Zagazig University Medical Journal
www.zumj.journals.ekb.eg

Volume 28, Issue 4, July 2022(728-733)

Manuscript ID ZUMJ-2009-1942 (R1)
DOI **10.21608/zumj.2021.42702.1942**

ORIGINAL ARTICLE

Polymorphism of 11 β-Hydroxysteroid Dehydrogenase Type 1 Gene in Hypertensive Patients

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Submit Date 2020-09-26
Revise Date 2021-05-01
Accept Date 2021-01-10

ABSTRACT

Background: Essential hypertension (EH) is the most well-known cardiovascular illness. It is estimated that (30% - 40%) of blood pressure (BP) is caused by genetic factor. This work aims to investigate the relation between 11 beta hydroxysteroid dehydrogenase type 1 (11β-HSD1) gene polymorphism and EH in patients attending Zagazig University Hospitals.

Methods: Thirty-one patients having EH and the control group of thirty-one apparently healthy individuals. 11β-HSD1 gene polymorphism (rs45487298) was analyzed by restriction fragment length polymorphism reaction.

Results: we found that A allele was significantly higher in hypertension (HTN) group and Subjects carrying A allele had a higher incidence to have HTN (OR = 2.5, 95 % CI = 1.04–5.8 and P = 0.04). Levels of TG, and total cholesterol, were significantly higher and levels of HDL cholesterol were significantly lower in A/A than in wt\wt genotype. Also, A allele and triglycerides are independent risk factor for EH development.

Conclusions: there was a significant relation between 11β-HSD1 gene polymorphism and EH.

Keywords: Essential hypertension; 11β-HSD1 gene; Polymorphisms; cardiovascular risk factors; genotypes.

INTRODUCTION

Hypertension is the most well-known cardiovascular disease around the world. It is estimated that nearly one billion people are affected by HTN and it is anticipated to increase to 1.5 billion by 2025 **[1].** About 20 to 50% of the developed countries' adults suffer from HTN . It is classified into primary type "also known as EH and secondary type due to other diseases. EH ia about 95% of all HTN cases **[2]**.

Hypertension related mortalities are mainly due to congestive heart failure (45%), coronary insufficiency (35%), cerebral vascular causes (15%) and chronic renal disease (5%) **[3]**.

Cortisol homeostasis is important for controlling BP and its excess is associated with HTN **[4]**. The cortisol regulation is mainly by 11β-HSD1enzyme and 11β-hydroxysteroid dehydrogenase type 2 (11β-HSD2) enzyme **[5]**.

It is present in many tissues, but it is mainly expressed in the hepatic and fat tissue, where the level of the active form of glucocorticoids is increased **[6]**. Also, in cases who suffer from EH, free cortisol is exercted in urine more than in cases with normal HTN **[7]**.

To our knowledge, No studies have been performed on the relation between 11β-HSD1 polymorphism and EH in Egyptian population. So we aimed to investigate the association between them.

METHODS

This is a case-control study performed on 62 participants (31 participants in two groups) recruited from the Zagazig University Hospitals. All cases were Egyptians. All participants signed an informed written consent before enrolment in the study and the study design was approved by the Ethical Committee of Faculty of Medicine, Zagazig University. The work has been carried out in accordance with The Code of Ethics of the World Medical Association (Declaration of Helsinki) for studies involving humans.

Group I (control group): This group comprised 31 normal apparently healthy volunteers with no previous history of treated HTN with BP less than 140/90 persistently on clinic visits and not suffering from any diseases that might interfere with the present study. No medications were received for the past four weeks.

Group II (HTN group): This group comprised 31 patients having EH.

Patients suffering from Diabetes mellitus, obesity, secondary HTN, endocrinal disorders or on hormonal therapy and smokers were excluded from the study.

Biochemical Measurements

Analyses of Lipid

Blood samples were drawn from all cases after fasting the night before. Separation of serum was done immediately and storage was at -20 C. total cholesterol (TC), high-density lipoprotein (HDL) and triglycerides (TG) levels were measured by spectrophotometer SUNDSTIK (SBA-733 PLUS), while low-density lipoprotein (LDL) was calculated according to the Friedewald formula **[8]**.

Isolation of DNA

DNA extraction from whole blood was done by using the commercially available G-spin TM Total DNA Extraction Kit (iNtron bio-tehnology, Korea).

