Relationship between Transforming Growth Factor-Beta 1 gene polymorphism and hypertension

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ABSTRACT

This study intends to evaluate the association between Transforming Growth Factor - Beta1 (TGF-β1) SNP codon 25 and hypertension in holy Kerbala city, Iraq. A case control study for one hundred and four subjects. Seventy four hypertensive patients (23 male and 51 female), already diagnosed with essential hypertension, and 30 control subjects (16 male and 14 female). The links between genotype and hypertension were examined then possible SNP related variances in the blood pressure were checked. The present study suggested that there is no significant association between transforming growth factor beta one (TGF-β1) and hypertension in its Iraqi population sample. However, there is a significant association between Arginine (Arg^{25}) and hypertension compared with control group clearly shown in the male gender ($p < 0.05$). In this study, the associations between the SNP of TGF-β1 codon 25 and hypertension was not significant while the SNP showed genotype-related differences in gene allele (Arg^{25}).

العلاقه بين الطفره لجين عامل النمو *TGF-β1* في الكودون 25 ومرض ضغط الدم

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الكلمات المفتاحية: ارتفاع ضغط الدم، عامل النمو الجيني TGF-β1، طفرة جينية

الخلاصة

تعتزم هذه الدراسة إلى تقييم العلاقة بين عامل النمو الجيني TGF-β1 المحمول في كودون 25 وارتفاع ضغط الدم لعينة بشرية من مدينة كربلاء المقدسة، العراق. تضمنت هذه الدراسه مائه واربعه عينه. اربعة وسبعون من مرضى ارتفاع ضغط الدم (23 من الذكور و 51 من الإناث)، تم تشخيصهم بارتفاع ضغط الدم مسبقاً، و 30 عينة من الا□حاء (16 ذكور و 14 إناث). تم فحص الروابط بين النمط الجيني وارتفاع ضغط الدم ثم تم فحص الفروق SNP ذات الصلة في ضغط الدم. وأشارت الدراسة أنه لا توجد علاقة ذات دلالة احصائية بين عامل النمو بيتا واحد (TGF-β1) وارتفاع ضغط الدم في عينة السكان العراقية. ومع ذلك، هناك علاقة وثيقة بين أرجينين (Arg25) وارتفاع ضغط الدم مقارنة مع مجموعة التحكم يظهر ذلك بوضوح في جنس الذكور ($p<0.05$).

1. INTRODUCTION

Essential hypertension defined as high blood pressure (BP) with no identifiable cause representing 95% of hypertensive patients [1].
It is considered to be the consequence of an interaction between environmental and genetic factors [2]. Despite the fact that there are many possible genes to be linked with essential hypertension yet transforming beta one is said to be one of them [3].
Transforming growth factor-Beta 1 (TGF-β1) is an extracellular polypeptide member of the TGF-β superfamily of cytokines. It is a secreted protein that does many cellular functions including control of cell growth, cell proliferation, cell differentiation and apoptosis.
On the other hand, TGF-β1 acts bi-functionally to elevate blood pressure:

a) Altering levels of vasoactive mediators.
b) Changing the vessel wall architecture to increase the peripheral resistance.

Also, TGF-β1 may play a key role in the regulation of the mechanical strain-induced matrix synthesis by the human vascular smooth muscle (VSM) cells in the cardiovascular system. Hence TGF-β1 may have an important role in the development of hypertension-induced cardiovascular fibrosis [4].

The family of transforming growth factor beta (TGF-β) belongs to a superfamily that involves over 25 diverse dimeric extracellular polypeptides of 110-140 amino acids [5]. It is located on the 19q13 chromosome and has 7 exons and 6 introns [6]. Furthermore, it is thought that there is a biologically rich and complex interaction occurs between the Renin-Angiotensin System (RAS) and TGF-β1 in which both act at various points to support the actions of each other. This interaction explains the vital roles that RAS and TGF-β1 play in essential hypertension development [7].

2. MATERIAL AND METHODS

Study population

The study included 74 hypertensive (23 male and 51 female) all of them diagnosed previously and some are taking treatment, along with 30 healthy controls (16 male and 14 female). Control subjects had to have systolic BP <135 mmHg and diastolic BP <85 mmHg, without using any blood pressure lowering remedies. Patients were selected from the out-patient department of one hospitals in holy Kerbala city, Iraq. All subjects' history and blood pressure have been recorded. The purpose of the study was thoroughly explained to all subjects, and informed consent was obtained and signed by all participants.

