Research Article *Soni et al.*, 10(4): April, 2019:6152-6162]
CODEN (USA): IJPLCP **ISSN: 0976-7126**

INTERNATIONAL JOURNAL OF PHARMACY & LIFE SCIENCES
(Int. J. of Pharm. Life Sci.)

Genetic polymorphism of SNP K121Q of ENPP1 gene and its association with Hypertension in Vindhyan population (India)

Neelam Soni*, Arvind Tripathi, Rishabh Dev Saket and R.M. Mishra
Centre for Biotechnology Studies, A. P. S. University, Rewa (M.P.) - India

Abstract

Hypertension is a major health problem throughout the world because of its high prevalence and its association with increased risk of cardiovascular disease. Advances in the diagnosis and treatment of hypertension have played a major role in recent dramatic declines in coronary heart disease and stroke mortality in industrialized countries. The ecto-nucleotide pyrophosphatase/phosphodiesterase 1 (ENPP1) gene is a member of the ecto-nucleotide pyrophosphatase/phosphodiesterase (ENPP) family. The encoded protein is a type II transmembrane glycoprotein.
No significant level of change has been seen in overall distribution of ENPP1 K121Q genotypes in HC group as compared to disease group although HC group showed little increase in 'KK' genotype as compared to Patients of diabetes type 2 (69.5% *vs 63.7*%). Similarly, mutant type 'QQ' genotype was present in low frequency in Diabetes type 2 patients group 1.58% and also in control group 0.96% (χ^2 =1.670, P=0.4339). '*KK'* genotype is higher in control group and may be protective in our population but statistically not significantly different between both groups. An odds ratio of KK genotype is 0.7687 which indicates little protective effect whereas an odds ratio of KQ genotype is 1.271 of Hypertension patients group respectively indicate little or no effect and association of this mutant genotype with the diabetes susceptibility. Overall allele 'K' was found little lower frequency in disease group as compared to HC group whereas allele '*Q'* was present in little high frequency in the disease group but the difference is nominal and was not significant (χ^2 = 1.461, P=0.2268). Carriage rate of allele 'Q' was slightly high in diabetic group as compared to healthy control (36.32% Vs 30.48%) whereas carriage rate of allele '*K*' was approximately similar in both control and disease group and no significant level of change has been seen. The pattern of genotype distribution, allele frequency and carriage rate in disease and control group suggests ENPP1 is not significantly associated with Hypertension in our population.

Key- words: Hypertension, ENPP1, BMI, PCR.

Introduction

Hypertension is a public health problem and a term used to describe HBP. It is a condition that occurs as a result of repeatedly elevated blood pressure exceeding 140 over 90 mmHg whereby a systolic pressure above 140 with a diastolic pressure above 90. However, normal blood pressure is below 120/80; readings between 120/80 and 139/89 is called pre-hypertension. Systolic blood pressure is the pressure in the arteries as the heart contracts and pumps blood forward into the arteries whereas diastolic represents pressure as a result to relation of the arteries after contraction (1-2).
It has been called a silent killer as it is usually without symptoms. Hypertension takes a long time before diagnosed thereby causing major health problems as stroke and other cardiovascular diseases.

*** Corresponding Author**

Damage to organs as the brain, heart, kidneys and eye and so on are the long term effect of high blood pressure disease. Hypertension is a term used to describe high blood pressure. Flow of blood is based on the beat of which the heart pumps blood. The pressure of the heart does not stay at the same level at all times. It varies based on activities at a particular point in time. Hypertension occurs as a result to long duration of abnormal pressure of the main arteries (3-5).
Hypertension is grouped into two main categories. These include primary and secondary hypertension. Primary hypertension is also known as essential hypertension and it affects ninety-five percent of persons suffering from the disease. Causes of hypertension are not yet known, however, factors as age, high salt intake, low potassium diet, sedentary lifestyle, stress as well as genes have been found as contributing to hypertension. High blood pressure occurring as a result to a consequence of another

disorder or a side effect of medication is referred to as secondary high blood pressure. Such disorders may include renal failure or renovascular disease. This type of blood pressure is evident in about five to 10% of cases (2,6).

Diagnosis of high blood pressure is usually measured with a device called sphygmomanometer. This consist of an inflatable rubber cuff, an air pump and a column of mercury or a digital readout reflecting pressure in an air column as well as electronic blood pressure machines. The readings are widely expressed in millimeters of mercury or mmHg. Diagnosis of high blood pressure is not based on a single reading except when it is extremely high (above 170-180/105-110). The cause of hypertension is not yet known unless it is unless is secondary high blood pressure. However, there are many underlying factors associated with the occurrence. These factors include: aging, excessive salt intake, sedentary lifestyle as well as genetic factors (6).

Salt is not a major cause of HBP. However, it is a contributing factor especially among salt sensitive persons. Excessive intake of salt accounts greatly to the occurrence of HBP and other cardiovascular diseases. Several studies conducted over the years recommend reduction of salt intake as the key to prevention and control of high blood pressure. Life expectancy has increased during the past three decades in Finland. This is attributed to the decrease in salt intake incorporated into the nationwide nutritional recommendation. New salt labeling regulations were passed by the Ministry of Trade and Industry in the conjunction with the Ministry of Social Affairs and Health (STM). This legislation affected all item categories that contributed high salt intake to the average Finnish meal. These included; bread, cheese, butter, sausage, sauces and so on. The fully implementation of this legislation since 1 June, 1993 contributed remarkably to decreasing high blood pressure. Even though obesity and alcohol consumption has increased, the decrease has contributed to other cardiovascular diseases as well (6-7).

