Genetic polymorphism in *CYP11B2* gene associated with hypertension in north Indian population.

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Abstract

This study was carried on genetic polymorphism of *CYP11B2* gene related to hypertension in north Indian population from Punjab region. Hypertension is associated with cardiovascular and kidney diseases. It is mainly caused by high blood pressure which exerts pressure on heart and in arteries which may cause heart failure in severe cases. High dietary intake of sodium is one of the main causative agents for high blood pressure. In adults, alcohol consumption is also associated with hypertension. Previous studies have shown that polymorphism in *CYP11B2* gene is associated with hypertension. This gene codes for an enzyme known as Aldosterone synthase which is present in adrenal gland of kidney. Aldosterone synthase is involved in the formation and breakdown of various molecules within the cell. However, the exact mechanism and its association with physiological parameters are lacking. In this study we have compared the genetic polymorphism -344C/T variation and hypertension by PCR-RFLP technique. The study showed that there was significant association between lifestyle and hypertension.

Keywords: *CYP11B2*, hypertension, cardiovascular diseases, RFLP, genetic polymorphism.

Introduction

Hypertension is very common diseases in persons who have high blood pressure. Person having hypertension are shown to be affected by cardiovascular or renal diseases. Due to various physiological conditions namely ageing, hormonal imbalance, sedentary life style there is increase chances of high blood pressure. In developed countries, 15-20% of grown-up people are affected by hypertension due to high consumption of alcohol (Park K 2015). Hypertension can be classified on the basis of three parameters: - (1) Malignant hypertension (2) Primary hypertension (3) Secondary hypertension. Malignant hypertension is associated with tissue and internal organ damage. People having blood pressures 180/120 are malignant hypertensive patients. Primary hypertension is affected by environmental factors such as lifestyle. Essential or idiopathic hypertensions are asymptomatic which causes 5% of chronic diseases (Bakris G 2015). It has been reported that 95% cases of hypertension in U.S are due to high blood pressure are essential hypertension. Although exact causes of essential hypertension are not known but it is associated with cardiovascular and kidney diseases.

Rennin angiotensin- aldosterone system is one of the key factors to increase blood pressure which lead to essential hypertension. There are different genes which are involved in high blood pressure. In this study we focused on *CYP11B2* gene involved in hypertension. This gene is located on chromosome 8q22 of humans. *CYP11B2* gene provides information for making an enzyme called aldosterone synthase which is found in the adrenal glands of the kidney. This enzyme involved in the formation and breakdown of various molecules within the cell. Aldosterone helps

in controlling the blood pressure by maintaining the proper salt and fluid concentration in the body. Aldosterone synthase deficiency (corticosterone methyloidase) causes imbalance in *CYP11B2* expression (Bassett MH, 2002). The C344T (cytosine to thymine) single nucleotide substitution in the promoter region of the gene is reported for polymorphism of the *CYP11B2* (Keavney B et al; 2005). The polymorphism of *CYP11B2* gene is associated with serum aldosterone and blood pressure (Keavy B et al; 2005, Russo P et al; 2002). It belongs to cytochrome P450 family. The C344T mutations lead to excessive release of sodium and chlorine ions from body into urine. This imbalance leads to nausea, vomiting, weak muscles and high blood pressure.

Hypertension is responsible for 57% of all stroke death and 24% heart diseases (Gupta R. 2004). World Health Organization rate hypertension as major cause of premature death worldwide. According to WHO, 46% of both male and female are affected by hypertension related to high blood pressure. Among both sexes' males are more prone due to alcohol consumption & smoking. In India high blood pressure is major risk problem and rapidly growing in both urban and rural populations. The pervasiveness of hypertension in India in last decade increased from 2% to 25% due to sedentary life style. Now it is considered to be the most common chronic disease in India. Reducing blood pressure also decreases the risk of cardiovascular diseases. In 2015, it was reported that hypertension and its risk factors in adults are caused due to consumption of alcohol in urban areas (Siraj Ahmad, 2005). Previous studies have shown that genetic polymorphism of *CYP11B2* gene is linked with hypertension in different populations. However, the exact mechanism is not clear. In this study, PCR-RFLP was used to detect the -344T/C in *CYP11B2* gene polymorphism in north Indian normotensive and hypertensive populace.

