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

Project Report

Submitted in partial fulfillment of the requirements for the degree of

Masters of Sciences (Biotechnology Honors)

Submitted By
Neha Rana (11510800)

Under the guidance of
M.Amin-ul Mannan.Ph.D.
Assistant Professor

SCHOOL OF BIOENGINEERING AND BIOSCIENCES
LOVELY PROFESSIONAL UNIVERSITY
PHAGWARA, PUNJAB- 144411

DECLARATION

I hereby declare that the project entitled ***"Genetic polymorphism in CYP11B2 gene associated with hypertension in north Indian population"*** is an authentic record of our own work carried out of School of Bioengineering and Biosciences, Lovely Professional University, Phagwara for the partial fulfillment of the award of Masters of Sciences in Biotechnology under the guidance of M.Amin-ul Mannan. Ph.D.

This work is our original work and has not been submitted for any degree/diploma in this or any other University. The information furnished in this report is genuine to the best of my knowledge and belief.

Place: Neha Rana (11510800)

Date:

CERTIFICATE

This is to certify that **Neha Rana** (11510800) have completed the project, entitled ***"Genetic polymorphism in* CYP11B2 *gene associated with hypertension in north Indian population"*** under my guidance and supervision. To the best of my knowledge, the present work is the result of her original investigation and study.

No part of the report has ever been submitted for any other degree at any university. The report is fit for submission and the partial fulfillment of the conditions for the award of M.Sc. Biotechnology.

Date: (Supervisor Signature)

ACKNOWLEDGEMENT

I would like to say my special thanks of appreciation to my mentor Dr. Mohammad Amin-ul Manan, Assistant Professor School of Bioengineering and Biosciences Lovely Professional University as well as our Head of School Dr. Neeta Raj Sharma who gave me the golden opportunity to do this magnificent project on the topic ***"GENETIC POLYMORPHISM OF* CYP11B2 *GENE ASSOCIATION WITH HYPERTENSION IN NORTH INDIAN POPULATION (IN JALANDHAR REGION OF PUNJAB)"*** which also helped me in doing a lot of research and I came to know new things.

I am really thankful to them.

Secondly, I would also like to express thank to my parents and friends who helped me a lot in this project within the partial time.

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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. Hypertension 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* gene, Aldosterone synthase, hypertension, high blood pressure, cardiovascular diseases, PCR-RFLP, genetic polymorphism.

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CHAPTER 1

INTRODUCTION

Hypertension is very common diseases in those persons who have high blood pressure. Person having hypertension are shown to be affected by cardiovascular or renal diseases Due to various physiological conditions, 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)**. When heart pumps the pressure exerted in blood vessels is called as systolic (left ventricle) and pressure exerted in blood when heart rests is called as diastolic (right ventricle). The different ranges of blood pressure are mentioned below.

Blood pressure category	***SYSTOLIC***	***DIASTOLIC***
Normal	120	80
Prehypertension	120-139	80-89
High Blood Pressure Stage 1	140-159	90-99
High Blood Pressure Stage 2	160	100
High Blood Pressure Stage 3	150	90

Table 1:-Blood pressure categories

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 **(Bakris G 2015)**.Essential or idiopathic hypertensions are asymptomatic. 5% of chronic diseases are caused by secondary hypertension **(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 certain risks to health. It is significantly influenced by diet and lifestyle **(Klodas E, 2015)**. Rennin angiotensin- aldosterone system is one of the key factors to increase blood pressure which lead 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 human. *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* gene expression.

The – 344CT (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, Bassett MH, 2002)**. The polymorphism of *CYP11B2* gene is associated with serum aldosterone and blood pressure**(Keavy B et al; 2005, Russo P et al; 2002, White PC et al; 1995)**. It belongs to cytochrome P450 family. The – 344CT mutations lead to excessive release of sodium and chlorineions 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, **Siraj Ahmed** reported that hypertension and its risk factors in adults are caused due to consumption of alcohol in urban areas. **Wasir HS et al;** revealed 3% prevalence of hypertension in Delhi.

