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Association Study TNF-α (G308A) Gene Polymorphism with Essential Hypertension in Vindhyan Population

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ARTICLE DETAILS

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ABSTRACT

Hypertension is one of the major risk factors for cardiovascular disease morbidity and mortality. In order to reduce events related to cardiovascular disease, control of hypertension is very important. The clinical phenotypes of hypertension are known to be affected by both lifestyle and genetic factors. Genetic approaches in humans encompass molecular investigations of rare inherited syndromes of human hypertension, and direct analysis of essential hypertension in humans. There is an accumulating body of evidence indicating that alterations of the immune system are involved in the cascade of events leading to essential hypertension (EH). Concentration of TNF-α level in hypertension patient (case) and healthy population (control) is showing elevated level during infection and it was statistically significant associated as P<0.0001*** . Overall distribution of TNF-α (G308A) genotypes was significantly different in healthy control group as compared to disease group (χ^2=13.01, P=0.0015**). HC group showed a decrease of mutant 'AA' genotype as compared to Patients of Essential hypertension (*1.11*% *vs.* 4.37%). Similarly, wild type '*GG*' genotype was present in significantly high frequency in HC as compared to Essential hypertension patients group (72.22% *vs.* 54.37%).

1. Introduction:

Essential hypertension is a chronic pathological state that afflicts about a third of the entire human population [**1**]. It is a major risk factor for premature cardiovascular disease, coronary and peripheral atherosclerosis, cardiac hypertrophy, heart failure, ischemic stroke, intracerebral hemorrhage and chronic and end-stage renal disease. More than ninety percent of the cases of hypertension do not have an identifiable cause and, therefore, are classified as essential or primary hypertension. Essential hypertension is thought to originate from the interaction between genetic and environmental factors. Finding a new gene associated with EH will help clarify the pathogenesis of EH and provide a new therapeutic strategy. The pathogenesis of EH is not limited to cardiovascular areas and is also related to immunology as well as heredity [1]. Inflammation plays a key role in the development of such cardiovascular diseases as atherosclerosis, diabetes, and so on [2]. The association of inflammation with EH has received increased attention for the past few years. Tumor necrosis factor-alpha (TNF a), the inflammation promoter, is secreted primarily by mononuclear phagocyte cells. TNF a induces endothelial cells to secrete vasoactive substances via the autocrine or paracrine pattern, which leads to vasorelaxation or vasoconstriction, and ultimately, to the regulation of blood pressure (BP) [3]. The TNF a gene is located in the major histocompatibility complex III region on chromosome 6p21.3. Recent research has revealed that TNF a gene polymorphisms are mostly focused on the probable

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influence of the promoter district on the expression of the TNFa gene. The TNFa gene polymorphism is also involved in infectious diseases, metabolic syndrome, stroke, hyperuricemia, and so on [1-4].

Essential hypertension is a common disorder affected by environmental and polygenic factors. In order to determine the pathogenesis of hypertension and to device a novel treatment method, it is essential to find new genes associated with hypertension. In addition to heredity and immunology, cardiovascular factors can be highly relevant to the pathogenesis of hypertension. Coronary atherosclerotic heart disease (CHD) characterizes as myocardial ischemia and hypoxia which arises from coronary atherosclerosis.[1] It is a worldwide medical problem and is still one of the leading causes of death in developed and developing countries.[2] At present, the occurrence and development of CHD is generally considered as a chronic inflammatory process characterized by highly specific cytokine response.[3] The regulation network formed by various proinflammatory and anti-inflammatory factors plays an immunomodulatory role in atherosclerosis.[4] Various proteins, cytokines, and adhesion molecules are involved in the development of coronary angiogenesis.[5] Among them, TNF-a and IL-6 have significant effects on the development of coronary heart disease.[6,7] It has been showed that both of them are capable to damage endothelium function and act on the plaque of the vessel wall, accelerating the rupture of the plaque and triggering the clinical coronary events.[8] As a complex disease, CHD results from the interaction between genetic and environmental factors.[9] Recent studies have suggested that the basic level and biological activity of TNF-a and IL-6 can be influenced by gene polymorphism, which may increase the risk of CHD[10,11] C863A of TNF-a and C174G of IL-6 are the mostly investigated but the results remain inconsistent. The TNF-a C863A gene polymorphism was associated with the pathogenesis of CHD through case-control study. The studies on IL- 6 gene polymorphism and risk of CHD are also inconsistent, similar to that research status of TNF-a [5-9].