Material and methods

Blood Sample collection: -

Blood sample were collected from different hypertension and normal patients in EDTA vials and sample were stored at -20°C for further use. Consent form was taken from all the patients. Sample collected from Pathankot district of Punjab. Biological parameters are tested for each individual including glucose, HDL, LDL, cholesterol, triglycerides and some clinical characteristics such as sex, age, drinking habits, and smoking. When Blood pressure of each individual is measured, they all are in sitting position. Hypertension is defined as systolic blood pressure more than 140mmHg and diastolic blood pressure more than 90mmHg.

Extraction of Genomic DNA: -

DNA from blood samples were extracted by using standard phenol chloroform method and by using Blood genome DNA extraction kit (GeNei). Precipitation of DNA was done with 500 µL absolute ethanol at 10,000 rpm for 5-10 minutes. DNA is stored in TE buffer at -20ºC for further use. DNA bands were observed on 1% agarose gel with 100 bp ladder. Concentration and purity of genomic DNA was determined using spectrophotometer by measuring O.D at 260 and 280nm.

PCR Amplification: -

PCR Amplification of DNA was done by using reaction mixture or master mix of 20 µL which contain most Taq polymerase 1 U, 3µL Template DNA sample, 10X PCR buffer with $MgCl_2$ 2µL, 2.5mM dNTPs 3µL, Forward Primer 1 µL, Reverse Primers 1 µL, Nuclease Free water 11 µL Conditions for PCR reaction are: -94ºC for 1 min, 32 cycles of

10 s at 94ºC, 15 s at 62.5ºC, 25 sec at 72ºC, followed by 5 min at 72ºC. Amplified PCR product was observed on 2% agarose gel under UV light.

RFLP and Genotyping: -

The Genotyping of C/T polymorphism of *CYP11B2* gene at -344 positions was performed by PCR-RFLP amplification. RFLP analysis was done by using restriction enzyme *Hae*III. Reaction was carried in reaction mixture of 10X H4 buffers 10 µL, 1 µL of *Hae*III enzyme and 8 µL of PCR amplified product at 37ºC for 1 hour. Digestions of products were loaded on 2% agarose gel electrophoresis.

Table 1: - List of primers used for PCR amplification.

Primers	**Sequence of Primer**
Forward Primer	5'-CAGGAGGAGACCCCATGTGAC-3'
Reverse Primer	5'-CCACCACCCTGTTCAGCCC-3'

Table 2: - The following reaction mixture is used for PCR amplification (20µl).

Components	**Concentration**	**Volume/Reaction In µl**
Nuclease Free Water		9
10X PCR buffer	1X	2
dNTP mix	0.1-0.2 mM	3
Forward primer	10 µM	1
Reverse primer	10 µM	1
Template DNA	50 ng	3
Taq-Polymerase	50 U	1

Result and Discussion

DNA Isolation and PCR amplification

The DNA was isolated from the collected blood samples using genomic DNA isolation kit (GeNei) as per the manufacturer's instruction. DNA was visualized in 1% agarose gel electrophoresis (data not shown). The concentration of DNA was measured using spectrophotometer (Table 3 and 4). The DNA was stored in TE buffer pH 8.0 at -20 ºC. PCR was used to amplify the targeted DNA fragment by using gene specific primers (Table 1). The fragment size amplifies the -344 T>C single nucleotide polymorphism (SNP) in the promoter region of the *CYP11B2* gene sequence. PCR product of 538 bp is visualized under UV light. The electrophoresis was done on 2% agarose gel (Figure 1).