Clinical data collection and blood sample analysis

On the day of enrolment, clinical data including demographic variables and medical history were recorded for all subjects. BP was measured by trained nurses at the right brachial artery using a mercury sphygmomanometer. All sample had 10 minutes of rest in the supine position before the measurements, and the average of at least three

measurements were used in the study. Venous blood samples were collected after an overnight fast, and samples were analysed within 4 hours of collection. All analyses were conducted by the hospital laboratory.

Genotyping

Genomic Deoxyribonucleic Acid (DNA) was extracted from 2 mL of peripheral venous blood by (Accuprep Genomic DNA Extraction Kit) from BIONEER. In order to detect allele Arg^{25}, allele Pro^{25} ARMS-PCR reaction were use. PCR was carried out using an Eppendorf Gradient Thermo cycler. The reaction mix was incubated and the following programme was used: 95°C for 5 minutes (initial denaturation), 95°C for 30 seconds (denaturation, 30 cycles), 61°C for 30 seconds (annealing, 30 cycles), 72°C for 30 second (extension, 30 cycles), and 72°C for 5 minutes (Final extension) and 4°C (hold phase). Primers that have been used to detect this polymorphism are shown in table (1).

Table (1): TGF-β1 codon 25 primers used [8]

Tgf_pro (p1)	***ACTGGTGCTGACGCCTGGCCC***
Tgf_arg (p2)	***ACTGGTGCTGACGCCTGGCCG***
Tgf_antisence (p3)	***TGCTGTTGTACAGGGCGAGCA***

Then we used Agarose Gel Electrophoresis to detect the polymorphism Arg^{25}/Pro^{25}. Bands for the required product sizes were obtained 196 bp for each allele and the gel was photographed using digital camera, as shown in figure (1).

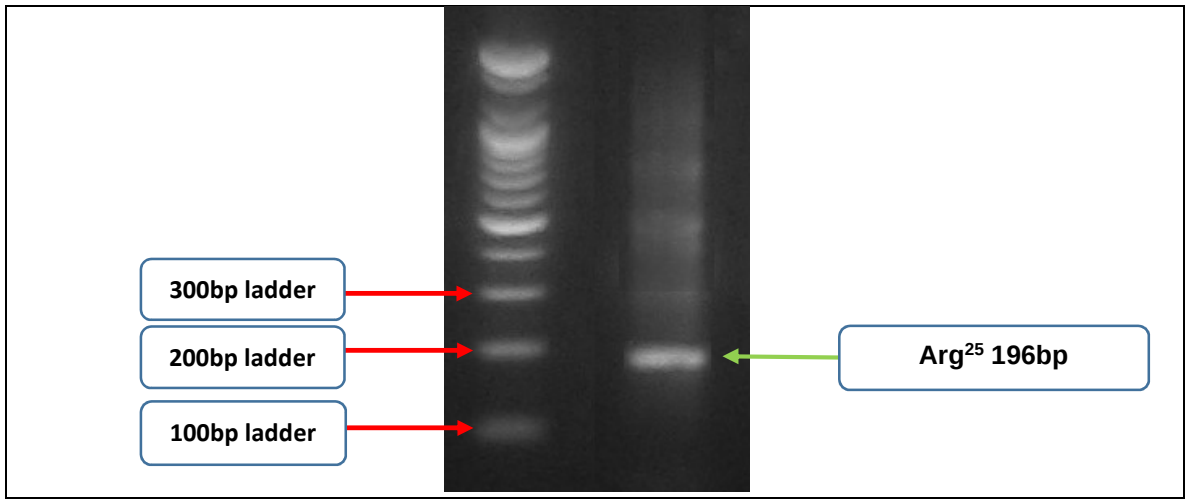

Figure (1): Electrophoresis band for Arg^{25} of TGF-β1 gene

Biochemical Parameters

Collecting **f**asting blood sample of (3ml) by venipuncture into a plain tubes and allowed to clot for 10-15 minutes, centrifuged and the separated serum used for further measurement of fasting blood sugar, urea, creatinine, albumin and lipid profile by colorimetric method .