2. Material and methods

2.1 Study population:

The study population consisted of 340 unrelated subjects comprising of 160 Essential Hypertension patients and 180 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's belong to Hindu religion in this region.

2.2 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. Hypertension was diagnosed in accordance with World Health Organization (WHO Expert committee 2003) criteria.

2.3 Inclusion and Exclusion criteria for Controls:

Control group composed of non-diabetic healthy individuals that were collected during "Diabetes Awareness Camps" organized in urban regions in and around SSMC Rewa and many volunteers were also included to collect control sample. 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)

*2.4 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

2.5 ELISA Analysis:

Biochemical parameters related to Essential Hypertension is immunological cytokine TNF-α selected. Antigen-antibody reaction based ELISA kit (Diaclone, cat.no. 950090096) for detection of human TNF-α in isolated blood serum were provide statistical data to stabilize association to Essential hypertension.

3. Molecular Laboratory Analysis:

3.1 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 then 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.

3.2 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.

3.3 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.

3.4 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). Lambda DNA *Eco*RI / *Hind* III double digest (Bangalore Genei, Bangalore, India) was used as molecular weight marker after completion of electrophoresis, the DNA bands were visualized and photographed using an UV transilluminator (312 nm) and gel documentation system (Vilber Lourmate, Cedex 1, France) respectively.

3.5 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 (0.5µg/ml) and subsequently visualized and photographed under UV transilluminator

3.6 Detection of TNF-α (G308A) Polymorphism:
The tumor necrosis factor-alpha (TNF-α) gene may play an important role in coronary heart disease and hypertension. Essential hypertension is associated with the genetic mutations in TNF-α (G308A) gene, SNP rs1800629, generate tow allele G (wild type) and A (Mutated).

PCR Primer: The oligonucleotides sequences (primers) used were those described by K Jamil ***(Jamil K, et. al. 2016).***

Forward primer- 5`- AGGCAATAGGTTTTGAGGGCCAT -3`

Reverse primer- 5`- TCCTCCCTGCTCCGATTCCG -3`

3.7 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).
3. 8 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. 107bp product will be generated after PCR.

3.9 Restriction Digestion by MspI:
Genetic polymorphism of TNF-α (G308A) gene at SNP rs1800629 was indicated two allele G and A. The PCR product was 107bp digested in to two fragments 87bp and 20bp. The PCR products when digested by restriction enzyme *MspI* produces allele A. The TNF-α (G308A) gene generates three genotype of GG, GA and AA where G was wild allele and A was rare mutant allele. A allele having restriction sit for enzyme *MspI* and give two fragments 87bp and 20bp.

4. Statistical Analysis of Genotype:

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. Only the biochemical parameters, for difference between obese diabetic patients and the obese non-diabetic controls were assessed using the student's t test. Similarly the biochemical parameters between the normal weight patients and normal weight non-diabetic controls were also assessed. The P-values calculated using t test along with the mean (inter-quartile range) were presented. Statistical analyses were performed using statistical package, Prism 3.0, Prism 5.1 version.