Table 3: -DNA Absorbance of normal patients

S. NO.	***Patients***	***A_{260nm}***	***A_{280nm}***	***A260/A280***	***Concentration ng/µL***
1	***N1***	1.742	0.962	1.81	92.62
2	***N2***	1.454	0.902	1.61	69.64
3	***N3***	1.264	0.752	1.68	52.46

4	***N4***	0.965	0.882	1.09	49.26
5	***N5***	1.352	1.157	1.16	65.62
6	***N6***	1.086	0.829	1.31	52.09
7	***N7***	1.054	0.758	1.39	69.99
8	***N8***	1.515	0.899	1.68	68.69

Table 4: - DNA absorbance of hypertension patients

S.NO.	***Patients***	A_{260nm}	A_{280nm}	***A260/A280***	***Concentration ng/µl***
1	***HY1***	0.893	0.59	1.51	44.67
2	***HY2***	0.965	0.626	1.54	48.24
3	***HY3***	4.334	2.341	1.85	216.72
4	***HY4***	7.088	3.883	1.83	354.39
5	***HY5***	1.551	0.863	1.8	77.53
6	***HY6***	1.373	0.847	1.62	68.66
7	***HY7***	1.384	0.8	1.73	69.19
8	***HY8***	1.399	0.812	1.72	69.96
9	***HY9***	1.042	0.723	1.44	52.08
10	***HY10***	0.74	0.439	1.69	37.02
11	***HY11***	0.371	0.432	0.86	18.55
12	***HY12***	2.377	1.755	1.35	118.84
13	***HY13***	1.029	0.63	1.63	51.46
14	***HY14***	1.164	0.659	1.77	58.21
15	***HY15***	1.226	0.879	1.4	61.32
16	***HY16***	0.595	0.372	1.6	29.73
17	***HY17***	2.116	1.187	1.78	105.8
18	***HY18***	0.349	0.223	1.57	17.47
19	***HY19***	1.312	1.053	1.25	65.58
20	***HY20***	0.865	0.464	1.86	43.26
21	***HY21***	2.635	1.416	1.86	131.77
22	***HY22***	6.234	3.471	1.8	311.69
23	***HY23***	1.855	0.98	1.89	92.73

Quantitative estimation of DNA was observed by NanoDrop 2000 at Guru Angad Dev Veterinary and Animal Sciences University, Ludhiana.