Sedentary lifestyle is a medical term used to describe lifestyle with little or no physical activity. Sedentary lifestyle is dangerous to health as smoking. This is due to the fact that it contributes to most death as a result from heart diseases. The high growing rate of sedentary lifestyle could be attributed to economic growth, modernization, urbanization as well as globalization of food. (Puska *et al.,* 2003). Advance in technology today has also reduced level of morbidity at work. Most jobs demand sitting behind the desks for long hours during the day. This is followed by long hours enjoying television or video games at leisure time. As a result to this, most diseases as high blood pressure are directly related to the lack of exercise (8)

Blood pressure is affected by various activities of the body throughout the day. The heart reacts differently to basic activities of the day such as eating and drinking. High consumption of alcohol has been related to the rise of blood pressure over the years, This is due to the fact that, the kidney and liver works extra hard in getting rid of waste from the bloodstream therefore, more pressure is exerted on the arteries. Excessive alcohol intake can also increase the chance of other medical issues as obesity that may lead to an increase in blood pressure (8-9).

The ecto-nucleotide pyrophosphatase/phosphodiesterase 1 (ENPP1) gene is a member of the ecto-nucleotide pyrophosphatase/phosphodiesterase (ENPP) family. The encoded protein is a type II transmembrane glycoprotein. This protein has broad specificity and cleaves a variety of substrates, including phosphodiester bonds of nucleotides and nucleotide sugars. The ENPP family is involved in a variety of processes that range from bone mineralization to insulin action signaling (10). Several polymorphisms are located in the ENPP1 gene the one most frequently analyzed being located in exon 4 and causing an amino acid change (K121Q). The variant allele (risk allele Q) is associated with a stronger interaction of the protein with the insulin receptor compared to the wild allele (allele K), resulting in a reduction of insulin receptor autophosphorylation . Due to its effect on insulin signaling, ENPP1 is a candidate gene for insulin resistance and/or development of diabetes mellitus (DM). The clinical manifestation of this polymorphism has not been well established. Even among Caucasian subjects, the effect of the presence of Q allele varies. Sicilian, Swedish and Finnish carriers of the Q allele have lower insulin sensitivity compared to noncarriers, but this is not the case for Danes or Spaniards (10-13).

Plasma cell glycoprotein 1 (PC-1, ENPP1) is a promising candidate gene for type 2 diabetes because it inhibits autophosphorylation of insulin receptor (IR) and impairs insulin signaling downstream of IR. PC-1 has been shown to interact directly with IR, and the 121Q variant (Gln121) in exon 4 has a greater inhibitory action on IR than does the 121K allele variant (Lys121). Additionally, PC-1 has enzymatic activity, and it plays a role in the regulation of signaling by nucleotides. Moreover, the K121Q

genotype has been shown to be associated with insulin resistance and high glucose and insulin levels. No data are available on the association of the PC-1 gene polymorphism with intrauterine growth (14). Therefore, the aim of our study was to investigate whether the impact of the K121Q polymorphism of the PC-1 gene on insulin sensitivity, and the occurrence of diabetes and hypertension, depends on size at birth (14-15).

Material and Methods

Study population

The study population consisted of 400 unrelated subjects comprising of 190 hyper tension patients and 210 ethnically matched controls of central Indian population were included in this study. In this region Hindu, Muslim and some Sikh peoples are mainly living but most people belong to Hindu religion in this region.

Inclusion and Exclusion criteria for Cases

Cases included consecutive patients who attended the Department of Medicine, Shyam Shah Medical College and Sanjay Gandhi Memorial Hospital, Rewa, Ayurveda Medical College, Rewa, Ranbaxy pathology Regional collection centre Rewa, District hospital Satna, Shahdol, Sidhi.

Inclusion and Exclusion criteria for Controls

Control group composed of healthy individuals that were collected from urban regions and around SSMC Rewa. The control subjects were recruited from the regions that from homogenous cluster in Vindhyan region India in accordance with a recent report of genetic landscape of the people of India. (Indian Genome Variation Consortium 2008) The inclusion criteria for control group were as follows:-

1) ≥40 years of age
2) HbA1c level ≤6.0
3) Fasting glucose level<110mg/dl
4) No family history of diabetes in first and/or second degree relatives.

All the participants were asked to fill a detailed questionnaire at the time of recruitment, seeking information regarding individual's age, sex, ethnicity, dietary habits, physical activity, and life style, personal and family medical history.

Anthropometric and Biochemical Measurements

Anthropometry

Height and Weight were measured in light clothes and without shoes in standing position as per standard guidelines. Body Mass Index (BMI) was calculated as weight in kilograms divided by height in meters squared. Waist circumference was measured in standing position midway between iliac crest and lower costal margin and hip circumference was measured at its maximum waist to hip ratio (WHR) was calculated using waist and hip circumferences. Systolic and diastolic blood pressures were measured twice in the right arm in sitting position after resting for at least 5 minute using a standard sphygmomanometer and the average of the two reading was used.

Biochemical Analysis

Biochemical parameters related to type 2 diabetes were estimated for both cases and controls subjects. Measurement of Serum levels of Total cholesterol (TC), Triglycerides (TG), HbA1c, High density lipoprotein-cholesterol (HDL-C), Low density lipoprotein-cholesterol (LDL-C), Urea, Uric acid, C-reactive protein (CRP) and Creatinine were measured based on spectrophotometric method using automated clinical chemistry analyzer Cobas Integra 400 plus (Roche Diagnostics, Mannheim, Germany).

Blood collection and plasma/serum separation

Venous blood samples were obtained from the subjects after 12 hours of overnight fasting in vacutainers with and without appropriate anti-coagulants. Immediately, plasma and serum from the respective vacutainers were separated by centrifuging the tubes at 1000 rpm for 10 min. at 4˚C.