5. Results:

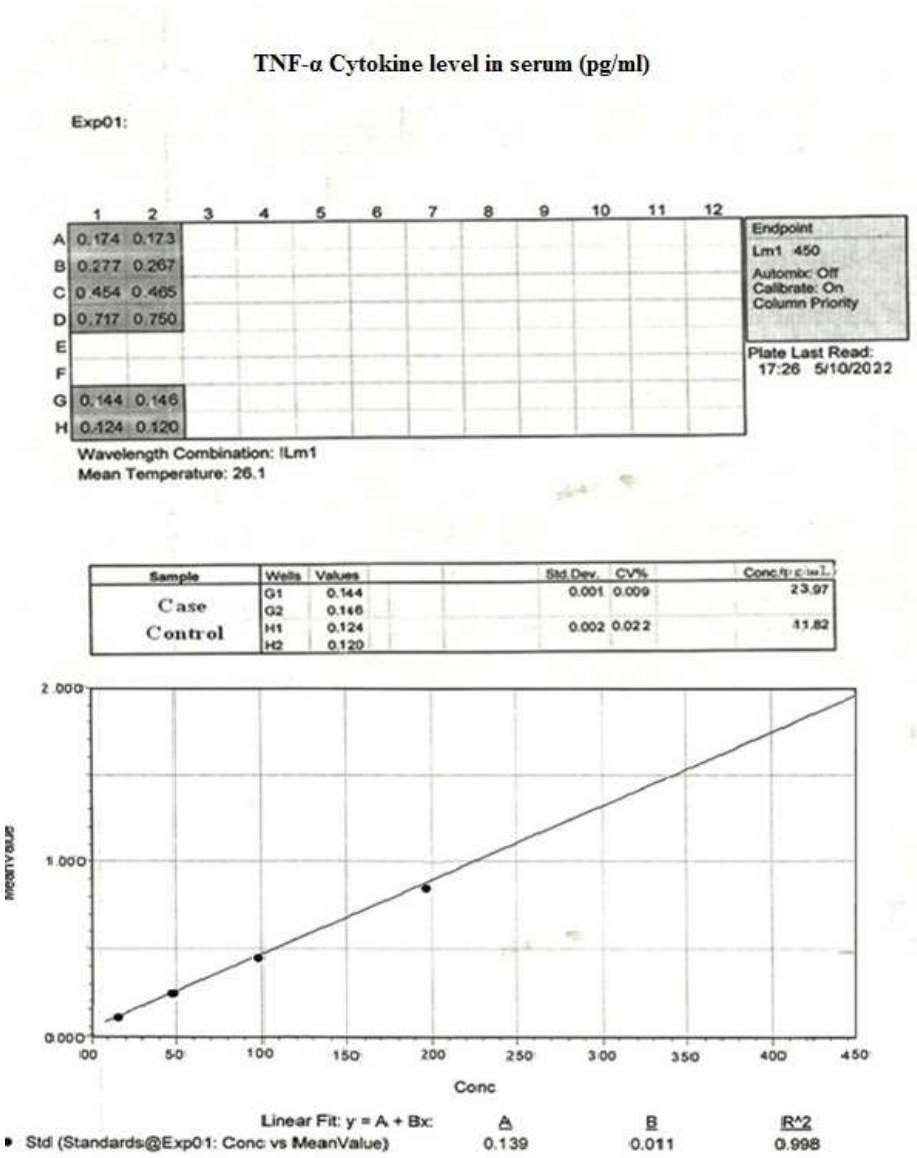
5.1 ELISA Analysis of TNF-α cytokine:
Tumor necrosis factor-α (TNF-α) is a pleiotropic cytokine that becomes elevated in chronic inflammatory states such as essential hypertension infection. Concentration of TNF-α level in hypertension patient (case) and healthy population (control) is depicted in table no.1, is showing elevated level during infection and it was statistically significant associated as P<0.0001***
.
This is kit based ELISA result reveals elevated TNF-α level in hypertension infection. In figure no. 4.3, two columns ELISA analysis having four standards A, B, C, D containing 25, 50, 100, 200 Pg/ml concentration respectively read absorbance at 450

nm. G for Patient (Case) and H for Healthy (control) showing differences in TNF-α level as 23.97 Pg/ml and 11.82 Pg/ml respectively.

Table 1. Comparison of Biochemical Factor between Essential hypertension Cases and Healthy Controls:

Biochemical Factor	Cases(160)	Controls(180)	P-value
TNF-α Cytokine Level in serum (pg/mL)	23.97 ± 9.52	11.82 ± 6.41	(P<0.0001)***

*(*Denotes level of significant change between malarial cases and healthy controls.)*



Graph 1; TNF-α Cytokine Level in both case and control. Straight line graph is showing concentration change accordance to absorbance.