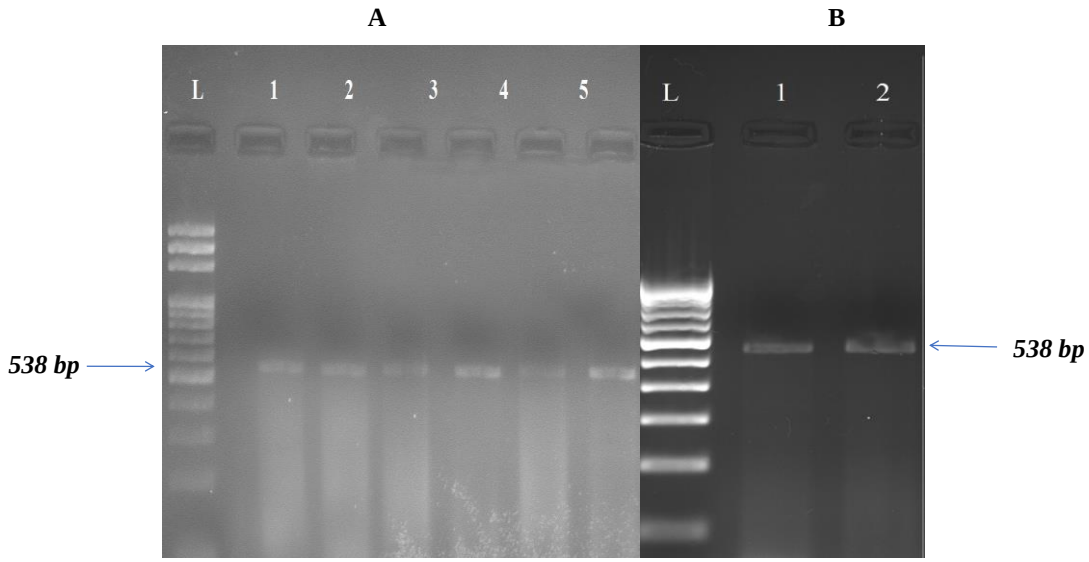

Figure 1: - PCR amplification of isolated genomic DNA. (A) PCR product of hypertension patients. Lane L= 100 bp ladder, Lane1= HY14, Lane2= HY15, Lane3= HY16, Lane4= HY12, Lane5= HY10. (B) PCR product of Normal Patients. Lane L= 100bp Lane1= N3, Lane2= N4.

Restriction Digestion

Analysis of restriction enzyme *Hae*III digest in the PCR product of the 538bp that contains -344 promoter region of the *CYP11B2* gene in which TT is wild type homozygous, TC is heterozygous mutant, CC is homozygous mutant.

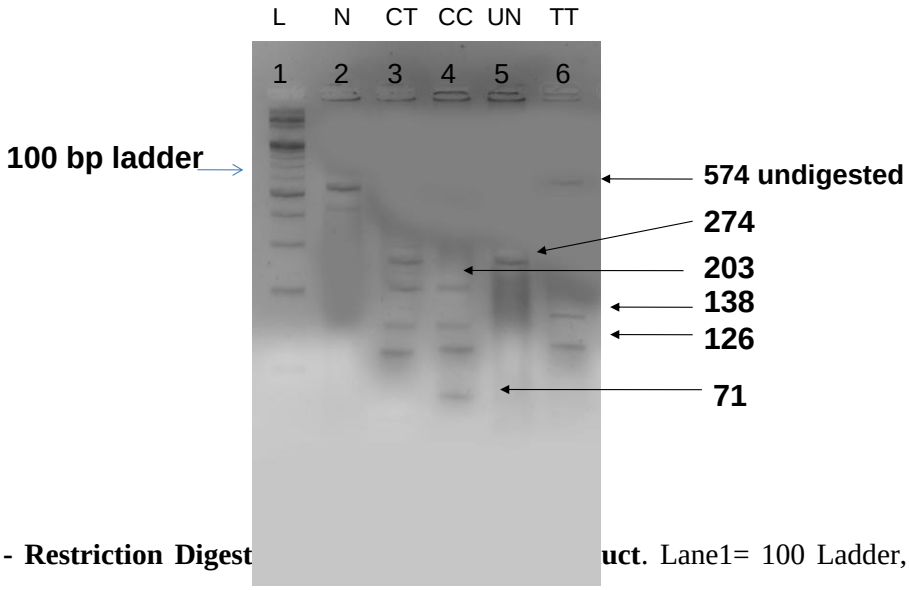

Figure 2: - Restriction Digest uct. Lane1= 100 Ladder, Lane2= HY17, Lane3= HY18, Lane4= N4, Lane5= N6, Lane6= N7. (In lane 6 the upper band is of undigested DNA and 274 band is not visible, but confirmed with other restriction digestion. The upper band is 574bp which is not digested properly). The product -344T>C of *CYP11B2* gene 274+138+126 (TT), 274+203+138+126+71 (TC), 203+138+126+71bp.

Restriction digestion was observed on 2% agarose gel, the percentage of genotype CC, TT and CT in hypertension and normal patients were calculated. We observed association of *CYP11B2* gene and hypertension in north Indian population. Out of 31 we could able to perform restriction digestion in 21 patients. However due to some experimental technical issues results were obtained only in 15 patients. Since sample sizes were small statistical significance could not be calculated. Statistical Analysis for various biochemical parameters was done for hypertension and normal patients. We found that there is no major difference between smoking and drinking habits, HDL of hypertension and

normal patients. Whereas the values of triglycerides and SBP, DBP, cholesterol, LDL were higher in hypertension patients than normal patients. There is huge difference in their values. SBP= Systolic Blood Pressure, DBP= Diastolic Blood Pressure.