Reducing blood pressure can reduce risk of cardiovascular diseases this can be done by some changes in lifestyle such as reducing dietary salt intake, increasing potassium and avoidance of alcohol and drugs usages. Kearney et al; had reported than more or less grownup adults are developing high blood pressure due to sedentary lifestyle and this figure is expected to reach 1.5 billion in years 2025 **(Kearney PM, 2005)**. Earlier reports suggest that the prevalence of hypertension is increased day by day in developing countries and leading causes of death and disability.

CHAPTER 2

TERMINOLOGY

Hypertension: - is defined as blood pressure when it is above 140 at 90 mmHg.

Blood Pressure: - A condition in which flow of blood in arteries is very fast.

Genomic: - Study of the whole set of genes within a single cell of an organism.

CYP11B2 gene: - Cytochrome P45 it gives information for making an enzyme Aldosterone synthase which maintain fluid and salt concentration inside body.

SNP: - Single Nucleotide Polymorphism.

HDL: - High Density Lipoprotein (good cholesterol).

LDL: - Low Density Lipoproteins (bad cholesterol).

SBP: - Systolic Blood Pressure.

DBP: - Diastolic Blood Pressure.

PCR: - is technique in molecular biology to amplify gene of interest, in which thermocycler is used to amplify a segment of DNA.

Taq Polymerase: - Enzyme that copies DNA using polymerase chain reaction.

dNTP:- Deoxynucleotide triphosphates contain sodium salts of dATP, dGTP, dCTP and dTTP.

RFLP: - Restriction Fragment Length Polymorphism.

CHAPTER 3

REVIEW OF LITERATURE

Hypertension is caused by high blood pressure. Blood pressure itself a pressure caused by blood on the walls of the blood vessels **(Quassy A 2015)**. Every one minute heart beats about 60-70 times to pumps blood into arteries **(Millar 1978)**.Hypertension is main risk factor of cardiovascular diseases which lead to 20-50% death in India. The Renin-angiotensin-aldosterone system is one of the key modulator of blood pressure in hypertension **(Poch E et al; 2001)**. Aldosterone is synthesized by the adrenal cortex of the adrenal glands, by the aldosterone synthase enzyme **(Freel EM et al; 2004)** which is prearranged by the *CYP11B2* gene which is to be found in chromosome number 8q22 **(Hilgers KF et al; 2005, Brand E et al; 1998)**.

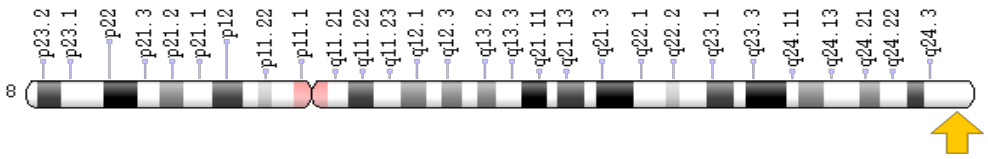


Figure 3.1: - Location of CYP11B2 gene on chromosome number (8q22)

Source- (National Centre of Biotechnology Information)

HYPERTENSION AND RISK FACTORS

As the individual grows older the possibilities of high blood pressure and hypertension consistently expanded. Risk of hypertension is higher in older persons with age group of 60 years or above. Risk of hypertension is different in different races African-American adults are at high risk of hypertension than Hispanic American adults. Smoking can also raise blood pressure because of its high risk of heart and the rest of the body **(European Heart Journal; 2013)**. Life style influences hypertension to a great extent*viz* highintake of sodium, less and no physical activity can contribute to increased risk of hypertension. Other risk factors include chronic stress and family history of diseases **(K Tsukada et al; 2002)**.

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 2000reported 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 being 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. Thepatients were dividedin 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 differentrisk 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.Relationbetween *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)**.

Hypertension in India – Meta examination of prevalence, control and its awareness have being led by **Anchala et al;** in 2014. They took particular region for their study in India and check its impact in different hypertensive patients and found that 29.8% prevalence for hypertension in India. Occurrence of hypertension and its risk factors along with adults within the age group 20 years in urban areas are led by **(Siraj Ahmad 2015)**. They found that hypertension caused in adults due to consumption of alcohol.

