Original article

The effect of serum angiotensin II and angiotensin II type 1 receptor gene polymorphism on pediatric lupus nephritis

Background: *Angiotensin II (Ang II) is found to perpetuate inflammation and visceral damage in systemic lupus erythematosus (SLE). It mediates most of its actions through Ang II receptor type I (AT1) whose gene polymorphism A1166C (CC genotype) seems to have pathogenic effects.* ***Objective:*** *To measure serum Ang II and the frequency of AT1 receptor CC genotype among a group of Egyptian patients with pediatric onset lupus nephritis (pLN).* ***Methods:*** *This is a case-control cross sectional study which included 24 patients with pLN and 24 age and sex-matched healthy subjects as controls. Clinical evaluation and routine laboratory markers for SLE patients were done. SLE disease activity index (SLEDAI) and the British Isles Lupus Assessment Group (BILAG)-2004 renal score were measured. Serum Ang II was measured by enzyme linked immunosorbent assay and detection of ATI receptor CC genotype by polymerase chain reaction were done for both patients and controls.* ***Results:*** *Patients had significantly higher serum Ang II than the controls (p=0.0001). The frequency of AT1 receptor CC genotype was significantly higher among patients as compared to the control group (p=0.008). Both serum Ang II and AT1 receptor CC genotype were comparable between patients with proliferative LN class III and IV and those with LN class II (p>0.05). Serum Ang II did not correlate significantly with SLEDAI or BILAG-renal score (p>0.05).* ***Conclusion:*** *Serum Ang II and AT1 receptor CC genotype seem to have pathogenic role in pLN but with no deleterious effects on the phenotype of LN for further assessment.*

Key words: Lupus nephritis, Angiotensin II, Angiotensin II type 1 receptor, Polymorphism, Pediatrics.

Shereen S. El-Sayed, Dalia H. El-Ghoneimy, Dina A. Soliman*, Marwa T. Mohamed, Shereen M. Gamal.

Departments of Pediatrics and Clinical Pathology, Faculty of Medicine, Ain Shams University, Cairo, Egypt.*

Correspondence:
Dalia H. El-Ghoneimy, 23 El-Shorta Street, Gesr El-Suez, 11321, Cairo, Egypt.
E-mail: *dalia.elghoneimy @gmail.com*

INTRODUCTION

Renin angiotensin system (RAS) has been considered one of the probable pathophysiologic mechanisms involved in SLE progression. However, the contribution of the RAS in SLE patients to the development of renal disease is less clear.[1]

Angiotensin II (Ang II) is the primary effector molecule of the RAS. It is an octapeptide that is produced both systemically and locally in various tissues, including the heart and blood vessel walls.[2] Moreover, Ang II has the ability to augment and perpetuate immune responses in several tissues and acts as strong candidate for the perpetuation of visceral damage, vascular autoimmunity and nephritis in SLE.[3]

There are two well-described subtypes of Ang II receptors, designated Ang II type 1 receptor (AT1) and Ang II type 2 receptor (AT2), both of which have a high affinity for Ang II.[4] The Ang receptors are a class of G protein-coupled receptors with Ang II as their ligands.[5] Most of the known physiological effects of Ang II are mediated by AT1 receptors, which are widely distributed in all organs, including liver, adrenals, brain, lung, kidney, heart, and vasculature.[6]

Ang II contributes to the recruitment of infiltrating cells into the kidney and causes the adhesion of circulating cells to endothelial and mesangial cells, and the migration of inflammatory cells into the kidney. This process is mediated by upregulation of adhesion molecules, cytokines and chemokines such as vascular cell adhesion molecule-1 (VCAM-1), intercellular adhesion molecule-1 (ICAM-1), interleukin-6 (IL-6) and monocyte chemoattractant protein-1 (MCP-1).[7] Based on the action of renal AT1 receptors to induce kidney damage in autoimmune nephritis, blockade of the RAS with angiotensin converting

enzyme inhibitors (ACEIs) or angiotensin receptor blockers (ARBs) may be useful in protecting the kidney in patients with lupus or other autoimmune disorders.[8]

With this as a background, we aimed in this study to measure serum Ang II and the frequency of AT1 receptor gene polymorphism A1166C (CC genotype) among a group of Egyptian children with lupus nephritis (LN). The relationship of serum Ang II and AT1 receptor gene polymorphism with clinical and laboratory markers of lupus nephritis was fully elucidated.

METHODS

Study design and population:

This is a case-control cross sectional study which included 24 patients with pediatric SLE (pSLE) fulfilling at least four of the revised American College of Rheumatology "ACR" classification criteria for SLE. [9] The study was approved by the Ethical Committee of the Department of Pediatrics, Ain Shams University, Cairo, Egypt. An informed consent was obtained from the parents or care-givers of the studied groups prior to enrollment.