Amplification of ins4436A (rs45487298) of the 11β-HSD1 gene Polymorphism:

The cases were genotyped for 11β-HSD1 gene polymorphism (rs45487298) by polymerase chain reaction–restriction fragment length polymorphism (PCR-RFLP) as described previously **[9]**. The polymorphism region was amplified with following Forward primers 5'AGACTGATGCCATTTCTGCTGT 3' and Reverse primer 5' GGT GATGTGGTTGAGAATGAGC 3'. PCR was performed at 94 °C for 5 min followed by 30 cycles of 95 °C for 30 s, 57 °C for 30s, and 72°C for 30s. Digestion of 300 bp amplified products with Xcm I yielded 300 bp for Wt allele and 201 and 101 bp fragments for A allele.

Statistical Analysis

Microsoft Excel software was used to enter data of history, clinical examination, laboratory investigations and outcome measures. Data then were analyzed into Statistical Package for the Social Sciences (SPSS version 20.0) software.

Qualitative data were expressed as number and percentage, but quantitative variables were expressed by mean ± SD. Difference of significance of qualitative variables is tested by Chi square test (X^2). Also, t test was used to asses difference of significance in Quantitative independent groups, P value was set at <0.05 for significant results.

RESULTS

The basic characteristics of control group and cases, Age and sex were examined and they were matched together without significant difference (P>0.05). But regarding BP (both systolic and diastolic), there was significant difference among HTN and control groups (P<0.001) as shown in (table 1).

Regarding the blood lipid levels among studies groups, we found significant higher levels of TG and cholesterol levels in patients' group as compared to control (P<0.001), Also, there was no significant difference in HDL levels (P= 0.103) and LDL levels (p= 0.14) between studied group as shown in (table 2).

The distribution of 11β-HSD1 gene polymorphism among control and HTN groups was assessed and it showed that incidence of A allele was significantly higher in HTN group (32.3%) compared to control (16.1%). Subjects carrying A allele were significantly more likely to have HTN (OR = 2.5, 95 % CI = 1.04–5.8 and P = 0.04) as shown in (table 3).

We found significant higher levels of TC, TG and lower level of HDLc in patients having the A/A genotype of 11β-HSD1 polymorphism as compared to those who have wt/ wt genotype as shown in table (4).

Multiple regression analysis was used to test independence of EH from other variables. The model included TC, TG, and 11β-HSD1 polymorphisms. We found A allele and triglycerides, to be independently related to EH as shown in (table 5).

https://dx.doi.org/10.21608/zumj.2021.42702.1942
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Table 1: Basic characteristics of the studied population.

	Variables		t-test	P
	Controls N =31	**Hypertensive N =31**		
Age:			0.43	0.112
Mean ± SD	60.2 ± 6.8	60.7 ± 5.2		
Range	45-60	49-70		
Sex:			1.38	0.501
Male N(%)	18 (58.1)	14 (45.2)		
Female N (%)	13 (41.9)	17 (54.8)		
Systolic blood pressure:			129.9	<0.001
Mean ± SD	118.6±8.96	155.2±11.6		
Range	100-135	140-180		
Diastolic blood pressure:			58.1	<0.001
Mean ± SD	79.5 ± 8.6	99.6± 8.7		
Range	60-90	90-120		

Table 2: Blood lipid levels among studied groups.

	Variables		t-test \ MW# test	P
	Controls N =31	**Hypertensive N =31**		
TG				
Mean ± SD	132.5 ±64.4	211.6 ± 70.6a	4.06#	<0.001
Median	120	220		
Range	49 – 275	88 – 320		
HDL				
Mean ± SD	45.7 ± 6.1	43.6 ± 6.3	1.63	0.103
Median	45	44		
Range	35 – 58	34 – 55		
LDL				
Mean ± SD	122.8 ± 41.5	138.1 ± 35.1	1.42#	0.14
Median	125	155		
Range	41 – 177	71 – 181		
Cholesterol				
Mean ± SD	195.2 ± 45.1	224.4 ± 41.7[a]	4.34	<0.001
Median	188	232		
Range	106 – 261	153 – 284		

Table (3): Different genotypes and allele distribution of 11BHSD1 gene among studied HTN cases and control.

Genotype	Controls (n=31)		Hypertensive (n=31)		OR (95% CI)	P
	N	%	N	%		
wt\wt	22	71	15	48.4	----------	
wt\A	8	25.8	12	38.7	2.2 (0.7-6.7)	0.2
A\A	1	3.2	4	12.9	5.9 (0.59-57.8)	0.12
A allele	10	16.1	20	32.3	2.5 (1.04-5.8)	0.04
wt allele	52	83.9	42	67.7		

https://dx.doi.org/10.21608/zumj.2021.42702.1942
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Table (4): Blood lipid levels according to 11BHSD1genotypes in HTN group.