Method for DNA isolation

Genomic DNA was extracted from whole blood by the modification of salting out procedure described by Miller and coworkers (Miller *et al*., 1988). Frozen blood sample was thawed at room temperature. 0.5 ml. of whole blood sample was suspended in 1.0 ml. of lysis buffer (0.32 M Sucrose, 1 mM Mgcl2, 12 mM Tris and 1% Triton-X-100) in a 1.5 ml. microcentrifuge tubes. This mixture was mixed gently by inverting the tube upside down for 1 min. The mixture was than allowed to stand for 10 min. at room temperature to ensure proper lysis of cells. The mixture was centrifuged at 11,000 rpm for 5 min. at 4˚C to pellet the nuclei. The supernatant was discarded carefully in a jar containing disinfectant, as pellet formed is loosely adhered to the bottom of centrifuge tube. The pellet was resuspended in 0.2 ml. of lysis buffer and recentrifuge at 11,000 rpm for 5 min. The pellet was than dissolved in 0.2 ml. of deionized autoclaved water and mixed thoroughly on vortexer. The mixture was centrifuged at 14,000 rpm for 1 min. at 4˚C. Supernatant was discarded to gain an intact pellet. To the above pellet, 80 µl. of proteinase K buffer (0.375 M Nacl, 0.12 M EDTA, pH 8.0) and 10 µl. of 10% SDS (10% w/v SDS, pH 7.2) was added. Mixture was well frothed with the help of micro tip to allow proper lysis of pelleted nuclei. After digestion was complete, 100 µl. of

saturated cold 5M Nacl was added and shaken vigorously for 15 sec. To the above mixture 0.2 ml. of deionized, autoclaved water and 0.4 ml. of phenol-chloroform (4:1 v/v) was added to remove most of the non nucleic acid organic molecules. Microcentrifuge tube was inverted upside down until the solution turned milky. Phases were separated by centrifuging the above mixture at 12,000 rpm for 10 min. at 4˚C. Aqueous (top) layer was saved and transferred in another microcentrifuge tube. Transferring of any interface layer was avoided. To the aqueous layer, 1 ml. chilled absolute ethanol was added and the tube was inverted several times until the DNA precipitated. DNA precipitates like thread. This was centrifuged at 14,000 rpm for 4 min. at 4˚C to pellet the DNA thread. Supernatant was discarded. The pellet was washed twice with 1 ml. of 70% alcohol. The mixture was again centrifuged at 14,000 rpm for 1 min. 4˚C. Supernatant was discarded and pellet was air dried for 10-20 min. The pelleted DNA was rehydrated in 100-200 µl. of TE buffer pH 7.4 (10 mM Tris-HCL pH 7.4, 1mM EDTA, pH 8.0). DNA was allowed to dissolve overnight at 37˚C before quantization.

Determination of quality and quantity of isolated DNA

The isolated DNA is to be used for PCR based study. Therefore its suitability for PCR along with its size heterogeneity is among the most important criterion for purity. As a matter of general practice all DNA preparations were tested for quality and quantity measures, as described in the following paragraphs.

Quantitation by UV spectrophotometry

The isolated genomic DNAs were then tested for purity by measuring their absorbance values at 230 nm, 260 nm, 280 nm and 300 nm using a UV visible spectrophotometer (Systronic, India). A DNA preparation was considered to be good if it had A 260 nm / A 280 nm ratio as approximately 1.8 and A 300 nm was 0.1 or lesser. The absorbance at 260 nm was used to calculate the amount of DNA, using the relationship that double stranded DNA at 50µg/ml concentration has an absorbance= 1.0 at 260 nm.

Agarose Gel Electrophoresis

Gel electrophoresis of the genomic DNAs was carried out for qualitative estimation of samples prepared. A good DNA preparation appears as single band. A horizontal agarose slab gel electrophoresis apparatus (Bangalore Genei, Bangaore, India) was used. In brief, 4-5 µl of each genomic DNA was loaded on 0.8 agarose (0.8 % w/v, Sigma) containing ethidium bromide solution (0.5 µg/ml) and electrophoresis was done at 80 V in 1x TAE buffer (40 mM Tris, 20 mM acetic acid, 1 mM EDTA).

Polymorphism screening

In general, the genomic DNA extracted from peripheral blood of healthy individuals and diseased individuals was subjected to PCR followed by restriction digestion and electrophoresis to genotype both the groups for relevant gene of interest. All the PCRs were carried out in a PTC 200 thermal cycler (MJ Research Inc. USA). PCR is a rapid, inexpensive and simple mean of producing relatively large copy number of DNA molecules from the small amounts of source DNA material, even when the source DNA is of relatively poor quality. Due to the extreme sensitivity, precautions were taken against contamination of the reaction mixture with the trace amounts of DNA, which could serve as an unwanted template. Appropriate negative control was included in each PCR run carried out for each gene, to monitor this contamination of PCR mix to avoid any false positive results. The negative control used for PCR contained whole PCR reaction mix except target DNA which was replaced by HPLC purified water free of RNase, DNase, and any contamination from any other source resembling the gene sequence.

Subsequently restriction enzyme digestion was performed by incubating the double stranded DNA with appropriate amount of restriction enzyme, in its respective buffer as recommended by the supplier and at optimal temperature for that specific enzyme. A typical digestion includes one unit of enzyme per microgram of starting DNA. One enzyme unit is usually defined as the amount of enzyme needed to completely digest one microgram of double stranded DNA in one hour at the appropriate temperature. Their biochemical activity of the restriction enzyme is the hydrolysis of phosphodiester backbone at specific sites in a DNA sequence. Precaution was taken to avoid star activity of restriction enzymes. When DNA is digested with certain restriction enzymes under non-standard conditions, cleavage can occur at sites different from the normal recognition sequence. Such aberrant cutting is called “star activity” which can be due to high pH (>8.0) or low ionic strength, extremely high concentration of enzyme (>100 U/µg of DNA) and presence of organic solvents in the reaction (e.g. ethanol, DMSO).

The PCR and restriction digestion conditions were optimized for specific locus of relevant segment of the gene to be studied. The PCR products as well as the digested products were separated on either agarose gel or polyacrylamide gel depending on their

size. Gels were stained with ethidium bromide solution (0.5 µg/ml) and subsequently visualized and photographed under UV transilluminator.