5.2 Detection of Genetic Polymorphism in TNF-α (G308A) gene:

The nucleotide position G308A polymorphism in TNF-α gene create restriction site for *HinII.* The PCR products when digested by restriction enzyme and wild type allele 107 bp segment which were generated by PCR but the mutant allele shows 87 and 20 bp segments. The product sizes are Wild type homozygote, 107 bp; mutant G308A homozygote, 87 and 20 bp; and heterozygote, 107, 87, and 20 bp respectively.

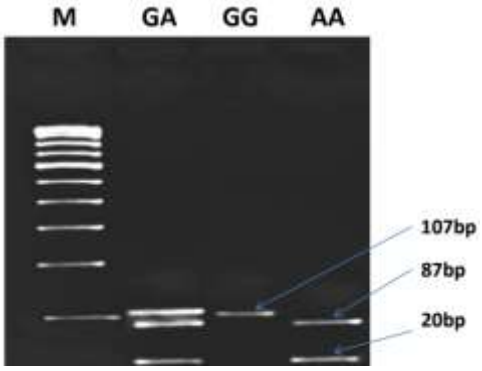


Fig-1: Representative gel picture of TNF-α (G308A) polymorphism. Lane M represents 50 bp molecular marker, Lane GG Wild type genotype, Lane GA heterozygous genotype and Lane AA variant genotype.

The distribution of polymorphic genotype was strongly under HWE. The observed genotype frequencies, allele frequencies and carriage rates for TNF-α (G308A) polymorphism are depicted in table 4.10 and table 4.11 and graph no. 4.9, 4.10, 4.11. Overall distribution of TNF-α (G308A) genotypes was significantly different in healthy control group as compared to disease group (χ^2=13.01, P=0.0015**). HC group showed a decrease of mutant 'AA' genotype as compared to Patients of Essential hypertension (*1.11*% *vs.* 4.37%). Similarly, wild type '*GG*' genotype was present in significantly high frequency in HC as compared to Essential hypertension patients group (72.22% *vs.* 54.37%). An odds ratio of 0.4584 in Essential hypertension group respectively for 'GG' genotype indicated a protective effect of this type genotype in our population whereas an odds ratio of 4.072 for Mutant TT Essential hypertension patients group respectively indicated a positive association of this wild type genotype with the disease, heterozygous is also significantly different but may be not protective because of odds ratio of 0.7094. Overall allele 'T' was found to be in significantly low frequency in disease group as compared to HC group whereas allele '*G*' was present in significantly high frequency in the healthy control group (χ^2 =12.07 P= 0.0005***). Overall G allele shows an odds ratio of 0.5065 which indicates its protective association. Carriage rate of allele 'G' was high in HC group whereas carriage rate of allele 'T' was high in disease group (χ^2 =6.180 P=0.0129*) but the values were not significant. .The pattern of genotype and allele distribution in disease and control group suggested a significant association of TNF-α (G308A) wild type allele 'G' carriage (carriage of 'GG') in Susceptibility to Essential hypertension and not show the protective effect.

Table 2. Frequency distribution and association of Genotype, allele frequency and carriage rate of TNF-α (G308A) gene polymorphism in population of Vindhyan region using Chi Square Test