Statistical analysis

Then statistical analysis was done by SPSS statistical software (SPSS 20 for Windows, standard version). The results were presented as mean ± standard deviation (SD).Continuous variables were tested by T-test and for genetic analysis Chi square has been used to compare the results. The correlation analysis was done using the Pearson test, a P. value of <0.05 was considered statistically significant.

3. RESULTS

Comparison between TGF-β1 Codon 25 alleles in hypertension and control groups

A significant association is shown between Arginine (Arg^{25} allele) and male gender of hypertension group compared with control group (p=0.02), shown in table (2). Moreover, Proline (Pro^{25} allele) is rare in both patient and control groups as shown in table (2).

Table (2): TGF-β1 codon 25 alleles in hypertension and control (females vs males)

Sex	Alleles	Result	Hypertension N (%)	Control N (%)	P. value
Female	Proline	negative	45 (77.6)	13 (22.4)	0.52
		positive	6 (85.7)	1 (14.3)	
Male	Proline	negative	17 (56.7)	13 (43.3)	0.44
		positive	6 (66.7)	3 (33.3)	
Female	Arginine	negative	3 (60.0)	2 (40.0)	0.29
		positive	48 (80.0)	12 (20.0)	
Male	Arginine	negative	0 (0.0)	4 (100.0)	0.02
		positive	23 (65.7)	12 (34.3)	

TGF-β1Codon 25 Genotype and other parameters

There is a significant association between this gene and Body Mass Index (BMI), systolic blood pressure, and total cholesterol in male gender particularly (p=0.02, p=0.04, and p=0.03) respectively, table (3). Also, there is a significant association between TGF-β1 genotype and STG in total sample and female gender as per table (3). However, there is no significant association between this gene and other parameters (p>0.05) table (3). On the other hand, the results reveal that the mean of allele Arg/Pro in total, female and male

gender is greater than other alleles Arg/Arg and Pro/Pro in relation to all parameters as shown in table (3).