CHAPTER 4

RATIONALE AND SCOPE OF THE STUDY

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. Studies had shown that positive association of *CYP11B2* gene with essential hypertension. Hypertension is caused due to high consumption of alcohol, drugs and high intake of sodium concentration. There are many variations in the study that still needs to be validated. Therefore present study was proposed to verify results of genetic variation *CYP11B2*with physiological and biochemical parameters such as age, glucose level, smoking, consumption of alcohol and cholesterol. HaeIII restriction enzyme was used to decipher the -344 promoter region genotyping of *CYP11B2* gene. We envisioned to expected CT, CC, & TT variations.

CHAPTER 5

OBJECTIVES OF THE STUDY

1) To amplify cytochrome P45 gene (CYP11B2) by PCR.
2) To study the relation between CYP11B2 gene polymorphism and hypertension in North Indian population.
3) To study association of various biochemical parameters to CYP11B2 gene polymorphism.

CHAPTER 6

MATERIALS AND RESEARCH METHODOLOGY

1) **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 study. Sample collected from Pathankot district of Punjab. Separation of individuals done according to their blood pressure normal or high blood pressure. 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 biological parameters are tested at that time my friend was present in hospital to take their record. 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.

2) **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.

3) **PCR AMPLIFICATION:-**

PCR Amplification of DNA was done by using reaction mixture or master mix of 20µl which contain most Taq polymerase 1 U, 3µl Template DNA sample, 10X PCR buffer with $MgCl_2$ 2µl, 2.5mM dNTPs 3µl, Forward Primer 1µl, Reverse Primers 1µl, Nuclease Free water 11µl. Conditions for PCR reaction are: -94ºC for 1min, 32 cycles of 10 sec at 94ºC, 15 sec at 62.5ºC, 25 sec at 72ºC, followed by 5min at 72ºC. Amplified PCR product was observed on 2% agarose gel under UV light.

4) **RFLP and GENOTYPING:-**

The Genotyping of T/C polymorphism of CYP11B2 gene at -344 positions was performed by PCR-RFLP amplification. RFLP analysis was done by using restriction enzyme HaeIII. Reaction was carried in reaction mixture of 10X H4 buffers 10 µl, 1µl of HaeII enzyme and 8 µl of PCR amplified product at 37ºC for 1 hour. Digestions of products were loaded on 4% agarose gel electrophoresis. The product -344T>C of *CYP11B2* gene 274+138+126 (TT), 274+203+138+126+71 (TC), 203+138+126+71bp.

CHAPTER 7

RESULT AND DISCUSSION

DNA ISOLATION

DNA isolation of from different blood samples is observed by using 1% agarose gel electrophoresis. Bands shows that genomic DNA is isolated from blood samples of hypertension and normal blood pressure patients. In some patients we see that band is very dull or light in color because concentration of genomic DNA is very less or concentration of $Mgcl_2$ is high some time that degrade DNA. When EDTA is added it inhibits the degradation of DNA. DNA quality can be affected by method of extraction.TAE buffer we use in this isolation as a running buffer or to make agarose gel. TE buffer used for the storage of DNA samples. TE differentiates from its components Tris: a general pH buffer, EDTA: that chelates cations. The main purpose of TE is to solublize DNA or RNA, while protecting them from dreadful conditions. With the help of UV spectrophotometer we can measure the concentration of genomic DNA in different samples by using different wavelength. The DNA were stored in TE buffer pH=8.0 at -20^{o}c till further use. Blood is collected in EDTA vials so that it does not coagulate.