SLE patients group:

They were 20 (83.3 %) females and 4 (16.7 %) males. Their ages ranged between 5 and 18 years with a mean ± SD of 13.9 ± 3.5 years. All patients had LN (biopsy –proven) classified according to the revised classification of WHO criteria of LN. [10] Ten (41.7 %) patients had LN class II, 13 (52%) had LN class III and IV and 1 (4%) patient had LN class V.

Hypertension was diagnosed according to the percentiles of blood pressure for age in pediatrics. [11] Eight (33.3 %) patients were hypertensive and 16 (66.7%) were normotensive.

Inclusion criteria:

- Age at enrolment younger than 18 years.
- Biopsy proven lupus nephritis according to revised classification of WHO criteria of lupus nephritis for patients with pSLE.

Exclusion criteria:

- Patients receiving angiotensin converting enzyme inhibitors (ACEIs) or angiotensin receptor blockers (ARBs) as they interfere with Ang II production and action as well as with 24-hours urinary protein measurement.

Control group:

It included 24 age and sex-matched healthy children. They were 18 (75%) females and 6 (25%) males. Their ages ranged between 5 and 16 years with a mean ± SD of 12.4 ± 3.4 years.

Study measurements:

Clinical evaluation:

Detailed history taking with special emphasis on age at onset of SLE, duration and presentation of LN. Cumulative doses of steroids, azathioprine and cyclophosphamide were calculated. Detailed antihypertensive drug history was taken. Global disease activity was measured by the SLE disease activity index (SLEDAI)[12] while the British Isles Lupus Assessment Group (BILAG)-2004 renal score was used to assess renal involvement[13] and Systemic Lupus International Collaborating Clinics/American College of Rheumatology (SLICC/ ACR) Damage Index (SDI) was used to assess SLE related damage.[14]

Laboratory Work:

Routine laboratory investigations for LN patients: Complete blood count by Coulter Max MUG–HL–CCl and erythrocyte sedimentation rate (ESR) by Westergren method. Biochemical parameters measured included serum creatinine, cholesterol, triglycerides, high density lipoproteins, and low density lipoproteins and 24-hours urine proteins and urine creatinine which were assayed by enzymatic procedures using Beckman Synchron CX7 Clinical System (Beckman Coulter Inc. California, USA), detection of anti-double stranded DNA (anti-ds DNA) by ELISA (quanta lite ds ELISA.www.nt-protons.com), and serum complement-3 (C3) using MININEPH TM (Binding Site Ltd Birmingham, UK).

Detection of serum angiotensin II and AT1 receptor CC genotype for patients and controls:

Serum angiotensin II was measured by quantitative ELISA#(supplied by Wuhan Eiaab Science Co., Ltd"A1710 Guangguguoji, East Lake Hi-Tech Development Zone, Wuhan 430079, China). According to manufacturer's instructions; a reference curve is used to ascertain the concentration of angiotensin II in unknown specimens by comparing the optical density (OD) of the samples to the standard curve. Detection Range is 31.2-2000 pg/mL.

Detection of AT1A receptor genotype A1166C by PCR-restriction fragment length polymorphisms (PCR RFLP) using thermal cycler (PCR EXPRESS-HYBAID):

- DNA was isolated from whole blood using Gentra Generation capture column kit according to the manufacturer's instructions (13355 10th Avenue North, suite 120 Minneapolis, Minnesota 55441 USA).
- The extracted DNA was amplified in a 25 µl mixture tube consisting of 5 uL of DNA extract

(40ng DNA), 4.5 uL of RNase-Free water, 12.5 ul of the ready to use master mix supplied by QIAGEN (www.qiagen.com/goto/TechSupportCenter), and 1.5uL of each of the forward primer '5-AATGC'TTGTAGCCAAAGTCACCT-3' and reverse prime5'-GGCTTTGCTTTGTCTTGTTG-supplied by Midland certified reagent company (www.oligos.com/thioIModified.htm).

- Thermo cycling consisted of an initial denaturation at 94°C for two minutes, then 40 cycles of: denaturation at 94°C for one minute, annealing at 60°C for one minute, extension at 72°C for two minutes. A final extension time of 72°C for 10 minutes. The 1166 C allele contains an additional recognition site for the restriction endonuclease DdeI that is absent in the A 1166 allele. Accordingly, PCR amplification products were digested at 37°Cwith DdeI supplied by NEW ENGLAND BioLabs (www.neb.com.) and electrically separated on a 2% agarose ethidium bromide gel supplied by Fermentas (www.fermentas.com).
- According to the band length of restriction fragment, genotypes defined for AT1 receptor were homozygous AA genotype if the band length equal 546 bp (uncut), homozygous CC genotype if the band is divided into 2 parts: 435 bp and 111 bp length and heterozygous AC genotype if it yield 3bands 546,435 and 111 bp.