Table 5: -Statistical Analysis of Biochemical Parameters of Normal and Hypertensive Patients

	No. of patients	**Age**	**SBP (mmHg)**	**DBP (mmHg)**	**HDL**	**LDL**	**Triglyceride**	**Cholesterol**
Normal Patients (n±S.D)	8	25-70	117.5±12.8	81.25±6.4	47±3.74	79.625±17.9	153.41±6.4	156.23 ±24.9
Hypertension Patients (n±S.D)	23	25-75	205±25.3	99.13±9.9	48.65 ±4.9	91.25±35.87	189.38±100.	180.78 ±46.

In men and women 8% prevalence is studied in different age groups such as 20 years and above and which belong to low social economic group (Mohan V et al; 2001). A study concluded in the urban areas of Chennai during 2000 reported a higher prevalence of hypertension (54%) among low income group and 40% pervasiveness among high-salary group (Ramachandran et al; 2002). Positive Association of *CYP11B2* gene polymorphisms with genetic predisposition to essential hypertension study have been done (K Tsukada et al; 2002). The genotype of - 344C/T polymorphism was determined in essential hypertension subject and normotensive subject. Predisposition to essential hypertension and cardiovascular diseases are possibly associated with gene polymorphism of the rennin-angiotensin system. It had been shown that - 344 allele of the gene polymorphism is associated with genetic predisposition to develop essential hypertension.

Aldosterone synthase gene polymorphism and cardiac dimension relation is studied in essential hypertension subjects in 2004. Relation between M-mode echocardiographic cardiac dimensions and aldosterone synthase - 344C/T polymorphism was studied. The patients were divided in different group's 210 never-treated, middle aged patients affected by mild to moderate essential hypertension. It was concluded that among all patients, 48 had the genotype C344CG, 97 had C344T, and 65 had T344T. Patients in the three groups were similar in term of age, gender and blood pressure (Stella et al; 2004).*CYP11B2* gene polymorphism has been reported that it is associated with serum aldosterone level, urinary aldosterone excretion, blood pressure and left ventricle size and mass. It was also shown that genotyping distribution of *CYP11B2* gene polymorphism did not differ among controls and ESRD patients (Lee et al; 2009). In 2009 (S. Rajan et al;) has shown the important association between *CYP11B2*gene polymorphism and hypertension. The different risk factors were confirmed in hypertension, for example, age, height, obesity, lifestyle, and excessive intake of sodium. Aldosterone synthase gene (CYP11B2) 344C/T polymorphism has been reported in association with serum aldosterone level, urinary aldosterone excretion, blood pressure. Relation between *CYP11B2* polymorphism and end-stage renal diseases was studied in Korean population. From this study it was concluded that *CYP11B2* polymorphism may not be a genetic marker for cardiovascular diseases in Korean ESRD patients (Lee et al 2009). Occurrence of hypertension and its risk factors along with adults within the age group 20 years in urban areas is led by (Siraj Ahmad 2015). They found that hypertension caused in adults due to consumption of alcohol.

Conclusion

Blood samples collected from high blood pressure patients and from healthy patients in EDTA vials. We measure various biological parameters. There is difference in blood glucose level of normal and high blood pressure patients. We found that there is no major difference in smoking and drinking habits, HDL of normal and hypertension patients. However, we found that triglycerides and cholesterol are higher in hypertension patients than normal patients. Genomic DNA is isolated from blood by using phenol chloroform method. To confirm genomic DNA gel electrophoresis was performed. Concentration of genomic DNA is calculated by using spectrophotometer at 260/280nm wavelength. In some cases, DNA is not digested due to some reagents not working properly. Apart from *Hae*III other enzyme like *Pst*I can also be tried for future studies. Moreover, to increase the significance level of study more samples can be tested. The study can lead to designing and identification of efficient drug and find out the vulnerability groups of people based on genotypes.

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