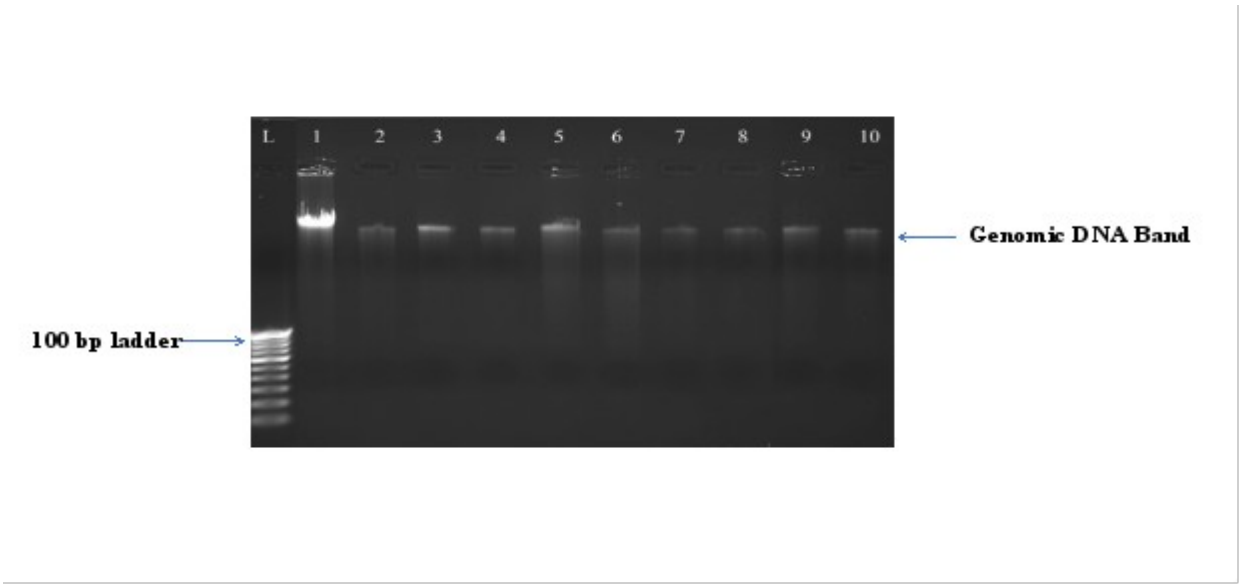


Figure 7.1:- Agarose gel electrophoresis of isolated genomic DNA. Lane1=100 bp ladder, Lane2= HY3, Lane4= HY4, Lane5= HY5, Lane6= HY7, Lane7= HY8, Lane8= HY9, Lane9= HY10, Lane10= HY12, Lane11= HY13. DNA isolated by using kit protocol. (The figure was adapted from Shikha's thesis Registration number 11510782, isolation was performed jointly)

PCR AMPLIFICATION

PCR amplification of patients was done to amplify the targeted DNA fragment by using gene specific primers. The fragment size amplifies the -344 T>C SNP was 538 bp 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.