Statistical methods:

Statistical analysis was performed using Statistical Package for Social Sciences, Version 17.0 (SPSS, Inc., Chicago, III., USA) for Windows. Continuous variables were analyzed as mean values ± standard deviation (SD) or median (range) as appropriate. Rates and proportions were calculated for categorical data. Kolmogorov-Smirnov test of normality was done to assess normality of continuous variables before starting the analysis especially in groups with small numbers. Differences among continuous variables with normal distribution were analyzed by Student's T-test. Mann-Whitney test for non-parametric quantitative data and Chi-square test for comparing categorical variables. Pearson's Correlations and non-parametric analogue Spearman Rho were used. Receiver Operating Characteristic (ROC) Curve was designed which is a plotting of sensitivity versus 1-specificity at different cutoff values of the studied variable. P value of <0.05 was considered statistically significant and less than 0.001 was considered as highly significant.

RESULTS

The clinical and the laboratory data of the studied patients are shown in table 1:

Inactive SLE was reported in 6 (24%) patients with SLEDAI = 0. Twelve (48%) patients had mild SLE activity (SLEDAI= 2– 4 with a median of 2) and 6 (24%) had moderate lupus activity (SLEDAI = 6–10 with a median of 6).

Assessment of LN using BILAG–renal score revealed that 15 (60%) patients had inactive LN with BILAG D score, 5 (20%) patients had mild LN activity with BILAG C score and 5 (20%) had moderate renal activity with BILAG B score.

Lupus related damage as assessed by SDI was absent in 23 (92%) patients where SDI was zero. Only 2 (8%) patients had a SDI of 2. The first one was an 18-years old female with LN class III who experienced persistent pericardial effusion and pulmonary fibrosis. The other patient was a 12-years old female with LN class II who had cataract and pulmonary fibrosis.

All (100%) patients were on steroid therapy at a dose of (0.1-0.5 mg/kg/day). Ten (40%) patients were on pulsed intravenous cyclophosphamide at a dose of (500-750 mg/m^2/month) and 13 (52%) patients were on azathioprine at a dose of (1.5-3 mg/kg/day).

Serum Ang II and AT1 receptor CC genotype among patients and controls:

Patients had significantly higher serum Ang II than the controls (Z= -6.3, p=0.0001) (figure 1). The best cut-off value of serum Ang II was 155 pg/ml with 100% sensitivity and 100 % specificity. So, levels above 155 pg/ml were considered abnormally high (figure 2). The frequency of AT1 receptor CC genotype was significantly higher (58.3%) among patients as compared to the control group (28.5%) (X^2= 7.1, p=0.008) (figure 3).

Variation of serum Ang II in relation to AT1 receptor genotype:

Patients with AT1 receptor CC genotype had comparable serum Ang II to those with wild AT1 receptor (AA genotype) (table 3).

Variation of Serum Ang II and AT1 receptor CC genotype in relation to the age and gender of the patients and controls:

There was no significant correlation between serum Ang II and the age of the patients or controls (p>0.05). Similarly, serum Ang II was comparable among male and female patients and controls (p>0.05). No gender difference was observed in the frequency of AT 1 receptor CC genotype of patients and controls (p>0.05).

The relationship between serum Ang II and AT1 receptor CC genotype and the age at onset of SLE:
There was no significant correlation between serum Ang II and the age at onset of SLE (r= 0.007, p>0.05). Also, patients with AT1 receptor CC genotype had comparable age at onset of SLE to those with wild AT1 receptor (AA genotype) (Z=-1.35, p>0.05).

The variation with the histological class of LN:
Serum Ang II and the frequency of AT1 receptor CC genotype were comparable in patients with proliferative LN (LN class III and IV) and those with LN class II (p>0.05).

The effect of serum Ang II and AT1 receptor CC genotype on the blood pressure:
Serum Ang II was comparable between hypertensive and normotensive LN patients (t=0.47, p=0.05). Also, the frequency of AT1 receptor CC genotype did not vary significantly between these patients (X^2 =1.37, p>0.05).

The relation to the studied laboratory indices:
Serum Ang II did not correlate significantly with serum C3, ESR and anti-dsDNA as well as creatinine clearance, 24 hours urinary proteins and serum lipids (table 2). Similarly, patients with AT1 receptor CC genotype and those with wild AT1 receptor (AA genotype) were comparable concerning the same laboratory markers (table 3).

The effect on SLE activity and related damage:
Serum Ang II did not correlate significantly with SLEDAI (r = 0.15, p>0.05), BILAG-renal score (r=-0.19, p>0.05), moreover, serum Ang II did not vary significantly between different grades of SLEDAI (F=0.22, p>0.05). Serum Ang II did not correlate significantly with SDI for lupus related damage (r=-0.02, p>0.05). Also, patients with AT1 receptor CC genotype and those with wild AT1 receptor (AA genotype) had comparable SLEDAI (Z=-0.27, p>0.05), BILAG-renal score (Z=0.51, p>0.05) and SDI (Z=-0.24, p>0.05). The frequency of AT1 receptor CC genotype was comparable among patients in remission and those with mild and moderate SLEDAI (X^2=0.68, p>0.05).

