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DISTRIBUTION OF TNF- ALLELES IN POPULATION OF VINDHYA REGION

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ABSTRACT

Tumor necrosis factor-alpha (TNF-) is a pleiotropic inflammatory cytokine. The cytokine possesses both growth stimulating properties and growth inhibitory processes, and it appears to have self regulatory properties as well. The cytokine is produced by several types of cells, but especially by macrophage. low levels of TNF-A promote the remodeling or replacement of injured and senescent tissue by stimulating fibroblast growth. The primary role of TNF is in the regulation of immune cells.

The study included 100 samples from urban area and 100 sample of Tribal area which is not infected with any other disease. The aim of the study to evaluate the function of TNF- alleles in population of vindhya region. The diagnosis based on DNA extraction from blood by salting out method. Polymorphism was examined by PCR and RFLP.

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INTRODUCTION

The Tumor Necrosis Factor (TNF) is a proinflammatory cytokine that has a key function in various autoimmune diseases, such as rheumatoid arthritis (RA), psoriasis, psoriatic arthritis, ankylosing spondylitis and Crohn's disease.

It plays an important role in human immune response to infections [1,2]. As regards infectious diseases, it encourages the release of other inflammatory cytokines, particularly interleukins IL-1, IL-6 and IL-8, and stimulates the output of protease, thus participating in the formation and maintenance of granulomas, a component of intracellular pathogen-defence [2,3,4]. Over expression of TNF have been observed in inflamed and normal intestinal mucosa and in the serum of patients with IBD [5]. TNF is a multifunctional cytokine involved in the promotion of inflammatory responses and plays a critical role in the pathogenesis of inflammatory, autoimmune, and malignant diseases [6,7,8]. TNF- is also known to play a key role in cell-mediated immunity and elevated serum levels of TNF- have been demonstrated in IBD [8]. The TNF- has a large number of polymorphisms-most of them located in its promoter region-of which some of them have effect on the expression level of the gene [9].

Disease related with TNF-

TNF- and neurologic diseases- Both TNF- and its receptors are expressed by microglial cells in the brain. Through activation of NF- B, TNF- plays an essential role in the survival of these cells. In the brain, TNF- has been shown to induce pro-inflammatory signals that have been linked with depression, bipolar disorder [10, 11].

TNF- and cardiovascular diseases-TNF- , along with other inflammatory molecules, is known to play a role in the initiation and progression of cardiovascular diseases, Although normal heart does not express TNF- , the failing heart produces massive amounts of TNF- [12]. There is now growing evidence that the immune system is an important source for TNF production in failing heart. However, myocardium may also synthesize TNF- denovo in failing heart [12].

TNF- and pulmonary diseases- TNF- has been shown to play a major role in various pulmonary diseases, including asthma, chronic bronchitis, chronic obstructive pulmonary disease, acute lung injury, and acute respiratory distress syndrome [13].TNF- is expressed in asthmatic airways and has been shown to play a role in amplifying asthmatic inflammation through the activation of NF- B, AP-1, and other transcription factors [14].

METHODS

Study population: The study included population of Urban area and Tribal area normal individuals of in population of Vindhyan region population. We have taken 100 sample from

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Urban area and 100 sample of Tribal area which is not infected with any other disease.

Sample collection – Approx. 5ml. of blood samples of each control were collected in storage vials containing EDTA as anticoagulant (50µl. EDTA for 1ml. blood), from the paediatric department of Sanjay Gandhi hospital and Singhal nurshing home Pathalogy Rewa.

Laboratory Procedure

Genomic DNA was purified from peripheral blood cells of the subjects using salting out extraction method. Gene polymorphisms were studied using PCR and RFLP.

In this study -308 (G/A) polymorphism was analyzed using forward primer, 5 - AGGCAATAGGTGGTTTGAGGGCCAT-3 and reverse primer,
5 - TCCTCCCTGCTCCGATTCCG-3. The amplified PCR product were separated on 1% agarose gel and visualized by UV illuminator. The amplification was accomplished by an initial denaturation at 94^0C for 3 min, and 35 cycles of denaturation at 94^0C for 1 min, annealing at 60^0C for 1min, extention at 72^0C for 1 min, followed by final extention at 72^0C for 5 min. A ladder with amplified product has been run on 1% agarose gel. The amplicon had a size of 107 bp.

The restriction digestion by Nco I included 0.25µl of 10000U/ ml NcoI (final concentration 2.5 U), 2µl of 10X NE buffer 4 (final conc 1X), 12 µl of PCR product and 5.75 µl of sterile water. Reaction mixture was incubated for 16hrs at 37^0C. The resultant products were analyzed on 2% agarose gel stained with ethidium bromide. A 1000 bp gene DNA ladder was run as molecular weight marker. The products were visualized using an ultraviolet trans illuminator.