Variables	HTN Group			X^2\ F test*	P
	Wt\wt N =15	Wt\A N =12	AA N=4		
SBP	142.3 ± 19.9 140 - 175	143.9 ± 14.2 145 – 180	149.9 ± 15.2 140 – 180	0.302	0.74
DBP	95.7 ± 11.7 90 – 110	96.2 ± 12.6 90 – 110	99.9 ± 14.9 90 – 120	0.184	0.83
TG Mean ± SD Range	197.6± 51.4 88 - 320	201.5 ± 44.7 145 – 254	300.3 ± 20.7 276 – 320	8.28	0.008
HDL Normal Low	45.7 ± 6.1 35 - 55	34.8 ± 2.4 34 – 44	33.8 ± 1.71 34 – 36	22.8*	<0.001
LDL Normal High	134 ± 36.8 71 - 181	130.8 ± 31.1 93 - 171	170.8 ± 4.99 166 – 176	2.43	0.106
Cholesterol Normal High	217.3± 44.1 153 - 247	217.7 ± 24.1 189 - 277	271.5 ± 9.29 262 – 284	4.1*	0.03

Table (5): Logistic multivariate regression analysis of significant predictors for HTN occurrence.

	Unstandardized coefficients		Standardized coefficients	T	Sig.
	B	**Std. Error**	**Beta**		
HTN cases					
A allele	0.211	0.005	0.022	3.12	0.01
TG	0.011	0.022	0.422	2.92	0.03
Cholesterol	0.001	0.008	0.121	0.934	0.532

DISCUSSION

Essential hypertension is a complex disease that results from an interaction between many environmental and genetic factors playing a great role in the variation of BP. It does not follow the Mendelian mode of transmission. It is estimated that (30% - 40%) of BP is caused by genetic factor. Recently, many genes have been supposed to have effect on the BP mechanism. Some associations between these genes had been found but not all of them were significant **[10-12]**.

11β-Hydroxysteroid dehydrogenase type 1 has a great importance in cortisol regulation. In addition, it is considered as a candidate gene for EH **[12]**, diabetes mellitus, metabolic syndrome **[13]** and obesity **[14]** through altered glucocorticoid production.

Therefore, the current study was done to assess the relation between 11β-HSD1 gene polymorphism and EH. Regarding basic characteristics of the studied patients, there was no statistically significant differences in age and sex, while there was highly statistically significant difference in both BP (either systolic or diastolic) (P<0.001).

On the other hand, **Hejduk *et al.* (2015) [9]** revealed higher incidence of EH in female cases as compared to male ones (M/F ratio was 34/71). They explain such increase among female sex may be explained by loss of protective action of estrogen. after 55 years so the prevalence of HTN increases in female than in male.

This study revealed that there were highly statistically significant differences regarding (TG and cholesterol) (P<0.001), while there was No statistically significant difference as regards HDL and LDL (P>0.001) .

In this study A allele was significantly higher in HTN cases (32.2%) in comparison with control cases

(16.1%). Subjects carrying A allele had higher incidence to have HTN (OR = 2.5, 95 % CI = 1.04–5.8 and P = 0.04).

Hejduk *et al.*, (2015) [9] revealed that ins4436A in the 11β-HSD1 gene is related to the presence of EH in the Polish population (OR 2.44; 95% Cl: 1.24-4.82). In accordance, Franks et al at 2004 **[15]** concluded that, there was an association between genetic variability at 11β-HSD1 with the development of HTN in Pima Indians.

In the same line, a Chinese study performed by **Ruan *et al.* (2014) [16]** displayed that, the rs4393158 in 11β-HSD1 was associated with the HTN (P = 0.037).

On the other hand, a Deutch study performed by **Smit *et al*. (2007) [17]** showed no evidence for effects of polymorphism in the 11β-HSD1 gene on BP. Also, **Rahman *et al.* (2011) [18]** did not reveal any relations between 11β-HSD1 genotypes (rs846910) and BP .

Draper *et al.* (2003) [19] found that adenine insertion in position 4436 in intron 3 of the 11β-HSD1 gene (rs45487298) may affect the gene transcription, supposing that this gene region acts as an intronic enhancer of 11β-HSD1 expression. This insertion might increase the activity of the 11β-HSD1 enzyme **[20]** resulting in an increase in the level of cortisol which has a higher affinity to the mineralocorticoid receptors than aldosterone and thus EH can be caused by this mechanism.