The effect of the immunosuppressive therapy:
Serum Ang II was not affected significantly with the types or the doses of the immunosuppressive therapy namely steroids (r=0.09, p>0.05), cyclophosphamide (r=-0.0006, p>0.05) and azathioprine (r=-0.09, p>0.05). Patients with AT1 receptor genotype A1166C and those with wild AT1 receptor (AA genotype) received comparable doses of steroids (Z=-0.84, p>0.05), and azathioprine (Z=-0.5, p>0.05).

Table 1. The clinical and the laboratory data of the studied patients.

N=24	Mean (range)	SD	Median (IQR)
Age (years)	13.9 (5-18)	3.6	15 (3)
Age at onset of SLE (years)	10.3 (3-16)	3.1	11(3.3)
Duration (years)	3.6 (1-12)	2.6	3(1.8)
C3 (mg/dl)	103 (37-205)	41.4	96.7(168)
Anti-ds DNA (IU/ml)	368.8 (45-1365)	360.8	200.5(565.5)
ESR (mm/hr)	26.5 (5-75)	16.3	22(23.8)
Serum creatinine (mg/dl)	0.5 (0.3-0.9)	0.15	0.45(0.18)
Creatinine clearance (ml/ min / 1.73m^2)	116.3 (22-323)	63.7	102(35.3)
24 hours urinary proteins (gms)	0.36 (0-4)	0.81	0.1(0.2)
Triglycerides (mg/dl)	147.8 (47-250)	61.5	157(107.3)
Low density lipoproteins (mg/dl)	140.7 (45-250)	47	138(66.3)
High density lipoproteins (mg/dl)	49.5 (10-150)	27.9	40(24.3)
Cholesterol (mg/dl)	201 (115-259)	44.4	210(67.8)
Serum Ang II (pg/ml)	307.5 (180-500)	81.6	300 (130)
SLEDAI	3.1 (0-10)	2.8	2(5)
BILAG-renal score	0.8 (0-3)	1.2	0(1)
SDI	0.2 (0-2)	0.6	0
Cumulative dose of steroids (gms)	16.3 (4.9-32.3)	8.7	13.5(16.2)
Cumulative dose of cyclophosphamide (gms)	4.1 (1.5-10)	3.1	2.75(4.5)
Cumulative dose of azathioprine (gms)	47.9 (8-94)	24.4	45.6(31)

N: number, **SD:** Standard Deviation**, IQR:** interquartile range, **SLE:** Systemic lupus erythematosus, **C3:** complement-3, **Anti-dsDNA:** Anti-double stranded deoxyribonucleic acid, **ESR:** Erythrocyte sedimentation rate, **hr:** hour, **Ang II**: angiotensin II, **SLEDAI:** Systemic lupus erythematosus disease activity index, **BILAG:** British Isles Lupus Assessment Group, **SDI:** Systemic Lupus International Collaborating Clinics/American College of Rheumatology (SLICC/ACR) damage index.

Table 2. The relation of the studied laboratory markers to serum Ang II

LN patients n (%) = 24 (100%) Studied immunological markers and routine laboratory data	**S.Ang II** (pg/ml)	
	r	**P**
C3	-0.2	0.33
Anti-dsDNA	0.19	0.38
ESR	0.25	0.24
Serum creatinine	-0.54	0.22
Creatinine clearance	0.3	0.15
24hrs. Urinary proteins	0.2	0.3
S. Chol	0.06	0.7
S. TG	-0.11	0.6
HDL	-0.2	0.3
LDL	-0.16	0.4

LN: Lupus nephritis, S.Ang II: serum angiotensin II, n: number, %: percentage, SD: standard deviation, C3: Complement 3, Anti-dsDNA: Anti double stranded deoxyribonucliec acid, ESR: erythrocyte sedimentation rate, hrs: hours, S: Serum, Chol: Cholesterol, TG: Triglyceride, HDL: High density lipoprotein, LDL: Low density lipoprotein, P>0.05: non-significant.