Detection of ENPP1 Single Nucleotide Polymorphism via PCR-RFLP

The K121Q (substitution of A base to C at 121codon) polymorphism of ENPP1 gene has been amplified by PCR. This polymorphism is a functional polymorphism causing change of Amino acid from lysine to glutamine. Primer sequences oligonucleotide sequence (primers) were designed to amplify the gene wild type gene is lack of restriction site for AvaII enzyme but mutant allele contains a restriction site.

…GGWCC…
…CCWGG…

Forward primer- 5`-GCAATTCTGTGTTCACTTTGGA-3`
Reverse primer- 5`-GAGCACCTGACCTTGACACA-3`.

PCR Mix

The PCR was carried out in a final volume of 25 µl, containing 100 ng of genomic DNA(4-5 µl), 2.5 µl of 10X *Taq* polymerase buffer (10 mM Tris HCl pH 8.8, 50 mM KCl, 1.5 mM MgCl2, 0.01% gelatin, 0.005% Tween-20, 0.005% NP-40; final concentration 1X; Genetix Biotech Asia Pvt. Ltd.,India), 1 µl of 10 mM dNTPs (Banglore Genei, Bangalore, India), 1 µl of 25 pmol/µl of forward and reverse primers specific for and 1 µl of unit of 1U/ µl Red Taq DNA polymerase (Bangalre genei).

PCR Thermal Program

After an initial denaturation of 5 min at 94˚C, the samples were subjected to 35 cycles at 94˚C for 1 min, at 55˚C for 40 s, and 72˚C for 40 s, with a final extension of 10 min at 72˚C in a thermal cycler. A 100bp ladder with amplified product has been run under 1 % agarose gel electrophoresis. 238bp product will be generated after PCR.

Restriction Digestion

Restriction Digestion The 238-bp product was digested with AvaII enzyme (New England Biolabs, overly, MA) for 16 h at 37˚C. The wild-type genotype (KK) was not digested, whereas the mutated homozygous genotype (QQ) was cut as a doublet of 148 and 90 bp. The heterozygous genotype (KQ) was represented as 3 fragments of 238, 148, and 90 bp. Samples were analyzed by electrophoresis using 2.5% agarose gels to analyze the genotype pattern of the gene.

Statistical Analysis

Statistical analysis was done by comparing the distribution of genotype frequencies, allele frequencies and carriage rates of all the four polymorphism in diseased and control group. Disease group included Diabetic patients whereas control group included all healthy controls (HC) enrolled in the study. The proportions of different genotypes for a gene in a population are known as genotype frequencies. The proportion of genotype in a sample will be the ratio of the number of individuals having that genotype to the total number of individuals in the sample. The proportions of different alleles for a gene present in a population are known as allele frequencies. The proportion of an allele in a sample will be the ratio of number of occurrences of the investigated allele in the population to the total number of alleles. The carriage rate was calculated as the number of individuals carrying at least one copy of the test allele divided by the total number of individuals. Data was analyzed using Microsoft Excel 2002, Microsoft corporation.

Results and Discussion

Anthropometric results

The descriptive data and comparison of anthropometric and biochemical parameters of hypertension patients versus controls are presented in Table 2(A). The age, sex, BMI, WHR were the parameters. As expected the diabetic patients had markedly higher levels of weight of women (P=0.0024), Men (P=0.0157) and BMI of Women (P=0.0388), Waist circumference in women (P<0.0001), WHR in Women (P<0.0001) and WHR in Men (P=0.0147). Other results were not found significantly different between case and control group. **(See Table 2A)**

Table 1: Comparison of anthropometric parameters of Hypertension patients and controls

Characteristics	*Cases*	*Controls*	*P-value*
n(Men/Women)	190(126/64)	210(114/96)	
Age(years)	52.5±12.5	53.0±14.2	0.7100
Height(m)	160.50±13.40	162.2± 12.000	0.1815
Weight (Kg)			
Women	62.5 ±5.70	60 ± 4.50	0.0024 **
Men	68±5.60	66.0±7.1	0.0157*
BMI (kg/m^2)			
Women	26.4±3.1	25.1 ± 4.3	0.0388*
Men	24.6±4.7	24.1± 5.1	0.4301
Waist circumference (cm)			

Women	92.5±6.2	84.5±6.7	P<0.0001***
Men	90.0±7.0	89.0±6.0	0.2383
Hip (cm)			
Women	95.0±5.0	96.5±6.0	0.178
Men	91.0±4.0	90.5±5.5	0.4183
WHR			
Women	0.97±0.05	0.88±0.08	P<0.0001***
Men	0.99±0.05	1.00±0.03	0.0147*

* denotes level of significant change between case and control

Biochemical and clinical findings

Biochemical test performed in the blood sample for following clinical parameters and the findings were tabulated. Statistical analysis was done by using students t test and p value obtained suggest the level of significant changes here. The descriptive data and comparison of biochemical parameters of diabetic patients versus controls are presented in Table 3. As expected the diabetic patients had markedly higher levels of fasting plasma glucose (P<0.0001) and HbA1c (P<0.0001) and Post prandial glucose (P<0.0001) compared to that of control subject. Nominal difference was also observed for LDL-C (P=0.0462), triglyceride (P=0.0024), systolic blood pressure (P=0.0447). creatine value, blood urea level, HDL-C level and diastolic pressure was not significantly different between two groups and all the clinical test results are tabulated in table no. 2.