However, 11β-HSD1 expression occurs in systems with a significant effect on the homeostasis of BP, as vascular smooth muscle cells. It is known that glucocorticoids increase the vasoconstrictor response to catecholamine **[21]**.

New studies demonstrate that 11β-HSD1 in perivascular fat, increased in metabolic syndrome, can affect vascular tone. Also sympathetic over stimulation enhances 11β-HSD1 activity in perivascular fat. This results in endothelial dysfunction in underlying vessels by activation of Mineralocorticoid Receptor by the resultant increased cortisol **[22]**.

The discrepancies among studies may be explained by that BP like other complex traits is a function of interactions between genetic and environmental factors **[23, 24]**.

The results of our study showed a positive influence of rs45487298: insA variation on BP. However, these positive results have to be repeated in a bigger cohort of cases as multiple genetic related studies performed so far, yielded different results. The discrepancy might be also affected by the ethnic and environmental differences among studied cohorts. Moreover, other, unknown genetic variants in 11β-HSD1 gene, the gene encoding enzyme needed for 11β-HSD1 reductase activity, can also affect expression of 11β-HSD1. Nevertheless, the 11β-HSD1 role in HTN is complex and may be due to its tissue-specific expression forms.

Limitations of study:

The relatively small sample size was the most important limitation of this study. Another important limitation was many associated risk factors which may interfere with net results of the study and was difficult even impossible to be excluded.

Conclusions:

In conclusion, this is the first study done on Egyptian population, in which subjects carrying A allele were significantly more likely to have HTN. Also, our study indicated that A allele and triglycerides are independently related to EH. More investigations having larger cohorts of subjects, and studies in different populations are needed to support these results.

Conflict of interest: None

Financial disclosure: None

REFERENCES

1. **Balakumar P, Maung-U K, Jagadeesh G.** Prevalence and prevention of cardiovascular disease and diabetes mellitus. *Pharmacol Res*. 2016;(113):600-9.
2. **Shah S, Iqbal T, Qamar R, Rafiq M, Hussain S.** SARG1 Gene Polymorphisms and Their Association in Individuals with Essential Hypertension: A Case–Control Study. *DNA Cell Biol*. 2018;7(37):609-16.
3. **Chen J, Budoff MJ, Reilly MP, Yang W, Rosas SE, Rahman M, et al.** Coronary artery calcification and risk of cardiovascular disease and death among patients with chronic kidney disease. *JAMA cardiol*. 2017;2(6):635-43.
4. **Hammer F, Stewart PM.** Cortisol metabolism in hypertension. *Best Pract Res Clin Endocrinol Metab*. 2006;20(3):337-53.
5. **Chapman K, Holmes M, Seckl J.** 11β-hydroxysteroid dehydrogenases: intracellular gate-keepers of tissue glucocorticoid action. *Physiol Rev*. 2013;93(3):1139-206.
6. **Ku YH, Koo BK, Kwak SH, Cho YM, Shin HD, Lee HK, et al.** Regulatory effect of common promoter polymorphisms on the expression of the 11β-hydroxysteroid dehydrogenase type 1 gene. *Horm Res Paediatr*. 2009;72(1):25-32.
7. **Kearney PM, Whelton M, Reynolds K, Muntner P, Whelton PK, He J.** Global burden of hypertension: analysis of worldwide data. *The lancet*. 2005;365(9455):217-23.