Table 3. Variation of the studied laboratory markers in relation to AT1 receptor genotype A1166C

	AT1 receptor gene (A allele) n (%): 10 (41.7%)	**AT1 receptor gene (C allele) n (%): 14 (58.3%)**	**test**	**P**
Serum Ang II (pg/ml) Mean (SD)	282(72.5)	325.7 (85.2)	1.3	0.2
C3 (mg/dl) Median (range)	105.7 (55-205)	83.5 (37-152)	Z=-1.1	0.25
Anti-dsDNA (IU/ml) Median (range)	600 (90-882)	200 (45-1365)	Z= -1.3	0.2
ESR Median (range)	20 (5-40)	24 (8-75)	Z=- 1.1	0.3
Serum creatinine (mg/dl) Median (range)	0.4 (0.3-0.7)	0.5 (0.3-0.9)	Z= - 0.36	0.7
Creatinine clearance (ml/min/1.73 m^2) Median (range)	105 (22-273)	101 (72-323)	Z= - 0.3	0.7
24hrs. Urinary proteins (g/24h) Median (range)	0.1 (0-1)	0.1 (0.05-4)	Z= -1.7	0.1
S. Chol (mg/dl) Mean± SD	203 ± 43.4	200 ± 46.7	t = - 0.2	0.85
S. TG (mg/dl) Mean± SD	152 ± 69.4	145 ± 57.8	t = - 0.3	0.8
HDL (mg/dl) Median (range)	47 (35-150)	40 (10-85)	Z= -1.1	0.3
LDL (mg/dl) Median (range)	139 (45-200)	138 (70-250)	Z= - 0.23	0.8

AT1A: Angiotensin II type 1A, n: number, %: percentage, SD: standard deviation, C3: Complement 3, Anti-dsDNA: Anti double stranded deoxyribonucliec acid, ESR: erythrocyte sedimentation rate, hrs: hours, S: Serum, Chol: Cholesterol, TG: Triglyceride, HDL: High density lipoprotein, LDL: Low density lipoprotein, P >0.05: non-significant.

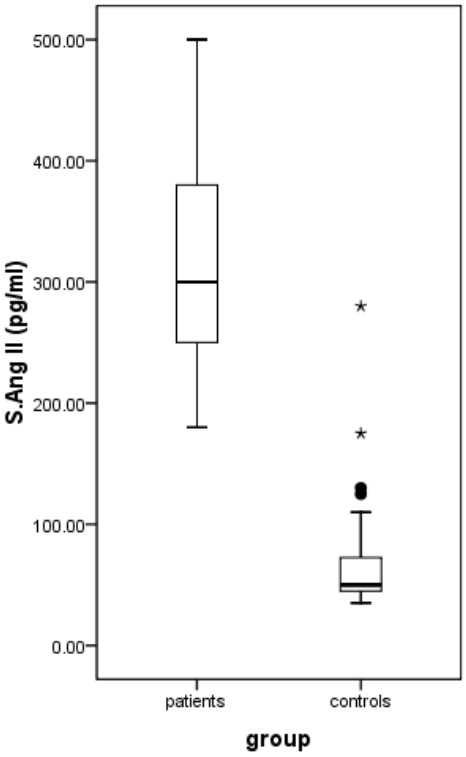

Figure 1. Serum angiotensin II in the studied patients and the control group.

Ang II: angiotensin II.

Boxes enclose the interquartile ranges (IQR) (the 25th and 75th percentiles). The horizontal line inside the box represents the median (50th) and whiskers represent maximum and minimum values (Range). The closed small circles and stars represent extreme values (more than IQR).

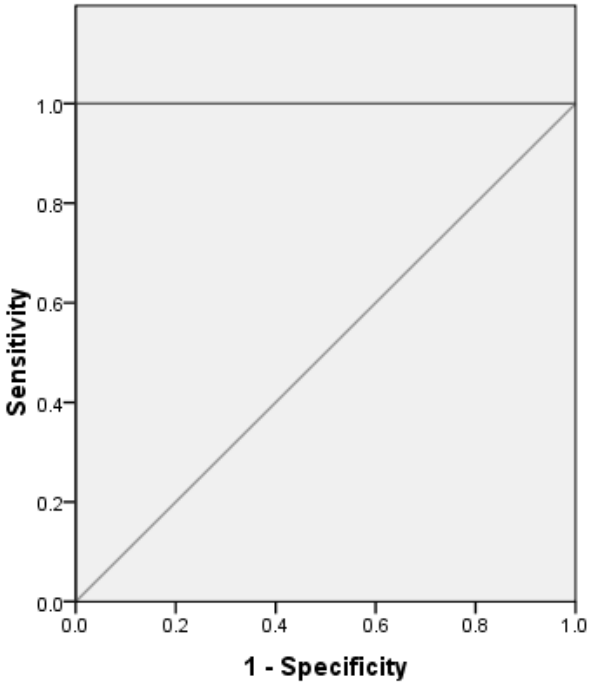

Figure 2. Receiver Operating Characteristic (ROC) curve to define the best cutoff value of serum angiotensin II to differentiate between the studied patients and the control group.

It was found that the best cut-off value of serum Ang II was 155 pg/ml with both sensitivity and specificity of 100 %. So, levels above 155 pg/ml were considered abnormally high.