Table 2: Comparison of Biochemical and clinical findings of diabetic patients and controls

Characteristics	*Cases*	*Controls*	*P-value*
FPG(mg/dL)	117.4±17.6	92.1±7.5	P<0.0001***
Post-Prandial Glucose (mg/Dl)	151.7±22.4	119.5±12.1	P<0.0001***
HbA1C(%)	6.9±0.8	5.3±0.6	P<0.0001***
HDL-C(mmol/L)	112.2±14.8	109.8±11.6	0.0705
LDL-C (mg/dL)	42.1±4.3	41.3±3.7	0.0462*
TG(mg/dL)	131.1±13.2	126.9±14.2	0.0024**
Systolic BP (mmHg)	130.20±8.1	128.8±5.7	0.0447*
Diastolic BP (mmHg)	87.1±5.8	86.5±6.0	0.3109
Blood Urea(mg/dL)	9.1±1.6	8.8±1.8	0.0801
Creatinine(mg/dL)	1.08±0.14	1.06±0.10	0.0986

(* denotes the level of significant change between case and control)

Detection of Genetic Polymorphism of ENPP1:

PCR amplification with specific primers gave 238-bp product which was digested with AvaII enzyme (New England Biolabs, Boverly, MA) for 16 h at 37˚C. The wild-type genotype (KK) was not digested, whereas the mutated homozygous genotype (QQ) was cut as a doublet of 148 and 90 bp. The heterozygous genotype (KQ) was represented as 3 fragments of 238, 148, and 90 bp as depicted in figure no. 11.

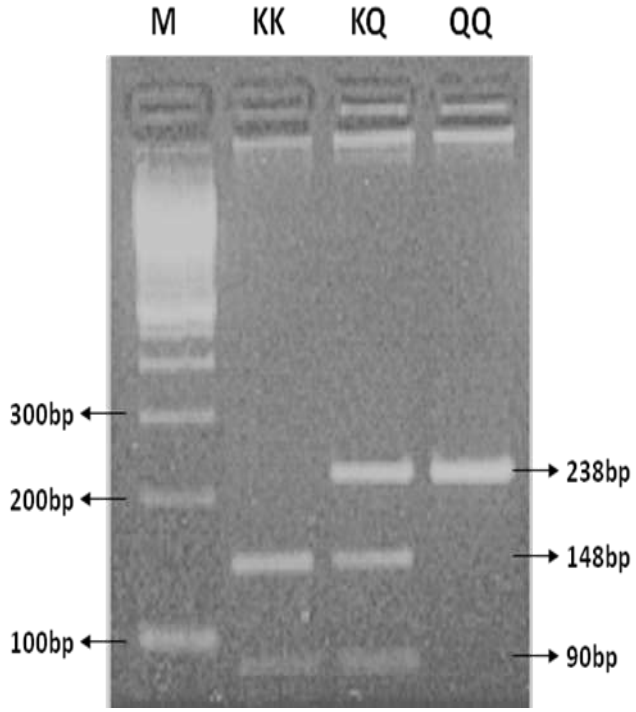


Fig. 1: Representative gel picture of *ENPP1 K121Q* polymorphism.

The distribution of the polymorphisms of ENPP1 was consistent with Hardy- Weinberg equilibrium (HWE) in healthy controls. The observed genotype frequencies, allele frequencies and carriage rates for ENPP1 K121Q polymorphism are depicted in table 4 and table 5 and Graph 1, 2, 3.

Soni et al., 10(4): April, 2019:6152-6162]
ISSN: 0976-7126

Table 3: Frequency distribution and association of Genotype, allele frequency and carriage rate of ENPP1 K121Q polymorphism in population of Vindhyan region using Chi Square Test

ENPP1 GENOTYPE	CASE N= 190 N	%	CONTROL N=210 N	%	CHI SQUARE VALUE χ^2 (P Value)
KK	121	63.68	146	69.52	1.670, (0.4339)
KQ	66	34.74	62	29.52	
QQ	3	01.58	2	00.96	
Allele					
K	308	81.05	354	84.29	1.461, (0.2268)
Q	72	18.94	66	15.71	
Carriage Rate					
K	187	98.42	208	99.05	0.8203, (0.3651)
Q	69	36.32	64	30.48	

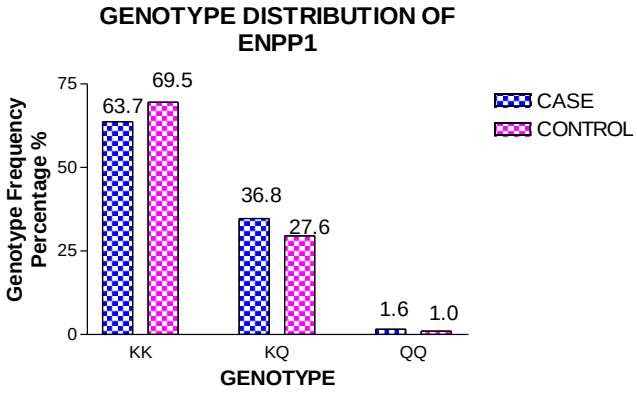
N – Number of individuals in study group
%- Genotype allele frequency and carriage rate expressed in percentage
* denotes the level of significant association between case and control

Table 4: Fisher Exact Test values of ENPP1 polymorphism

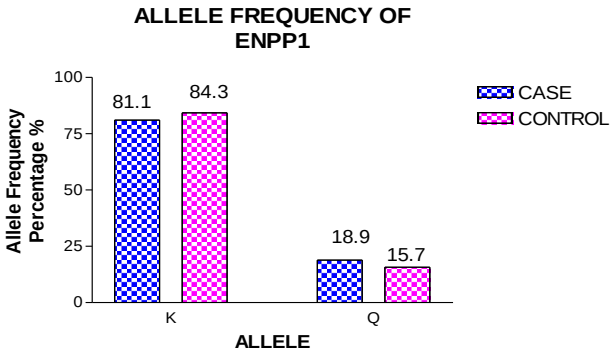
ENPP1 GENOTYPE	CASE N= 190 n	%	CONTROL N=210 n	%	P Value	Odds Ratio
KK	121	63.68	146	69.52	0.2427	0.7687, (0.5066-1.166)
KQ	66	34.74	62	29.52	0.2841	1.271, (0.8340- 1.936)
QQ	3	1.58	2	00.96	0.6717	1.668, (0.2757- 10.10)
Allele						
K	308	81.05	354	84.29	0.2608	0.7976, (0.5524-1.152)
Q	72	18.94	66	15.71		1.254, (0.8684 -1.810)
Carriage Rate						
K	187	98.42	208	99.05	0.3689	0.8339, (0.5627-1.236)
Q	69	36.32	64	30.48		1.199, (0.8091-1.777)