Statistical Analysis: Statistical analysis was done by comparing the distribution of genotype frequencies, allele frequencies and carriage rates. Statistical analysis were performed using statistical package, Prism 3.0, Prism 5.1 version.

RESULT

Hardy Weinberg Equilibrium Test

Gene	^{2} value for Urban population	^{2} value for Tribal population
TNF-	**1.8**	**0.08**

Table No-1 Frequency distribution and association of Genotype allele frequency and carriage rate of TNF alpha polymorphism in population of Vindhya region using chi square test.

GENOTYPE	POPULATION URBAN PAPULATION N = 100		TRIBAL PAPULATION N = 100		CHI SQUARE VALUE 2 (P Value)
	n	%	n	%	
GG	70	70.27	76	76.12	
GA	25	24.12	22	22.68	1.724 (P = 0.4224)
AA	5	4.5	2	2.20	
ALLELES					
G	165	82.5	174	87	1.567 (P =0.2107)
A	35	17.5	26	13	
CARRIAGE					
RATE	95	95	98	98	
G	30	30	24	24	0.6770 (P = 0.4106)
A					

Table NO. 2 Fisher Exact values of TNF- alpha polymorphism.

Genotype	Population Urban population N = 100		Tribal population N = 100		P Value
	n	%	n	%	
GG	70	70.27	76	76.12	0.4448
GA	25	24.12	22	22.68	0.4260
AA	5	4.5	2	2.20	0.7390
ALLELES					
G	165	82.5	174	87	0.2658
A	35	17.5	26	13	
CARRIGE RATE	95	95	98	98	
G	30	30	24	24	0.4440
A					

Restriction fragment length polymorphism

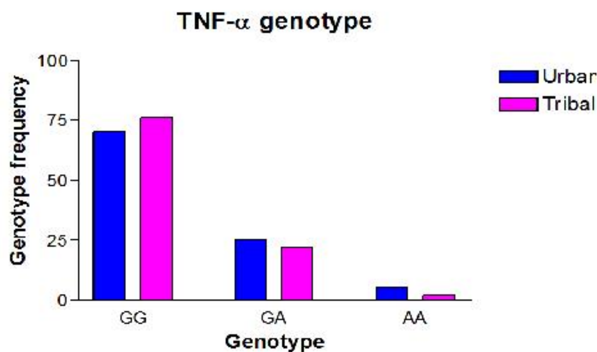
The PCR products were digested using the restriction enzyme Nco I and the products were run on 2% agarose gel stained with ethidium bromide.

Restriction site for Nco I is 5' C˅CATGG…..3'
3'GGTAC˄C…..5'

genotype of gene in each study group were tested to be in accordance with Hardy Weinberg equilibrium using chi square (2) test for independence.(2) value of Urban population (1.8) was more than (2) value for tribal population(0.08).

Genotype allele frequency and carriage rate expressed in percentage. (*Denotes the significant association among population), N = number of individual in study group,%

Genotype allele frequency and carriage rate expressed in percentage.



Overall distribution of TNF– genotypes was significantly different between Urban population and Tribal population (2-1.724 , P-0.4224).We have found that in our study urban population AA genotype more than in comparison of Tribal population (4.5% Vs 2.20%). Heterozygous genotype GA significantly not more different between Urban population and tribal population (24.12Vs 22.68).

In Urban population GG Genotype frequency was lesser than Tribal population. Allele G frequency was lower in Urban population as compare to Tribal population (82.5% Vs 87 %). Were as allele A was found slightly more in Urban population as compare to Tribal population. Allele frequency between Urban population and tribal population was no significantly and 2 1.567 and P Value is 0.2107. Carriage rate of G allele was not more different in Urban population and Tribal population (95% Vs 98%). Similarly carriage rate of a allele is slightly more in Urban population compare to Tribal population (30%Vs 28%). Carriage rate of both allele G and A was statically non significantly between Urban population and Tribal population (2 = 0.6770, P = 0.4106).

DISCUSSION

TNF- is a modulator of cell immune response, there is a risk of opportunist infections through suppression of endogenous TNF-a, an important cytokine for the formation of granulomas [2,3,15,16]. Over expression of TNF- have been observed in inflamed and normal intestinal mucosa and in the serum of patients with IBD [5].

As regards infectious diseases, it encourages the release of other inflammatory cytokines, particularly interleukins IL-1, IL-6 and IL-8, and stimulates the output of protease, thus participating in the formation and maintenance of granulomas, a component of intracellular pathogen-defence [2,3,4].

Results of this study indicate overall distribution of TNF-a allele in Urban and Tribal population, allele A was found slightly more in Urban population as compare to Tribal population. Allele frequency between Urban population and tribal population was no significantly and 2 1.567 and P Value is 0.2107. It is possible that the distribution of TNF-a alleles in the population of vindhya region is different.

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