8. **Edmondson AC, Brown RJ, Kathiresan S, Cupples LA, Demissie S, Manning AK, et al.** Loss-of-function variants in endothelial lipase are a cause of elevated HDL cholesterol in humans. *J. Clin. Investig*. 2009;119(4):1042-50.
9. **Hejduk P, Sakowicz A, Pietrucha T.** Association between ins4436A in 11β-hydroxysteroid dehydrogenase type 1 gene and essential hypertension in Polish population. *Postepy Hig Med Dosw*. 2015;69(0):1245-50.
10. **Forman JP, Fisher ND, Pollak MR, Cox DG, Tonna S, Curhan GC.** Renin-Angiotensin System Polymorphisms and Risk of Hypertension: Influence of Environmental Factors. *J. Clin. Hypertens* 2008;10(6):459-66.
11. **Tsezou A, Karayannis G, Giannatou E, Papanikolaou V, Triposkiadis F.** Association of renin-angiotensin system and natriuretic peptide receptor A gene polymorphisms with hypertension in a Hellenic population.*JRAAS*. 2008;9(4):202-7.
12. **11. Dhanachandra Singh K, Jajodia A, Kaur H, Kukreti R, Karthikeyan M.** Gender Specific Association of RAS Gene Polymorphism with Essential Hypertension: A Case-Control Study. *Biomed Res Int*. 2014;2014:1-10.
13. **Thieringer R, Hermanowski-Vosatka A.** Inhibition of 11β-HSD1 as a novel treatment for the metabolic syndrome: do glucocorticoids play a role? *Expert Rev Cardiovasc Ther*. 2005;3(5):911-24.
14. **Olza J, Gil-Campos M, Leis R, Rupérez A, Tojo R, Cañete R, et al.** A gene variant of 11β-hydroxysteroid dehydrogenase type 1 is associated with obesity in children. *Int J Obes*. 2012;36(12):1558-63.
15. **Franks PW, Knowler WC, Nair S, Koska J, Lee Y-H, Lindsay RS, et al.** Interaction between an 11β-HSD1 gene variant and birth era modifies the risk of hypertension in Pima Indians. *Hypertension*. 2004;44(5):681-8.
16. **Ruan LL, Xu J, Wang CL, Zou CC.** Variants of 11β-hydroxysteroid dehydrogenase (HSD11B) gene type 1 and 2 in Chinese obese adolescents. *J Endocrinol Invest*. 2014;37(6):565-73.
17. **Smit P, Dekker MJ, de Jong FJ, van den Beld AW, Koper JW, Pols HA, et al.** Lack of association of the 11β-hydroxysteroid dehydrogenase type 1 gene 83,557 insA and hexose-6-phosphate dehydrogenase gene R453Q polymorphisms with body composition, adrenal androgen production, blood pressure, glucose metabolism, and dementia. *J. Clin. Endocrinol. Metab*. 2007;92(1):359-62.
18. **Rahman TJ, Mayosi BM, Hall D, Avery PJ, Stewart PM, Connell JM, et al.** Common variation at the 11-β hydroxysteroid dehydrogenase type 1 gene is associated with left ventricular mass. *Circ Cardiovasc Genet*. 2011;4(2):156-62.
19. **Draper N, Walker EA, Bujalska IJ, Tomlinson JW, Chalder SM, Arlt W, et al.** Mutations in the genes encoding 11β-hydroxysteroid dehydrogenase type 1 and hexose-6-phosphate dehydrogenase interact to cause cortisone reductase deficiency. *Nat. Genet*. 2003;34(4):434-9.
20. **Gelernter-Yaniv L, Feng N, Sebring N, Hochberg Z, Yanovski J.** Associations between a polymorphism in the 11 beta hydroxysteroid dehydrogenase type I gene and body composition. *Int J Obes*. 2003;27(8):983-6.
21. **Hadoke PW, Christy C, Kotelevtsev YV, Williams BC, Kenyon CJ, Seckl JR, et al.** Endothelial cell dysfunction in mice after transgenic knockout of type 2, but not type 1, 11β-hydroxysteroid dehydrogenase. *Circulation*. 2001;104(23):2832-7.
22. **Victorio JA, Clerici SP, Palacios R, Alonso MJ, Vassallo DV, Jaffe IZ, et al.** Spironolactone prevents endothelial nitric oxide synthase uncoupling and vascular dysfunction induced by β-adrenergic overstimulation: role of perivascular adipose tissue. *Hypertension*. 2016;68(3):726-35.
23. **Paterson JM, Morton NM, Fievet C, Kenyon CJ, Holmes MC, Staels B, et al.** Metabolic syndrome without obesity: hepatic overexpression of 11β-hydroxysteroid dehydrogenase type 1 in transgenic mice. Proceedings of the National Academy of Sciences. 2004;101(18):7088-93.
24. **Masuzaki H, Yamamoto H, Kenyon CJ, Elmquist JK, Morton NM, Paterson JM, et al.** Transgenic amplification of glucocorticoid action in adipose tissue causes high blood pressure in mice. *JCI*. 2003;112(1):83-90.

Mohamed, R., Elzaiat, A., Elfeky, H., Al-Karamany, A. Polymorphism of 11 β-Hydroxysteroid Dehydrogenase Type 1 Gene in Hypertensive Patients. *Zagazig University Medical Journal*, 2022; (728-733): -. doi: 10.21608/zumj.2021.42702.1942