(* denotes the level of significant association between case and control)
N – Number of individuals in study group.
%- Genotype allele frequency and carriage rate expressed in percentage

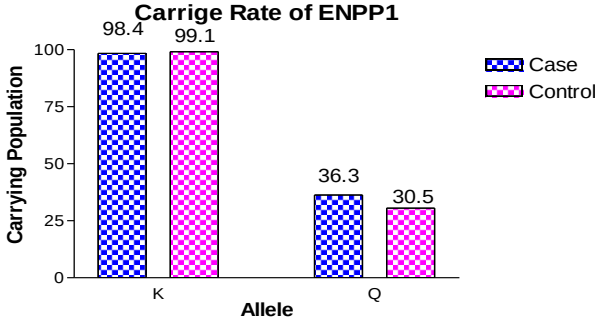
Soni et al., 10(4): April, 2019:6152-6162]
ISSN: 0976-7126



Graph 1 Genotype Frequency of ENPP1 gene



Graph No. 2: Allele Frequency of ENPP1 gene.



Graph No.2; Carriage rate of ENPP1 gene.

Conclusion

Genetic association as well as Functional genomics has now opened the door of new era to understand the possible role of genes in many disorders including diabetes, cancer, many other autoimmune and life threatening diseases. The disorders in metabolism could be due to lack of normal expression of gene and their functional proteins. This abnormality could arise in the genes due to the presence of specific polymorphic allele of particular gene. Many genetic studies already conducted which clearly indicate the role of genetic factors in the susceptibility of disease causation. Genes have widely studied and found to be associated with vast range of metabolic disorders including diabetes and many type of cancers (16-18).

In our study we had taken samples from both urban and rural population living in Vindhyan region. During the sample collection we prepared a questionnaire to gather the information about environmental and life style factors such as physical activity level and smoking habits. These factors have been previously found to be associated with increased diabetes risk in different other populations.

BMI indexing is a tool used for documentation of obesity. In our present investigation we found that BMI was significantly higher in females. BMI of diabetic females were as compared to healthy females (P=0.0388). Weight Height Ratio (WHR) was also shown to be higher in male and female both. Sedentary lifestyle is strong factor behind the surprising rise in the prevalence of both obesity and diabetes. In the past decade, we have witnessed an epidemic of both type 2 diabetes and obesity. The prevalence of type 2 diabetes has increased by 33% in the United States, and 62% of Americans are classified as obese (BMI $\geq$ 30 kg/m^2) or overweight (BMI 25–29.9 kg/m^2). The recent increase in the prevalence of obesity is closely paralleled by the increase in the prevalence of hypertension. In India our data also suggests that obesity and higher BMI can be an important factor which can affect the susceptibility to hypertension (19-22).

In hypertension case lower number of physical active persons was seen as compared to control (41.57% Vs 53.33%). The significance level was sufficiently strong to reveal the protective association of physical activity (χ^2 =5.524, P Value 0.0188). An odds ratio of 0.62 clearly indicates the positive association of physical activity with prevention of diabetes and active life style could be concluded as a very important factor which can prevent pathophysiology of hypertension (*23*).

Smoking is an established modifiable risk factor which is associated with many diseases such as CVD *and cancer*. To some extent, the effects in physical conditions of smoking and diabetes are similar, which brings question if there is any association between smoking and diabetes. Many studies evidenced that chronic smokers have a higher risk for insulin resistance, and to develop type 2 diabetes mellitus (DM2). Our result shows that percentage of smokers in case and control is not more different and there is lack of statistically significant association but

an odds ratio of 1.205 shows a little higher risk of diabetes type 2 in smokers as compared to nonsmokers. Our results indicate that smoking may increase the risk of hypertension but relation with disease susceptibility was not established. It was previously reported in a metaanalysis that heavy smokers (at least 20 cigarettes daily) had a 61% higher risk, while less than 20 cigarettes daily were correlated to a 29% increase of the risk (24-26).

ENPP1 gene is known to susceptible gene because of its affinity to bind with alpha subunit of insulin receptor and may inhibit the tyrosine kinase activity which is essential for glucose metabolism (Maddux BA *et al*., 2000). Metformin, a biguanide oral antidiabetic agent, was shown to affect insulin resistance by decreasing enzymatic activity of over expressed PC-1 molecules in hypertension. In functional studies, the 121Q allele variant binds more strongly to the insulin receptor and inhibits its protein kinase activity more effectively than the K variant (*25).*

Our finding shows that genotype pattern and allele frequency of ENPP1 K121Q is not significantly different between case and healthy controls. With odds ratio of 0.7687 KK genotype may indicate the little protective role but neither KK nor other genotypes were not significantly different, although the higher allele frequency of mutant 121Q allele in diabetic population has been seen but this difference was also not significant because the higher number of Heterozygosity (27). The frequency of homozygous mutant 121Q allele is very low in case as well as in control. Previous studies suggest that the frequency of the 121Q allele carriers in the ethnic Chinese study was 18.8%, Caucasians (23.2% - 36.4%), South Asian Indians (specially Chennai population living in Chennai and Dallas USA (27.5% - 34.2%), African-Americans (67.0%), and Dominicans (78.4%) Caucasian population of USA and Finland showed the significant association of 121Q allele with diabetes type 2. In addition to diabetes, the PC-1 Q121 allele has recently also been reported to influence the risk of obesityMany association studies have been done but the results are different in different race and population. Other many Caucasian including Sweden and Denmark shows that ENPP1 polymorphism is not associated with susceptibility to hypertension..While the same finding of no association has been reported from Chinese population (Miao-Pei Chen *et al*., 2006), North Indian Sikh population, another study in Spanish population has shown that ENPP1 K121Q polymorphism is not significantly associated with hypertension. Our findings are also consistent with Caucasian and African- American Oji Cree and Mexicans. It is possible that the susceptibility induced by the ENPP1 K121Q gene polymorphism is modulated by interactions with other ethnic specific genetic or environmental factors (24-26).