Table (3): Relation between TGF- β1 Codon 25 genotype and other parameters

Parameter		Total mean ±SD	Female mean ±SD	Male mean ±SD
Age (years)	p. value	0.22	0.54	0.37
	Arg/Arg	49.13 ± 11.31	49.90 ± 10.93	47.63 ± 12.06
	Arg/Pro	51.44 ± 11.26	51.00 ± 12.77	51.67 ± 11.72
	Pro/Pro	57.17 ± 10.80	57.00 ± 6.08	57.33 ± 15.95
BMI	p. value	0.46	0.85	0.02
	Arg/Arg	28.14 ± 5.36	28.30 ± 6.00	27.84 ± 3.88
	Arg/Pro	28.48 ± 4.23	26.41 ± 5.63	29.51 ± 3.47
	Pro/Pro	25.42 ± 5.75	28.93 ± 6.23	21.91 ± 2.62
Systolic BP	p. value	0.09	0.73	0.04
	Arg/Arg	137.61 ± 23.87	139.69 ± 24.89	133.50 ± 21.54
	Arg/Pro	142.22 ± 12.02	140.00 ± 17.32	143.33 ± 10.33
	Pro/Pro	117.50 ± 13.32	128.33 ± 7.64	106.67 ± 5.77
Diastolic BP	p. value	0.59	0.43	0.08
	Arg/Arg	83.43 ± 13.35	83.14 ± 14.08	84.00 ± 11.99
	Arg/Pro	87.78 ± 6.67	86.67 ± 5.77	88.33 ± 7.53
	Pro/Pro	81.67 ± 14.72	93.33 ± 5.77	70.00 ± 10.00
FBS	p. value	0.84	0.77	0.24
	Arg/Arg	120.33 ± 56.23	129.20 ± 66.05	102.87 ± 19.85
	Arg/Pro	131.78 ± 62.91	155.67 ± 104.21	119.83 ± 38.39
	Pro/Pro	120.33 ± 40.53	141.67 ± 51.07	99.00 ± 11.53
S. Urea	p. value	0.75	0.73	0.25
	Arg/Arg	28.65 ± 8.09	27.36 ± 8.18	31.20 ± 7.39
	Arg/Pro	26.67 ± 7.16	24.67 ± 11.72	27.67 ± 4.84
	Pro/Pro	27.67 ± 4.18	30.00 ± 4.36	25.33 ± 2.89
S. Creatinine	p. value	0.72	0.44	0.49
	Arg/Arg	0.68 ± 0.25	0.59 ± 0.20	0.86 ± 0.23
	Arg/Pro	0.74 ± 0.27	0.63 ± 0.21	0.80 ± 0.30
	Pro/Pro	0.72 ± 0.21	0.74 ± 0.31	0.70 ± 0.11
S. Albumin	p. value	0.84	0.98	0.92
	Arg/Arg	4.36 ± 0.24	4.33 ± 0.24	4.41 ± 0.23
	Arg/Pro	4.40 ± 0.18	4.30 ± 0.30	4.45 ± 0.08
	Pro/Pro	4.38 ± 0.21	4.33 ± 0.06	4.43 ± 0.32
S. Cholesterol	p. value	0.74	0.32	0.03
	Arg/Arg	180.56 ± 37.51	186.71 ± 38.48	168.47 ± 32.87
	Arg/Pro	187.22 ± 36.79	204.33 ± 30.66	178.67 ± 39.06
	Pro/Pro	190.83 ± 47.78	157.67 ± 41.48	224.00 ± 26.23
S.TG	p. value	0.04	0.04	0.52
	Arg/Arg	166.43 ± 89.43	157.96 ± 82.27	183.10 ± 101.46
	Arg/Pro	250.33 ± 182.28	301.33 ± 268.39	224.83 ± 148.34
	Pro/Pro	137.00 ± 48.49	131.33 ± 74.70	142.67 ± 14.19
S. HDL-C	p. value	0.47	0.88	0.59
	Arg/Arg	40.85 ± 22.30	44.97 ± 25.08	32.75 ± 12.15
	Arg/Pro	31.78 ± 8.78	37.63 ± 11.58	28.85 ± 6.22
	Pro/Pro	40.77 ± 11.70	44.27 ± 7.14	37.27 ± 15.95
S. LDL-C	p. value	0.77	0.57	0.07
	Arg/Arg	108.42 ± 38.79	112.47 ± 39.66	100.47 ± 36.35
	Arg/Pro	105.16 ± 40.95	105.77 ± 71.42	104.85 ± 25.34
	Pro/Pro	119.33 ± 46.63	87.13 ± 32.56	151.53 ± 35.57
VLDL-C	p. value	0.71	0.81	0.63
	Arg/Arg	39.53 ± 55.23	39.57 ± 65.92	39.45 ± 23.74
	Arg/Pro	50.07 ± 36.46	60.27 ± 53.68	44.97 ± 29.67
	Pro/Pro	27.40 ± 9.70	26.27 ± 14.94	28.53 ± 2.84

BMI (Kg/m^2), SBP & DBP (mmHg), FBS, S.urea, S.creatinine, S.albumin, S.cholesterol, S.TG, S.HDL, S.LDL, and VLDL (mg/dl), Vitamin D_3 (ng/ml)