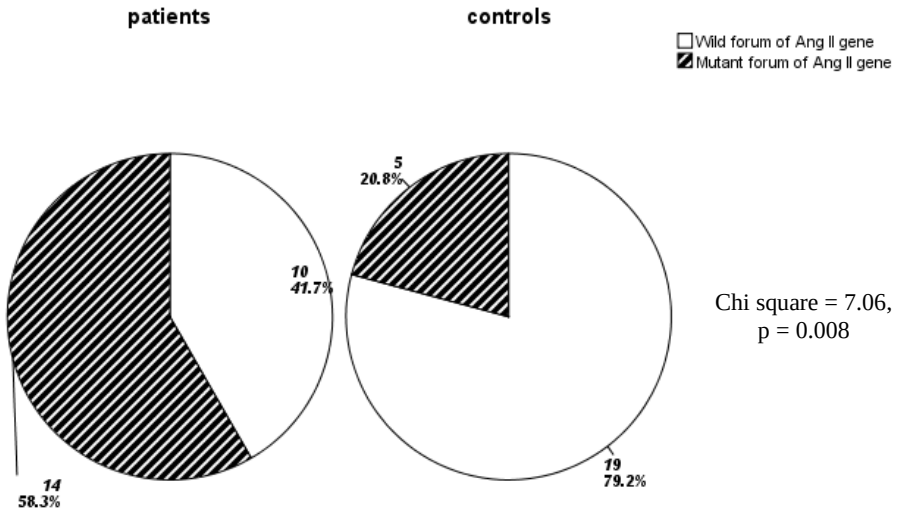

Figure 3. The frequency of AT1 receptor CC genotype in patients and controls.

DISCUSSION

The inflammatory role of Ang II and the effect of AT1 receptor polymorphism A1166C (CC genotype) in the pathogenesis of SLE and particularly LN is still unclear. Furthermore, their effect on the clinical phenotype of LN is rather undefined. In this study, the significantly increased serum Ang II and the frequency of AT1 receptor CC genotype among patients supports the inflammatory role of Ang II in SLE and may indicate that AT1 receptor CC genotype has a pathogenic effect rather than a normal variation. However, this needs to be verified on a wider scale. Previous studies investigated the role of ACE activity in SLE and RAS activation has been reported in SLE patients.[2,3,15] ACE catalyses the formation of Ang II from its inactive precursor, Ang I.[16] It has been reported that AT1 receptor CC genotype was more frequent in adult SLE patients as compared to healthy controls. This might increase the susceptibility to SLE, however the susceptibility to SLE, its nephropathy and progression to renal failure was only performed by studying the genetic polymorphism of ACE which showed controversial results.[17] In this series, AT1 receptor CC genotype did not affect serum angiotensin, this implies that the polymorphism might not affect the sensitivity of the receptor or have significant feedback on serum Ang II.

The presence of AT1 receptor CC genotype did not affect significantly the age at onset of SLE and this has been previously reported.[17] SLE is a complex disease with the interplay of multiple factors resulting in different lupus presentations. Also, we did not find a specific gender predilection for AT1 receptor CC genotype. This has been reported in an earlier study.[18] The lack of significant effect of age and sex on serum Ang II in both patients and controls suggests that it is rather regulated by hemodynamic factors as well as the inflammatory milieu.

In this series, LN class III and IV were not associated with significantly higher serum Ang II or frequency of AT1 receptor CC genotype. The limited number of patients in each class of LN would not allow for making solid conclusions as to the role of AT1 receptor CC genotype and serum Ang II in the pathogenesis and progression of LN.

Other investigators also reported that there was no significant correlation between AT 1 receptor CC genotype and LN classes.[17,19] It has been suggested that the influence of this polymorphism on SLE may be related to the interaction of the genotype variables of the different components in the RAS system.[17,20,21]

Ang II is an important contributor to blood pressure regulation and plays a significant role in hypertension and in the pathophysiology of vascular damage during the course of hypertension.[22] However, in this study, there was no significant association between serum Ang II or the frequency of AT1 receptor CC genotype and hypertension demonstrating the multifactorial nature of hypertension in these patients including the use of long term steroids therapy and hyperlipidemia. A previous study reported that this polymorphism had no significant effect on the development of hypertension in adult SLE patients,[17] whereas, several other studies reported that reported that AT1 receptor CC genotype had a significant role in the development of essential hypertension.[18,23-25]

In the present study, we failed to demonstrate a significant association between serum Ang II and AT1 receptor CC genotype and indices of lupus activity whether SLEDAI, serum anti-dsDNA titre or complement-3. The fact that the majority of patients had mild activity or were in remission (72%) could explain this result especially that patients with moderate and high lupus activity had higher serum Ang II, albeit insignificant, compared to those with low grade activity or in remission.