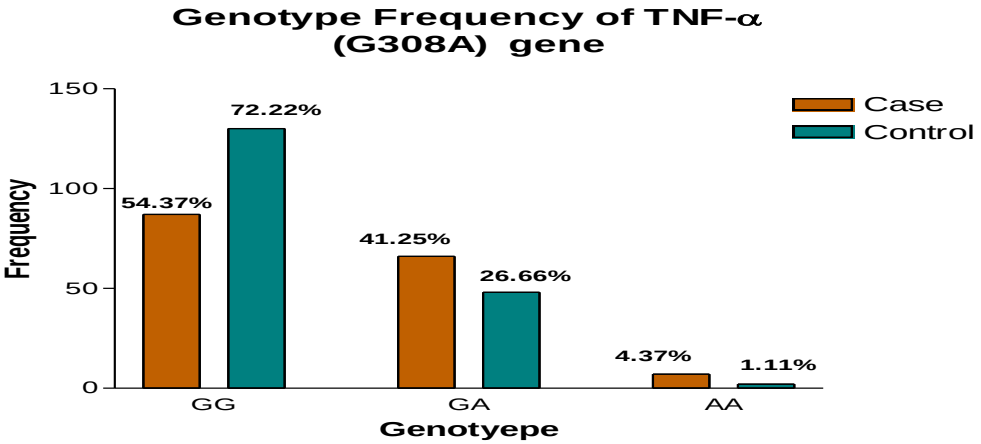
TNF-α (G308A) GENE	**CASE N= 160**		**CONTROL N=180**		**CHI SQUARE VALUE χ^2 (P Value)**
	N	**%**	**N**	**%**	
Genotype					
GG	87	54.37	130	72.22	
GA	66	41.25	48	26.66	13.01 (0.0015**)
AA	7	4.37	2	1.11	
Allele					
G	240	75.00	308	85.55	
					12.07 (0.0005***)
A	80	25.00	52	14.44	
Carriage Rate					
G	153	67.69	178	78.07	
					6.180 (0.0129*)
A	73	32.30	50	21.92	

(- 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.)

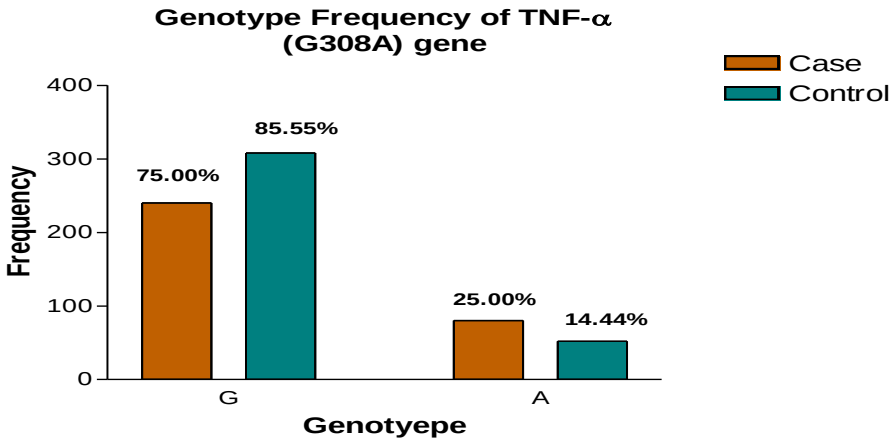
Table 3. Fisher Exact Test values of TNF-α (G308A) gene polymorphism

TNF-α (G308A) GENE	CASE N= 160 N	%	CONTROL N=180 N	%	P Value	Odds Ratio (95% confidence interval)
Genotype						
GG	87	54.37	130	72.22	0.0007***	0.4584 (0.2920 to 0.7195)
GA	66	41.25	48	26.66	0.0057**	1.931 (1.223 to 3.048)
AA	7	4.37	2	1.11	0.0894ns	4.072 (0.8332 to 19.90)
Allele						
G	240	75.00	308	85.55	0.0006***	0.5065 (0.3437 to 0.7465)
A	80	25.00	52	14.44		1.974 (1.340 to 2.910)
Carriage Rate						
G	153	67.69	178	78.07	0.0151*	0.5887 (0.3869 to 0.8958)
A	73	32.30	50	21.92		1.699 (1.116 to 2.585)

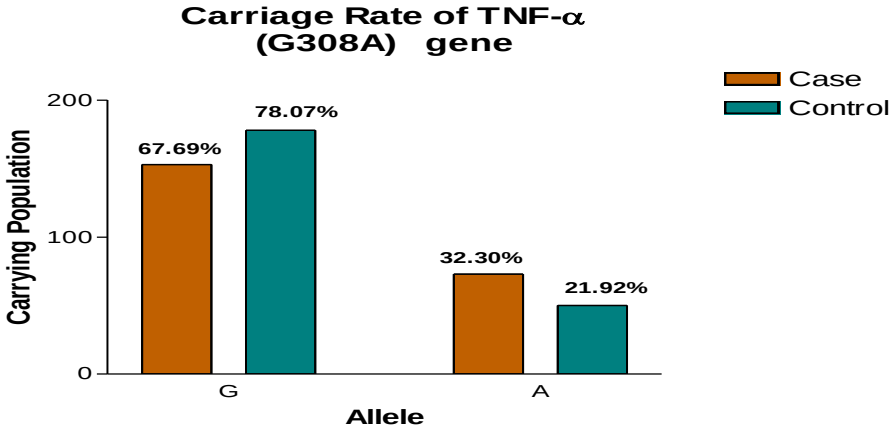
(- denotes the level of significant association between case and control.)*
(N – Number of individuals in study group.)
(% - Genotype allele frequency and carriage rate expressed in percentage.)