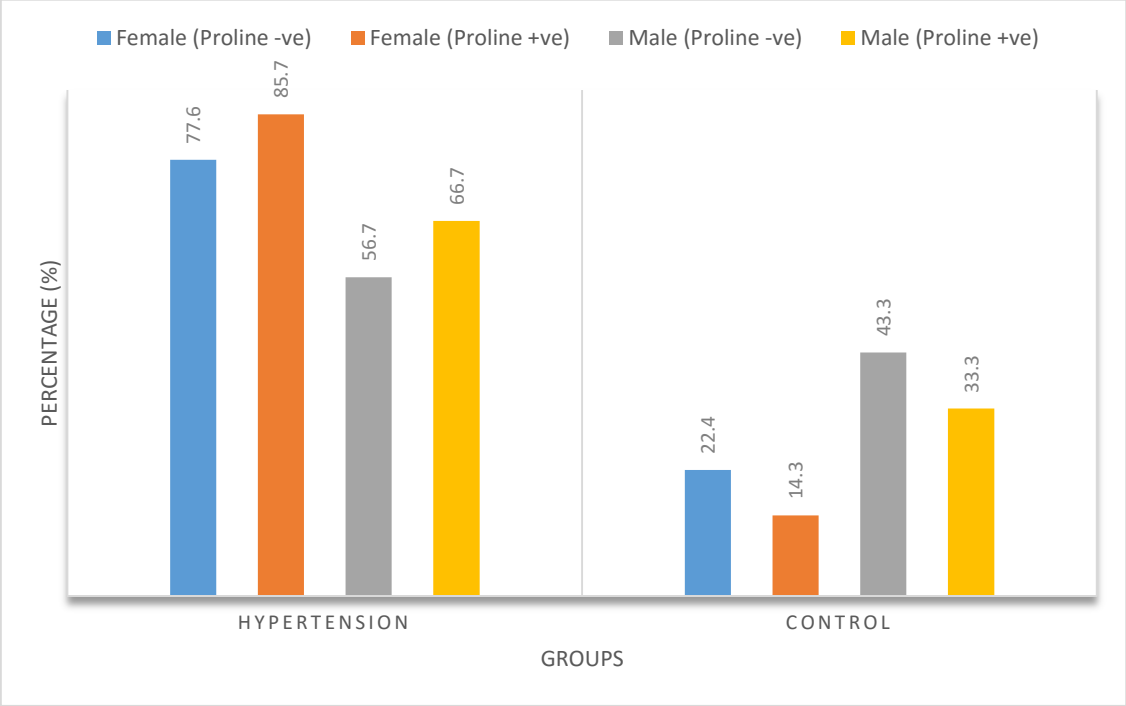

Figure (2): Proline distribution in hypertension and control groups (females vs males)

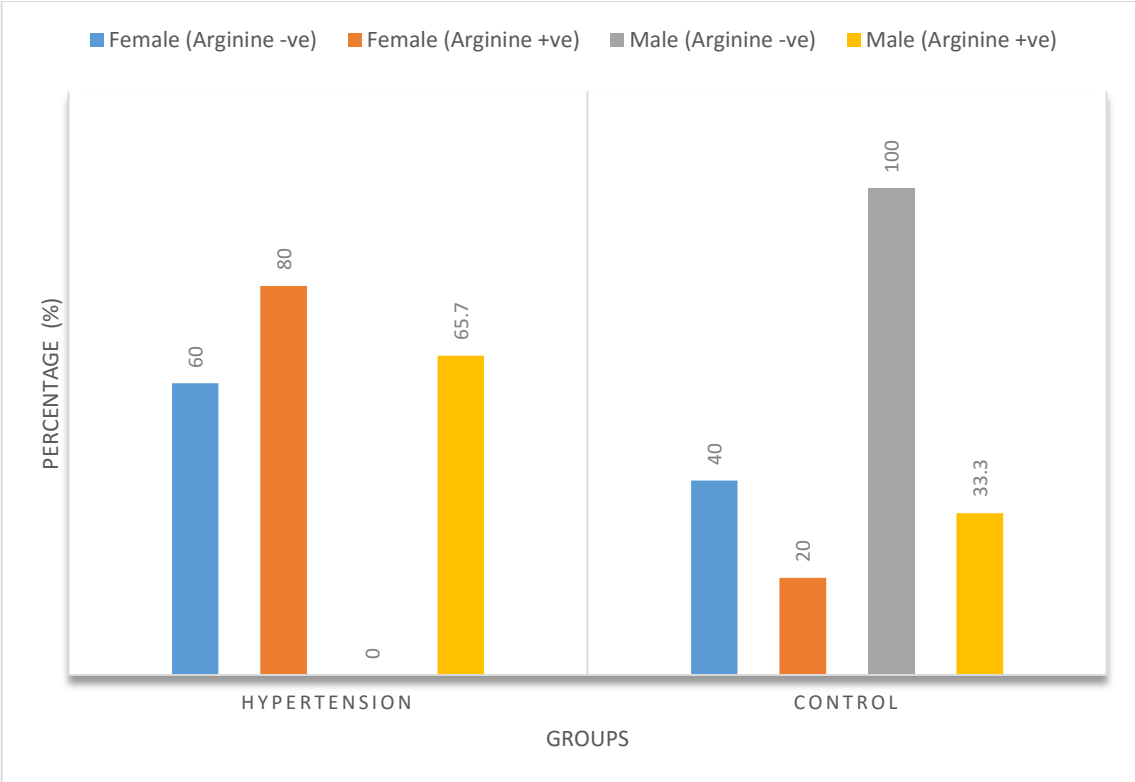

Figure (3): Arginine distribution in hypertension and control groups (females vs males)