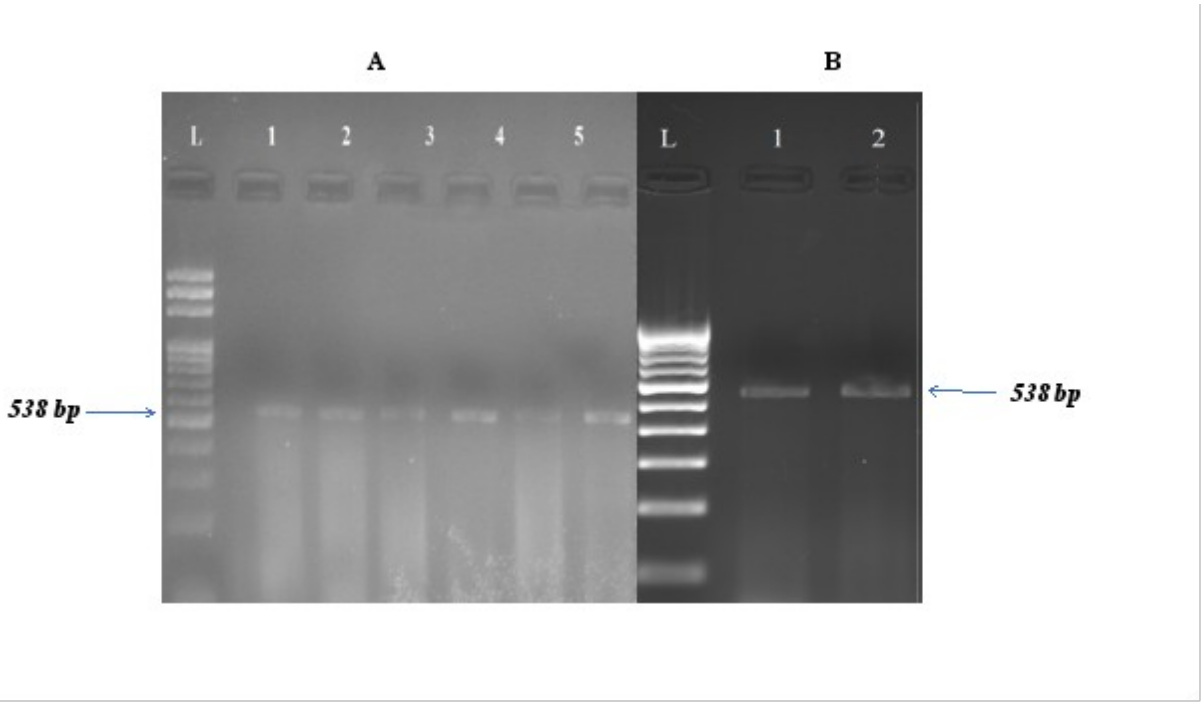
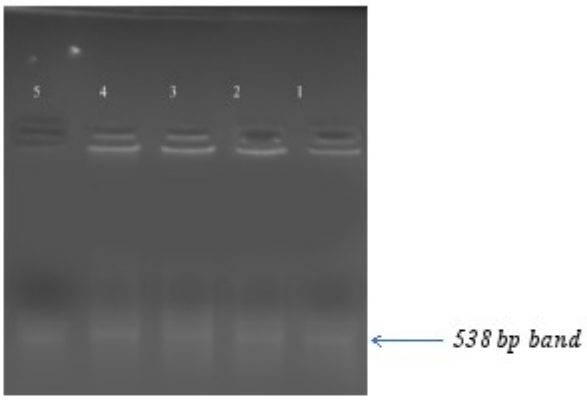


Figure 7.2:- PCR amplification of isolated genomic DNA.

(A) PCR product of hypertension patients. Lane1= 100 bp ladder, Lane2= HY14, Lane3= HY15, Lane4= HY16, Lane5= HY12, Lane6= HY10.
(B) PCR product of Normal Patients. Lane1= 100bp Lane2= N3, Lane3= N4.



F igure 7.3:-PCR amplification of isolated genomic DNA. Lane1= HY3,Lane2= HY4, Lane3= HY5, Lane4= HY6, Lane5= HY7, Lane6= HY8.

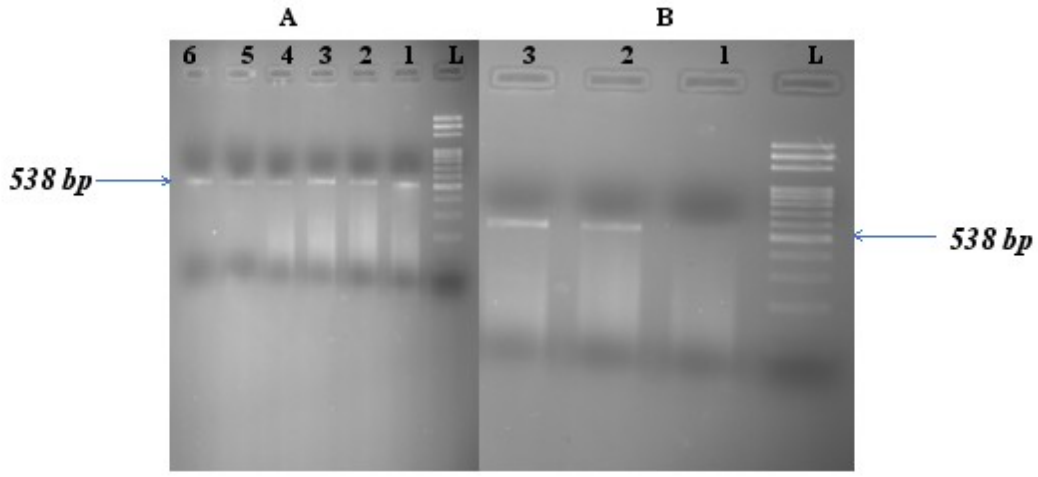


Figure 7.4 :- (A) Amplified PCR product of hypertension patients. Lane1= ladder, Lane2= HY19, Lane3= HY20, Lane4= HY21, Lane5= HY22, Lane6= HY23, Lane7= HY18.