Interestingly, serum Ang II and the frequency of AT1 receptor CC genotype did not vary with BILAG-renal score as well as renal function tests. Intra-renal Ang II may better correlate with LN and its presentation as it has been reported that renal tubules and interstitial compartments contain significantly high levels (1000-fold) of Ang II compared with the plasma level.[7] Intra-renal Ang II contributes to the recruitment of inflammatory cells into the kidney and the adhesion of circulating cells to endothelial and mesangial cells.[8] Furthermore, sustained elevation of intra-renal Ang II induces proteinuria accompanied by progressive injury of the glomerular filtration barrier.[26-28]

A previous study found that there was no significant difference between patients with AT1 receptor CC genotype and those with AA genotype as regards SLEDAI and BILAG-renal score.[17] There are reports that AT1 receptor CC genotype was associated with the progression of chronic kidney diseases to end stage renal failure.[29,30] To the best of the authors knowledge, there are no previously published studies concerning serum Ang II in LN.

In this study, serum Ang II and AT1 receptor CC genotype did not affect significantly lupus related damage. This finding could be explained by the lack of significant effect of serum Ang II on lupus activity in terms of SLEDAI and BILAG-renal score which are eventually reflected as lupus related damage.

Lack of significant association between serum Ang II and AT1 receptor CC genotype and serum lipids needs to be verified on a wider scale. Ang II may provide a link between atherosclerotic risk factors such as hypercholesterolemia and hypertension, since high cholesterol levels have recently been shown to increase angiotensinogen and Ang II.[31] However, a previous study reported that AT1 receptor genotype A1166C had no effect on serum lipids.[32]

In conclusion, serum Ang II and AT1 receptor CC genotype seem to have pathogenic role in LN being significantly higher among patients than the healthy subjects. However, elevated serum Ang II and AT1 receptor CC genotype did not show a significant effect on the histological and clinical consequences of LN. Further studies on a wider scale are needed to evaluate serum Ang II and AT1 receptor CC genotype linking them to the intra-renal Ang II for a better understanding of their role in the etiopathogenesis of LN and to define the therapeutic value of ACEIs and ARBs in LN without hypertension.

REFERENCES

1. **Prkacin I, Novak B, Sertic J, Mrzljak A.** Angiotensin-converting enzyme gene polymorphism in patients with systemic lupus. Acta Med Croatica 2001; 55:73–6.
2. **Rabbani MA, Mahmood MS, Mekan SF, Frossard PM.** Association of angiotensin-converting enzyme gene dimorphisms with severity of lupus disease. Saudi J Kidney Dis Transplant 2008; 19 (5):761-6.
3. **Klahr S, Morrissey JJ.** The role of vasoactive compounds, growth factors and cytokines in the progression of renal disease. Kidney Int 2000; **57** (75): S7–S14.
4. **Smith W, Morgan P.** Actions of angiotensin II on the heart. Cited at http://www.uptodate.com. Last updated on June 18th 2012. Accessed on September 18th 2012.
5. **De Gasparo M, Catt KJ, Inagami T, Wright JW, Unger T.** "International union of pharmacology. XXIII. The angiotensin II receptors". Pharmacol Rev 2000; 52 (3): 415–72.
6. **Mehta PK, Griendling KK.** Angiotensin II cell signaling: physiological and pathological effects in the cardiovascular system. Am J Physiol Cell Physiol 2007; 292: C82–97.
7. **Esteban V, Ruiz-Ortega M, Rodríguez-Vita J, Sánchez-López E, Carvajal G, Egido J.** Angiotensin II: a key factor in the inflammatory and fibrotic response in kidney diseases. Nephrol Dial Transplant 2006; 21: 16–20.
8. **Crowley SD, Vasievich MP, Ruiz P, Gould SK, Parsons KK, Pazmino AK, et al.** Glomerular type 1 angiotensin receptors augment kidney injury and inflammation in murine autoimmune nephritis. J Clin Invest 2009; 119: 943-53.
9. **Hochberg MC.** Updating the American College of Rheumatology revised criteria for the classification of systemic lupus erythematosus. Arthritis Rheum 1997; 40: 1725.
10. **Weening JJ, D'Agati VD, Schwartz MM, Seshan SV, Alpers CE, Appel GB, et al.** The classification of glomerulonephritis in systemic lupus erythematosus revisited. Kidney Int 2004; 65: 521–30.