Comparison between TGF-β1 Codon 25 Genotype in Hypertension and Control Groups in Both genders

According to this study, the results of alleles distribution for TGF-β1 codon 25 are either homozygous (Arg/Arg or Pro/Pro) or heterozygous (Arg/Pro) mutation. It has been found that there is no significant differences in TGF-β1 gene present in hypertensive patients compared with control group, male or female genders.

However there is a significant association between SBP>140 mmHg and SBP<140 mmHg with TGF-β1 alleles (p=0.013). Whereas no significant association with DBP has been shown in table (4).

Table (4): TGF-β1 Codon 25 Genotype in hypertension and control groups (females vs males)

Parameters		Arg/Arg N (%)	Arg/Pro N (%)	Pro/Pro N (%)	p. value
Sex	Female	59 (90.8)	3 (4.6)	3 (4.6)	0.12
	Male	30 (76.9)	6 (15.4)	3 (7.7)	
Chronic Diseases	Hypertension	63 (85.1)	8 (10.8)	3 (4.1)	0.26
	Control	26 (86.7)	1 (3.3)	3 (10.0)	
Systolic BP	SBP≥140 mmHg	44 (86.3)	7 (13.7)	0 (0.0)	0.01
	SBP<140 mmHg	45 (84.9)	2 (3.8)	6 (11.3)	
Diastolic BP	DBP≥90 mmHg	43 (82.7)	6 (11.5)	3 (5.8)	0.58
	DBP<90 mmHg	46 (88.5)	3 (5.8)	3 (5.8)	

