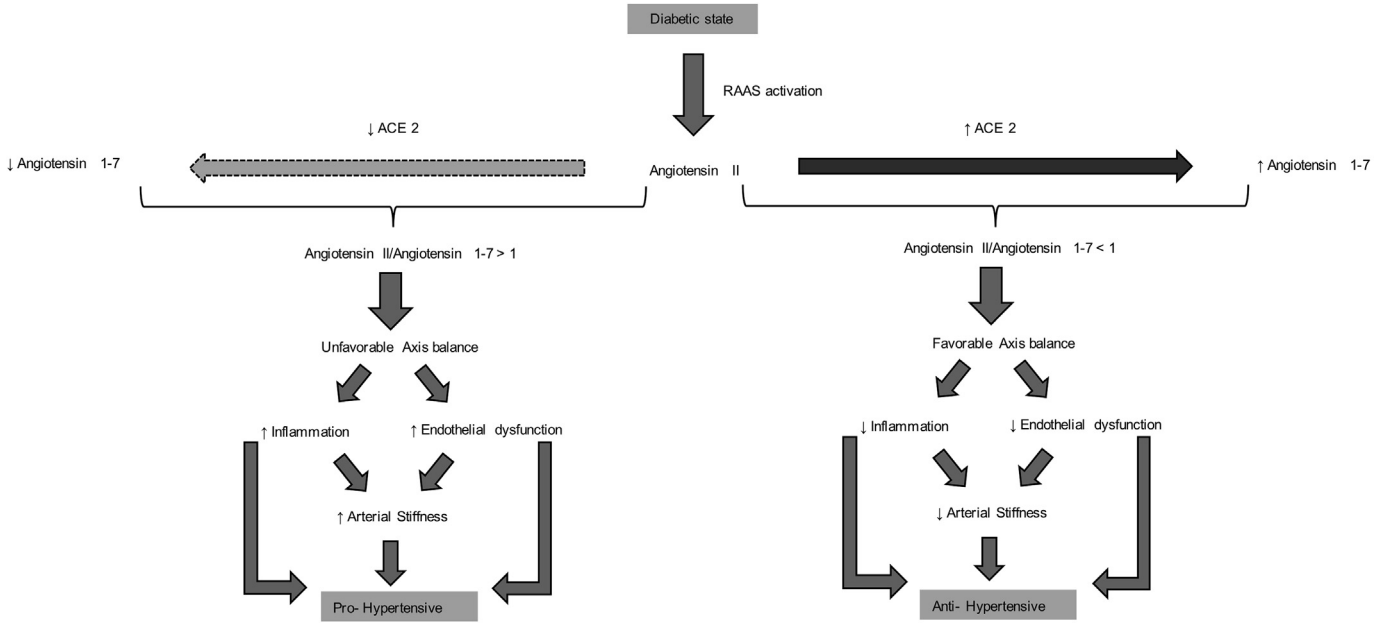

Fig. 4. Simplified diagram showing the favourable and unfavourable axis of renin angiotensin aldosterone system and its effects on vascular function and inflammation. Decrease in Angiotensin Converting Enzyme 2 (ACE 2) levels results in a decrease in concentration of Angiotensin 1-7 which would cause a shift of balance towards unfavourable axis leading to endothelial dysfunction, inflammation and increased arterial stiffness.

in human endothelial cells resulted in a decrease in Ang II induced production of VCAM-1, Monocyte Chemoattractant Protein-1 (MCP-1) and E-selectin in-vitro and also decreased the levels of these factors in in-vivo conditions [25].

Structural stiffening of the vessels can result from the cumulative effects of endothelial dysfunction and inflammation. In the present study, both E-selectin and FMD showed a positive and negative correlation respectively with CIMT. In addition, E-selectin also showed a positive correlation with cf-PWV. The inflammatory markers, hsCRP and VCAM-1 correlated positively with CIMT and cfPWV respectively while IL-10 showed a negative correlation with both cfPWV and CIMT.

The net effect of RAAS imbalance, impaired vascular function, inflammation and arterial stiffening can contribute towards the development of hypertension in diabetics (Fig. 4). The results of the current study show positive association between Renin and pulse pressure and negative associations of Ang 1–7 and ACE 2 with central and peripheral pressures. E-selectin, AIx@75 and cfPWV also correlated positively with multiple peripheral and central blood pressure indices. Additionally, IL-10 showed a negative correlation with both peripheral and central SBP. While no difference in blood pressure values was observed in group-wise comparison of favourable and unfavourable RAAS axis, in the multiple regression models, Ang II emerged as a significant predictor of central mean blood pressure, while Ang II/Ang 1–7 ratio was a significant predictor of central pulse pressure.

The current study assesses associations in a cross-sectional design which restricts the mechanistic interpretation of the data and is a limitation of the study. The small sample size is another limitation in the current study. We only assessed the levels of circulating RAAS components and so the role of local RAAS activation cannot be commented upon. However, To the best of our knowledge, this is the first study in humans to comprehensively evaluate both arms of the renin-angiotensin-aldosterone system and their differential effects on inflammation, vascular structure and function. The study provides valuable insight into the associations of the opposing arms of RAAS with vascular function and inflammation, the effect of RAAS imbalance on these factors and their possible role in the development of hypertension in diabetes.

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Declaration of conflicting interest

The author(s) declare(s) that there are no potential conflicts of interest with respect to the research, authorship and/or publication of this article.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.dsx.2019.04.042.

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