11. National Heart, Lung, and Blood Institute. Report of the Second Task Force on Blood Pressure Control in Children-1987. Pediatrics 1987;79:1.
12. **Bombardier C, Gladman DD, Urowitz MB, Caron D, Chang CH.** Derivation of the SLEDAI. A disease activity index for lupus patients. The Committee on Prognosis Studies in SLE. Arthritis Rheum 1992; 35: 630-40.
13. **Isenberg DA, Rahman A, Allen E, Farewell V, Akil M, Bruce IN, et al.** BILAG 2004. Development and initial validation of an updated version of the British Isles Lupus Assessment Group's disease activity index for patients with systemic lupus erythematosus. Rheumatology 2005; 44: 902-6.
14. **Gladman D, Ginzler E, Goldsmith C, Fortin P, Liang M, Urowitz M, et al.** The development and initial validation of the Systemic Lupus International Collaborating Clinics/American College of Rheumatology damage index for systemic lupus erythematosus. Arthritis Rheum 1996; 39: 363-9.
15. **Abbas D, Ezzat Y, Hamdy E, Gamil M.** Angiotensin-converting enzyme (ACE) serum levels and gene polymorphism in Egyptian patients with systemic lupus erythematosus. Lupus 2011; 21: 103-10.
16. **Wharton J, Walsh DA, Catravas J.** Angiotensin converting enzyme in human synovium: increased stromal [^{125}I] 351A binding in rheumatoid arthritis. Ann Rheum Dis 2000; 59:125–31.
17. **Sprovieri SR, Sens YA.** Polymorphisms of the renin-angiotensin system genes in Brazilian patients with lupus nephropathy. Lupus 2005; 14: 356-62.
18. **Bonnardeaux A, Davies E, Jeunemaitre X, Féry I, Charru A, Clauser E, et al.** Angiotensin II type 1 receptor gene polymorphisms in human essential hypertension. Hypertension 1994; 24: 63–9.
19. **Sprovieri SR, Sens YA, Martini Filho D.** Association between polymorphisms of the renin-angiotensin system and more severe histological forms of lupus nephritis. Clin Nephrol 2005; 64: 20-7.
20. **Pei Y, Scholey J, Thai K, Suzuki M, Cattran D.** Association of angiotensinogen gene T235 variant with progression of immunoglobulin A nephropathy in Caucasian patients. J Clin Invest 1997; 100: 814-20.
21. **Marre M, Jeunemaitre X, Gallois Y, Rodier M, Chatellier G, Sert C, et al.** Contribution of genetic polymorphism in the renin-angiotensin system to the development of renal complications in insulin-dependent diabetes: Genetique de la Nephropathie Diabetique (GENEDIAB) study group. J Clin Invest 1997; 99: 1585-95.
22. **Fyhrquist F, Metsärinne K, Tikkanen I.** Role of angiotensin II in blood pressure regulation and in the pathophysiology of cardiovascular disorders. J Hum Hypertens 1995; 9 (Suppl 5): S19-24.
23. **Wang WY, Zee RYL, Morris BJ.** Association of angiotensin II type 1 receptor gene polymorphism with essential hypertension. Clin Genet 1997; 51:31–4.
24. **Szombathy T, Szalai C, Katalin B, Palicz T, Romics L, Csaszar A.** Association of angiotensin II type 1 receptor polymorphism with resistant essential hypertension. Clin Chim Acta 1998; 269: 91–100.
25. **Kainulainen K, Perola M, Terwilliger J, Kaprio J, Koskenvuo M, Syvänen A-C, et al.** Evidence for the involvement of the type 1 angiotensin II receptor locus in essential hypertension. Hypertension 1999; 33:844–9.
26. **Kobori H, Harrison-Bernard LM, Navar LG.** Urinary excretion of angiotensinogen reflects intrarenal angiotensinogen production. Kidney Int 2002; 61:579–85.
27. **Miller PL, Rennke HG, Meyer TW.** Glomerular hypertrophy accelerates hypertensive glomerular injury in rats. Am J Physiol 1991; 261:459-65.
28. **Hoffmann S, Podlich D, Hahnel B, Kriz W, Gretz N.** Angiotensin II type 1 receptor overexpression in podocytes induces glomerulosclerosis in transgenic rats. J Am Soc Nephrol 2004; 15:1475-87.
29. **Whaley-Connell A, Chowdhury N, Hayden MR, Stump CS, Habibi J, Wiedmeyer CE, et al.** Oxidative stress and glomerular filtration barrier injury: role of the renin-angiotensin system in the Ren2 transgenic rat. Am J Physiol 2006; 291: 1308-14.
30. **Buraczynska M, Ksiazek P, Drop A, Zaluska W, Spasiewicz D, Ksiazek A.** Genetic polymorphisms of the renin-angiotensin system in end-stage renal disease. Nephrol Dial Transplant 2006; 21 (4):979-83.
31. **Elshamaa MF, Sabry SM, Bazaraa HM, Koura HM, Elghoroury EA, Kantoush NA, et al.** Genetic polymorphism of ACE and the angiotensin II type1 receptor genes in children with chronic kidney disease J Inflamm 2011; 8: 20.
32. **Daugherty A, Rateri DL, Lu H, Inagami T, Cassis LA.** Hypercholesterolemia stimulates angiotensin peptide synthesis and contributes to atherosclerosis through the AT1A receptor. Circulation 2004; 110: 3849–57.