Graph -2: Genotype Frequency of TNF-α (G308A) gene



Graph -3: Allele Frequency of TNF-α (G308A) gene



Graph -4: Carriage rate of TNF-α (G308A) gene

6. Discussion:

Tumor necrosis factor α (TNF-α) and interleukin 6 (IL-6) are proinflammatory cytokines and known to be involved in many pathological processes. However, the association between serum levels of TNF-α, IL-6, and pregnancy-induced hypertension (PIH) is unclear [12]. The aim of the present study was to determine the serum levels of TNF-α and IL-6 and to investigate their potential correlation with PIH [11]. The serum concentrations of TNF-α and IL-6 in pregnant women who developed PIH and normal pregnant women were measured. We found that the serum concentrations of TNF-α and IL-6 were significantly increased in the patients with PIH compared to the normal pregnant women. In addition, elevated TNF-α and IL-6 concentrations were associated with pathological complications [10,13]. Moreover, in a hypoxia-induced PIH mice model, animals from the PIH group demonstrated higher TNF-a and IL-6 levels when compared to control, and serum TNF-α and IL-6 levels were positively correlated with right ventricular systolic blood pressure [10-14].

Our study on TNF-α associated with hypertension. Thus Tumor necrosis factor-α (TNF-α) is a also pleiotropic cytokine that becomes elevated in chronic inflammatory states such as essential hypertension infection. Concentration of TNF-α level in hypertension patient (case) and healthy population (control) is showing elevated level during infection and it was statistically significant associated as P<0.0001***. In case of TNF-α, two columns ELISA analysis also having four standards A, B, C, D containing 25, 50, 100, 200 Pg/ml concentration respectively read absorbance at 450 nm. G for Patient (Case) and H for Healthy (control) showing differences in TNF-α level as 23.97 Pg/ml and 11.82 Pg/ml respectively.

The tumor necrosis factor-alpha (TNFa) G308A gene polymorphism has been implicated in susceptibility to essential hypertension (EH), but study results are still controversial. The present meta-analysis is performed to investigate the relationship between the TNFa G308A gene polymorphism and EH [16,17]. Electronic databases were searched and seven separate studies on the association of the TNF a G308A gene polymorphism with EH were analyzed. The meta-analysis involved 1092 EH patients and 1152 controls. The pooled odds ratios (ORs) and their corresponding 95% confidence interval (CI) were calculated by a fixed or random effect model. A significant relationship between the TNFa G308A gene polymorphism and EH was found in an allelic genetic model [15,18]. Genomic DNA was extracted; PCR–RFLP was performed using TNF-a primers specific to detect the presence of SNPs. The PCR–RFLP studies showed that among the Snp238G/A types the GG genotype was 87%, GA genotype was 12% and AA genotype was 1%. Almost a similar pattern of results was obtained with TNF-a Snp308G/A polymorphism. The results obtained were evaluated statistically to determine the significance [15-19].