(B)PCR amplified product of normal patients. Lane1= ladder, Lane2= N6, Lane3= N7, Lane4= N8.

RESTRICTION DIGSTION

Analysis of restriction enzyme Hae111 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.

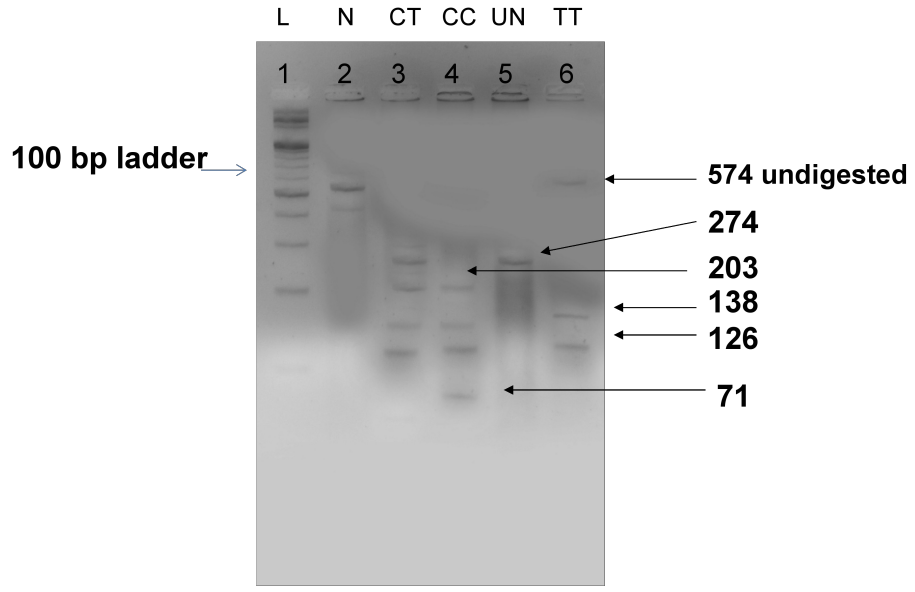


Figure 7.5:- Restriction Digestion of amplified PCR product. Lane1= 100 Ladder,

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)

Restriction digestion was observed on 4% 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 7.1:-Statistical Analysis of Biochemical Parameters of Normal and Hypertensive Patients

	Number of patients	**Age**	**SBP (mmHg)**	**DBP (mmHg)**	**HDL**	**LDL**	**Triglycerides**	**Cholestrol**
Normal Patients (n±S.D)	8	25-70	117.5±12.81	81.25±6.41	47±3.74	79.625± 17.93	153.41± 6.45	156.23 ±24.96
Hypertension Patients (n±S.D)	23	25-75	205±25.37	99.13±9.960	48.65 ±4.97	91.25±3 5.87	189.38± 100.9	180.78 ±46.9

Quantitative estimation of DNA was observed by NanoDrop 2000 at GADVASU, Ludhiana.

Table 7.3: -Absorbance of normal patients

S.NO.	***Patients***	***A260nm***	***A280nm***	***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 7.4: - Absorbance of hypertension patients

S.NO.	*Patients*	*A260nm*	*A280nm*	*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

CHAPTER 8

Experimental work

1. DNA isolation done through Kit protocol.

Blood sample collected in EDTA vials and lable each tube was properly label Normal or Hypertension blood sample.

Pipette 300µl of blood in 1.5ml tube. Centrifuge at 5000 rpm for 8 minutes at -4ºC.

Remove the supernatant very carefully. Do not mix pellet.

Resuspend the pellet (red) in 1ml 1X solution A (RBC Lysis). Gently mix the solution at room temperature. This will lead to clear solution. Mix intermediately about 2-4 times. Do not incubate more than 10 minutes.