The Study in central Indian population has shown that ENPP1 K121Q polymorphism is not associated with susceptibility to hypertension and the results are similar to previous study already done in north Indian as well as many Caucasians, Japanese and Chinese, Heterozygousity and rare Q allele homozygousity was closer to Caucasians and lower then Sikh population. Although the sikh population study shows maximum level of heterozygous but the statistical difference were not seen. Other studies done before in south Indian Chennai population and some Caucasian population shows that Q allele could be risk factor and in our study allele Q is present in higher percentage in the form of heterozygous in diabetes patients but its effect or association with hypertension was not significantly established. In studies of complex multifactorial disorders such as hypertension, discordant results in genotype-phenotype association are not uncommon. These discordant results suggest that differences in either the genetic and/or environmental backgrounds of the subjects studied or the recruitment procedures of the populations investigated are important factors in these analyses (19,21).

Our study can establish polymorphism of ENPP1 was not significantly different between case and control population so we can conclude that this polymorphism don't have contribution in diabetes susceptibility in vindhyan region population. The replication of this study with larger sample size will be expected in future. Despite those genes many other genes can have strong effect in diabetes pathophysiology so we can use genome wide association study to discover some novel genes which have strong association with hypertension.

Hypertension is one of the most important causes of the total disease burden in the world. According to large observational studies, hypertension is thus associated with high incidence of cardiovascular disease, such as stroke, ischemic heart disease, and other vascular diseases. An increased incidence of cardiovascular disease has in fact been seen in relation to blood pressure levels across the entire blood pressure distribution, also within the normal blood pressure range. The risk of becoming hypertensive in later life is considerable, as studies from almost all high-income countries have shown

that blood pressure rises with increasing age. The incidence of hypertension is likely to vary depending on the initial blood pressure and the intra-individual variation of blood pressure measurements.

This is the first study done in vindhyan region. The population size selected in present study was 400 (190 Case of diabetes and 210 healthy control). Anthropometric data has been collected during sample collection; a brief questionnaire has been done to study the life style as well as environmental factors which could be associated with hypertension susceptibility. Clinical and biochemical test of diagnosis has been carried out to recruit the patients as well as control samples. Many genes have been found to be associated with hypertension. It has been established by different genomic study approach that some genetic polymorphism can be associated with increased insulin resistance and sensitivity. ENPP1 have been selected in present investigation to study the impact of actual polymorphism and allelic variation among those genes and their distribution pattern between case and control samples in Vindhyan region population. On the basis of research work already done in many other populations we have prepared research plan in which we included following genes and tried to find out the effect of polymorphism in these genes in susceptibility to hypertension. It is widely accepted that many life style factors could be defined as causing factor for hypertension. In present study we also focused our research work on smoking, physical activity and obesity.

Genetic polymorphism study of ENPP1 has been done by using PCR-RFLP method and the observed genotype frequencies, allele frequencies and carriage rates for ENPP1 K121Q polymorphism and we found overall distribution of ENPP1 K121Q genotypes was not significantly different in HC group as compared to disease group. HC group showed a little increase in 'KK' genotype as compared to Patients of hypertension but genotypes were not significantly different. Mutant type 'QQ' genotype was present in very less frequency in diabetic patients and in control group. Our study revealed genotype, allele Frequency and carriage rate of ENPP1 is not different between case and control group which indicates lack of association of this genotype with disease. As it is a multifactorial disorder so we can conclude that in our population ENPP1 is not associated with hypertension.

References

1. Science Daily. Physical Activity Reduces Hypertension In Young Adults.(2007)
2. Kowalski, R.F.2007. Take The Pressure Off Your Heart. South Africa. New Holland Publishers.
3. Whiteworth JA. World health organization, international society of hypertension writing group (2003).2003
4. World Health Organization (WHO)/International Society of Hypertension (ISH) statement on management of hypertension. J. Hypertens. 2003; 21(11):1983-1992.
5. Olsen MH, Mallion JM, Rahn KM, Erdine S, Viigimae M, Laurent S, Agabiti-Rosei E, Mancia G, Schmieder RE,Cifkova R, Dominiczak A, Kjeldsen SE, Redon J, Zanchetti A, Nilsson P, Narkiewicz K. ESH Council. Agreement within Europe about antihypertensive treatment and education: results from the european society of hypertension questionnaire. J. Hypertens. 2010;28(7):1593-1594.
6. Ogihara T, Kikuchi K, Matsuoka H, Fujita T, Higaki J, Horiuchi M, Imai Y, Imaizumi T, Ito S, Iwao H, Kario K, Kawano Y, Matsuyama S, Kimura G, Matsubara H, Matsuura H, Naruse M, Saito I, Shimada K, Shimamoto K, Suzuki H, Takishita S, Tanahashi N, Tsuchihashi T, Uchiyama M, Ueda S, Ueshima H, Umemura S, Ishimitsu T, Rakugi H. The Japanese society of hypertension guidelines for the management of hypertension (JSH 2009). Hypertens. Res.2009; 32(1):3-107.
7. Eriksson J, Forsen T, Tuomilehto J, Osmond C, Barker D 2000 Fetal and childhood growth and hypertension in adult life. Hypertension 36:790 – 794 5. Forsen T, Eriksson J, Tuomilehto J, Reunanen A, Osmond C, Barker D 2000 The fetal and childhood growth of persons who develop type 2 diabetes. Ann Intern Med 133:176 –182.
8. Epstein M, Sowers JR 1992 Diabetes mellitus and hypertension. Hypertension 19:403– 418
9. Sowers JR, Epstein M, Frohlich ED 2001 Diabetes, hypertension, and cardiovascular disease: an update. Hypertension 37:1053–1059
10. Ferrannini E, Buzzigoli G, Bonadonna R, Giorico MA, Oleggini M, Graziadei L, Pedrinelli R, Brandi L, Bevilacqua S 1987 Insulin resistance in essential hypertension. N Engl J Med 317:350 – 357
11. Abate N, Chandalia M, Satija P, Adams-Huet B, Grundy SM, Sandeep S, Radha V, Deepa R, Mohan V: ENPP1/PC-1 K121Q polymorphism and genetic susceptibility to type 2 diabetes. Diabetes 54:1207–1213, 2005.
12. Meyre D, Bouatia-Naji N, Tounian A, Samson C, Lecoeur C, Vatin V, Ghoussaini M, Wachter C, Hercberg S, Charpentier G, Patsch W, Pattou F, Charles MA, Tounian P, Clement K, Jouret B, Weill J, Maddux BA, Goldfine ID, Walley A, Boutin P, Dina C, Froguel P: Variants of ENPP1 are associated with childhood and adult obesity