Our study on TNF-α gene suggested that the nucleotide position G308A polymorphism in TNF-α gene create restriction site for *HinII.* The PCR products when digested by restriction enzyme and wild type allele 107 bp segment which were generated by PCR but the mutant allele shows 87 and 20 bp segments. The product sizes are Wild type homozygote, 107 bp; mutant G308A homozygote, 87 and 20 bp; and heterozygote, 107, 87, and 20 bp respectively. The distribution of polymorphic genotype was strongly under HWE. The observed genotype frequencies, allele frequencies and carriage rates for TNF-α (G308A) polymorphism are depicted in table 4.10 and table 4.11 and graph no. 4.9, 4.10, 4.11. Overall distribution of TNF-α (G308A) genotypes was significantly different in healthy control group as compared to disease group (χ^2=13.01, P=0.0015**). HC group showed a decrease of mutant 'AA' genotype as compared to Patients of Essential hypertension (*1.11*% *vs.* 4.37%). Similarly, wild type '*GG*' genotype was present in significantly high frequency in HC as compared to Essential hypertension patients group (72.22% *vs.* 54.37%). An odds ratio of 0.4584 in Essential hypertension group respectively for 'GG' genotype indicated a

protective effect of this type genotype in our population whereas an odds ratio of 4.072 for Mutant TT Essential hypertension patients group respectively indicated a positive association of this wild type genotype with the disease, heterozygous is also significantly different but may be not protective because of odds ratio of 0.7094. Overall allele 'T' was found to be in significantly low frequency in disease group as compared to HC group whereas allele '*G'* was present in significantly high frequency in the healthy control group (χ^2 =12.07 P= 0.0005***). Overall G allele shows an odds ratio of 0.5065 which indicates its protective association. Carriage rate of allele 'G' was high in HC group whereas carriage rate of allele 'T' was high in disease group (χ^2 =6.180 P=0.0129*) but the values were not significant. .The pattern of genotype and allele distribution in disease and control group suggested a significant association of TNF-α (G308A) wild type allele 'G' carriage (carriage of 'GG') in Susceptibility to Essential hypertension and not show the protective effect.

The tumor necrosis factor-alpha (TNF-α) gene may play an important role in coronary heart disease (CHD) and myocardial infarction (MI) risk. Recently, controversial results regarding the association of the G-308 A (rs1800629) polymorphism of the TNF-α gene with CHD/MI have been reported [21,23]. AA genotypes in the G-308 A (rs1800629) polymorphism of the TNF-α gene did not occur more frequently in CHD/MI patients than in controls; odds ratios (95% confidence intervals) were 1.743 (0.325 to 1.423) for CHD and 1.731 (0.442 to 1.526) for MI, after adjusting for conventional risk factors. Further stratification for age, gender, and other cardiovascular risk factors did not alter the prior negative findings [22]. Pooled meta-analysis of 23 studies also found no statistically significant associations between the TNF-α polymorphism and CHD/MI risk in the genetic additive, dominant, and recessive models [24]. Subgroup analyses showed no association between the TNF-α polymorphism and CHD/MI in Asian and Caucasian populations. Our study showed no association between the G-308 A (rs1800629) polymorphism of the TNF-α gene (presence of A allele) [20-25].

7. Conclusion:

Our data revealed that association of G-308 A (rs1800629) polymorphism of the TNF-α gene with Essential hypertension. An association of *TNF*-α-*308* polymorphism, a G/A transition at -308-bp position, which may alter promoter activity, with TNF-α expression in vitro, as well as plasma concentrations. An increased serum concentration of TNF-α is an independent predictor of Essential hypertension. The observed genotype frequencies, allele frequencies and carriage rates for TNF-α (G308A) polymorphism suggest distribution of TNF-α (G308A) genotypes was significantly different in healthy control group as compared to disease group (χ^2=13.01, P=0.0015**) in vidhyan population. HC group showed a decrease of mutant 'AA' genotype as compared to Patients of Essential hypertension (*1.11*% *vs.* 4.37%). Similarly, wild type '*GG*' genotype was present in significantly high frequency in HC as compared to Essential hypertension patients group (72.22% *vs.* 54.37%). An odds ratio of 0.4584 in Essential hypertension group respectively for 'GG*'* genotype indicated a protective effect of this type genotype in our population whereas an odds ratio of 4.072 for Mutant TT Essential hypertension patients group respectively indicated a positive association of this wild type genotype with the disease, heterozygous is also significantly different but may be not protective because of odds ratio of 0.7094. Finally, we conclude that TNF-α , G-308 A (rs1800629) polymorphism was significantly associated with Essential hypertension in vindhyan population.

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