Centrifuge at 8000 rpm for 5 minutes at RT.

Remove the supernatant and repeat the step 4.

Remove the supernatant carefully. Keep the small white nucleated pellet intact.

Add 600µl of solution B (Nuclease lysis buffer) & keep the cells at RT for 5 minutes for complete lysis.

Centrifuge at 10,000 rpm for 10 minutes at RT.

Collect the supernatant in a fresh tube.

2.0 Or 2.5v of absolute chilled ethanol is added for precipitation of DNA. The DNA is precipitated by centrifuging at 10,000 for 20 minutes.

Wash DNA twice with 100 µl of 95% alcohol by giving short spins of 12000 rpm for 2 minutes.

Give a final wash with 100 µl of 75% ethanol. Air dries the DNA pellet only for 2 minutes at 37ºC.

Add 100 ul of solution C (TE buffer). Incubate at 55ºC for 10-15 minutes to improve the solubility.

Centrifuge at 10,000 rpm for 2 minutes to remove any insoluble material. Collect the supernatant this DNA can be used immediately or store at -20ºC for further use. (GeNei)

2. Concentration and purity of isolated DNA was checked spectrophotometrically by taking O.D at 260 and 280nm.

3. PCR amplification of DNA sample was done by using *TaqPolymerase* and specific forward and reverse primers. Thermocycler is used for three steps PCR amplification reaction.

Table 8.1:- List of specific primers used for PCR amplification.

PRIMERS	***SEQUENCE OF PRIMER***
Forward Primer	5'-CAGGAGGAGACCCCATGTGAC-3'
Reverse Primer	5'-CCACCACCCTGTTCAGCCC-3'

Table 8.2: - 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	50ng	3
Taq Polymerase	50µl	1

4. RFLP analysis of sample to be done by using specific restriction endonuclease enzyme for restriction digestion. Restriction was done at 37ºC for 1 hour.

Table 8.3:-Restriction Digestion Parameters

COMPONENTS	***VOLUME***
DNA	9***µ***l
HaeIIIenzyme	1***µ***l
DH4 buffer	10***µ***l

5. Statistical Analysis of Biological parameters are done.

CHAPTER 9

CONCLUSION AND FUTURE SCOPE

Hypertension is caused due to high blood pressure. Hypertension is major risk factor for cardiovascular diseases. Life style play important role in prevention and curing of high blood pressure. There are different parameters which affect hypertension. *CYP11B2* gene involved in high blood pressure which lead to high risk of cardiovascular diseases or affects organ damage. This gene located in 8q22 chromosome. 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 HaeIII other enzyme like Bstu1 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.

CHAPTER 1O

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CHAPTER 11

APPENDIX

EDTA 0.5M (pH-8) ,100ml

M= W ¿$mw \times 1000/V$

0.5=W/292.25× 1000/100

W=0.5×292.25/10, W=14.61g

NaCl 5M, 500ml

M= W ¿$mw \times 1000/V$

5=W/58.44×1000/500

W=58.44×5/2, W=146.1g

Tris-Hcl, 500ml

M= W ¿$mw \times 1000/V$

1=W/157.60×1000/500

W=157.60/2, W=78.8g

10% SDS

10g of SDS was dissolved in

100ml of distilled water

Sodium acetate 3M (ph-5.2), 500ml

M= W ¿$mw \times 1000/V$

3M=W/82.03×1000/500
W=3×82.03/2
W=123.045

NaOH 5M, 100ml

M= W ¿$mw \times 1000/$¿

5M=W/40×1000/100

W=20g

$MgCl_2$ 1M, 50ml

M= W ¿$mw \times 1000/V$

1M=W/203.31×1000/50 W=203.31/20 W=10.1655

Lysis Buffer 10X, 200ml

Ammonium Chloride- 16.58 gram
Potassium Bicarbonate- 24 gram
EDTA - 40 ul (0.5M)

TAE 50X, 500 ml

Tris base – 121 grams
Glacial Acetic Acid- 28.55ml
0.5M EDTA - 50ml
Adjust volume to 500ml with autoclaved distilled water.