and increase the risk of glucose intolerance and type 2 diabetes. Nat Genet 37:863–867, 2005
13. Leitao CB, Nabinger GB, Krahe AL, Bolson PB, Gerchman F, Gross JL, et al. The role of K121Q ENPP1 polymorphism in diabetes mellitus and its complications. Braz J Med Biol Res. 2008; 41: 229–234. PMID: 18176722.
14. Meyre D, Bouatia-Naji N, Tounian A, Samson C, Lecoeur C, Vatin V, et al. Variants of ENPP1 are associated with childhood and adult obesity and increase the risk of glucose intolerance and type 2 diabetes. Nat Genet. 2005; 37: 863–867. PMID: 16025115
15. Canani LH, Ng DP, Smiles A, Rogus JJ, Warram JH, Krolewski AS. Polymorphism in ecto-nucleotide pyrophosphatase/phosphodiesterase 1 gene (ENPP1/PC-1) and early development of advanced diabetic nephropathy in type 1 diabetes. Diabetes. 2002; 51: 1188–1193. PMID: 11916943
16. DeFronzo RA, Ferrannini E 1991 Insulin resistance. A multifaceted syndrome responsible for NIDDM, obesity, hypertension, dyslipidemia, and atherosclerotic cardiovascular disease. Diabetes Care 14:173–194
17. Capaldo B, Lembo G, Napoli R, Rendina V, Albano G, Sacca L, Trimarco B 1991 Skeletal muscle is a primary site of insulin resistance in essential hypertension. Metabolism 40:1320 – 1322
18. Guimaraes S, Albino-Teixeira A 1996 Hypertension due to chronic blockade of P1-purinoceptors. J Auton Pharmacol 16:367–370;
19. Kruger, S.H., Puoane, T., Senekal, M & Van Der Merwe M.T. 2005. Obesity In South Africa: Challenges For Government And Health Professionals.
20. Akinboboye Et Al.(2002:17,381-387). (Opie, L.H. 2004.) Fourth Edition. Heart Physiology. From Cell To Circulation. U.S.A. Maple Press.)
21. Kowalski, R.F.2007. Take The Pressure Off Your Heart. South Africa. New Holland Publishers.)
22. (Opie, L.H. 2004. Fourth Edition. Heart Physiology. From Cell To Circulation. U.S.A. Maple Press.)
23. Kubaszek A, Markkanen A, Eriksson JG, Forsen T, Osmond C, Barker DJP, et al. The association of the K121Q polymorphism of the plasma cell glycoprotein-1 gene with type 2 diabetes and hypertension depends on size at birth. J Clin Endocrinol Metab. 2004;89(5):2044-7.
24. Abate N, Carulli L, Cabo-Chan A Jr, Chandalia M, Snell PG, Grundy SM: Genetic polymorphism PC-1 K121Q and ethnic susceptibility to insulin resistance. J Clin Endocrinol Metab 88:5927–5934, 2003 3.
25. Wu LS, Hsieh CH, Pei D, Hung YJ, Kuo SW, Lin E. Association and interaction analyses of genetic variants in ADIPOQ, ENPP1, GHSR, PPARgamma and TCF7L2 genes for diabetic nephropathy in a Taiwanese population with type 2 diabetes. Nephrol Dial Transplant. 2009; 24: 3360–3366. doi: 10.1093/
26. Leitao CB, Nabinger GB, Krahe AL, Bolson PB, Gerchman F, Gross JL, et al. The role of K121Q ENPP1 polymorphism in diabetes mellitus and its complications. Braz J Med Biol Res. 2008; 41: 229– 234. PMID: 18176722
27. Bacci S, Ludovico O, Prudente S, Zhang YY, Di Paola R, Mangiacotti D, et al. The K121Q polymorphism of the ENPP1/PC-1 gene is associated with insulin resistance/atherogenic phenotypes, including earlier onset of type 2 diabetes and myocardial infarction. Diabetes. 2005; 54: 3021–3025. PMID: 16186408.

How to cite this article

Soni N, Tripathi A., Saket R. D. and Mishra R.M. (2019). Genetic polymorphism of SNP K121Q of ENPP1 gene and its association with Hypertension in Vindhyan population (India), *Int. J. Pharm. Life Sci.*, 10(4):6152-6162.

Source of Support: Nil; Conflict of Interest: None declared

Received: 05.03.19; Revised: 21.03.19; Accepted: 23.04.19