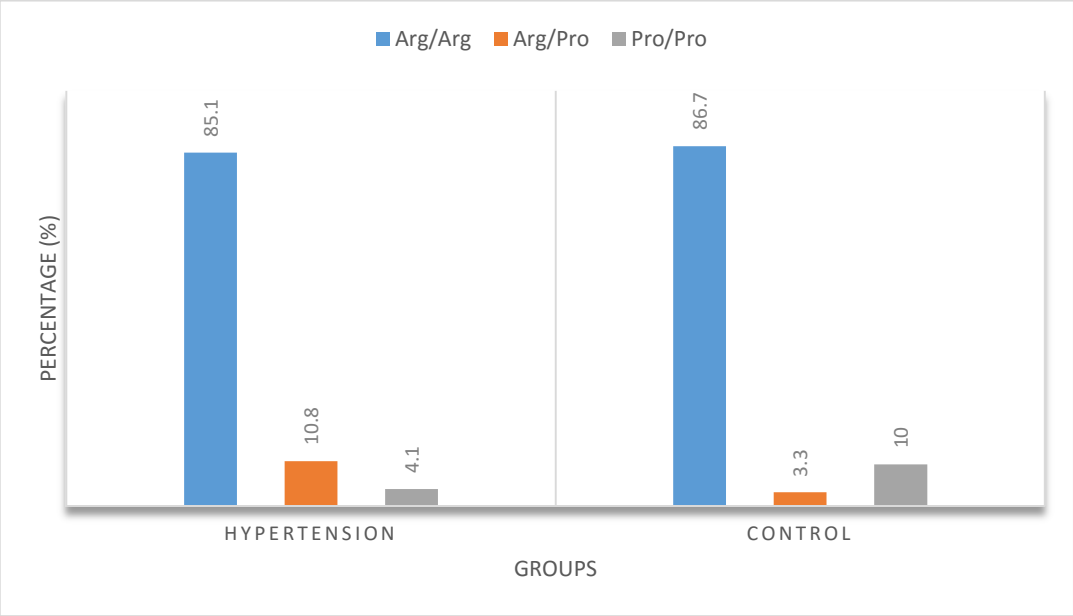

Figure (4): TGF-β1 Codon 25 alleles' in patients and control groups

4. DISCUSSION

The present study has evaluated the association between the single nucleotide polymorphisms at the TGF-β1 codon 25 and essential hypertension. This study showed that the GG genotype (Arg^{25} homozygotes) of TGF β1 +915 G->C at codon 25 was more prevalent than GC (Arg/Pro) heterozygotes and CC (Pro^{25} homozygotes). This important association was more obvious, statistically, in male gender with hypertension and in control group. Also, the study showed a significant differences between these variants genotype and the two groups of SBP>140 and SBP<140. These results support the idea of genetically determined TGF-β1 protein concentration may contribute to the regulation of blood pressure [8]. There are several functional polymorphisms in the TGF-β1 gene that had been determined previously. Some of these functional polymorphisms, e.g. (+869TC at codon 10 and +915GC at codon 25), are reported to be associated with cardiovascular disorders including myocardial infarction, artery stiffness and left ventricular hypertrophy in hypertensive patients [9,10].

Researchers, also, found clear association between single nucleotide polymorphisms at the TGF-β1 locus and AF in subjects with EH. They documented that GG genotype of TGF-β1 +915 G->C at codon 25 was more dominant in individuals with AF than those without. However the +869 T->C at codon 10 showed no positive relationship to AF. Nevertheless, higher levels of TGF-β1 have been observed in patients with hypertension associated with cardiac and/or renal complications [11,12].

Taken together, according to previous mentioned studies, GG genotype of TGF-β1 +915 G->C at codon 25 is associated with occurrence of AF in essential hypertension. However, to investigate the association of the common promoter polymorphism rs1800469 in the TGF-β1gene with the risk of AF and hypertension in Chinese Han population in more detail a study has been conducted and exposed no significant difference in the genotype or allele

frequencies of this SNP between AF patients and controls [13]. This result, also, confirmed by Wang *et al.* and others who noted no significant association between the rs1800473 and the risk of AF in subjects with essential hypertension [14,15].

In another study about rs1800471 polymorphism in unrelated Caucasian patients, a significant association between rs1800471 polymorphism of the TGF-β1gene and hypertension occurrence was identified [16]. The renin-angiotensin-aldosterone system is, also, involved and many researchers focused lately on the cytokine mediating angiotensin action, namely TGF-β1. This is a multi-potent cytokine involved in the process of epithelial-to-mesenchyme transition (EMT) and production of extracellular matrix (ECM) [17,18].

Enhanced expression of the TGF-β1gene is one of the most obvious molecular changes causing pathological tissue fibrosis. There is an opinion that overproduction of TGF-β, partly enhanced by angiotensin II, beside favouring progression of CKD is also linked with hypertension occurrence [19,20].

Similarly, Li *et al.* reported higher risk of hypertension occurrence among carriers of the G allele of the mentioned polymorphism [20]. The G allele is known to be part of highly productive genotypes predisposing to production of larger amounts of cytokine, which is thought to influence hypertension [21, 22].

It has been proved that in essential hypertension patients TGF-β1 protein as well as TGF-β1 mRNA levels are hyper-expressed. This can be attributed to several factors such as elevated angiotensin II, increased systemic blood pressure, increased fluid shear stress and the differential expression of TGF-β1 linked to DNA polymorphism in promoter [23]. This study, also, proved that Arg^{25} polymorphism in the TGF-β1 gene is associated with higher blood pressure [24]. It is, therefore, impossible to state a conclusion regarding the association between polymorphism of the TGF-β1 codon 25 gene and risk of hypertension occurrence. That is why it is necessary to perform further studies in this field to prove this relationship in the Iraqi populations. In this study, the results have shown that there are significant differences between male and female gender and this result reported by Giulia Dell'Omo *et al* which studied an all-male hypertensive group and the influence of gender on the TGF-β1 polymorphism [24]. Restriction to an all-male Southern European cohort may also influence the generalizability of this study to other genetic groups even within the same ethnicity. Much remains to be done, however, to fully understand many aspects of TGF-β biology in men [25].

Therefore, it is still impossible to state an unequivocal conclusion regarding the association between polymorphism of the TGF-β1 codon 25 gene and risk of hypertension occurrence. Hence further studies in this field are necessary.

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