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THE POLYMORPHIC ROLE OF TGF β (-509C/T) IN THE ETIOLOGY OF GASTRIC CANCER

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ABSTRACT

The present study is aimed at evaluating the role of Transforming growth factor β in the pathogenesis of gastric cancer (GC). The TGF β gene is present on chromosome 19 at 19q13.2 location and the site of gene polymorphism is -509C/T. TGF β 1 signals the target cell by binding to a heterodimeric complex of two transmembrane receptors and forms Smad4 complex. This translocates to the nucleus, wherein it interacts with the promoters of transcription factor to influence the expression of selective genes involved in cell proliferation. The present study highlighted the role of T allele at -509 C/T in the etiology of gastric cancer. Among 50 gastric cancer patients, 34 were males (68%) and 16 (32%) were females. Higher frequency was observed in the age group of above 40 yrs i.e.84% when compared to age below 40 yrs (16%). 50% patients were alcoholics and 50% were non-alcoholics. The genotyping of TGF β 1 has been carried out for all the subjects and the various genotypes are depicted by Agarose (1.5%) gel electrophoresis of ARMS-PCR products for TGF β 1 -509 C/T genotypes along with 100 bp ladder. We analyzed 50 gastric cancer cases and an equal number of controls for the TGF β 1 -509C/T gene polymorphism. Distribution of genotypes was CC 36%, CT 50% and TT 14% in GC cases and CC 44%, CT 46% and TT 10% in controls. Allelic frequencies of C and T alleles were 0.61 and 0.39 in GC cases and 0.67 and 0.33 in controls respectively. We observed higher frequency of TT genotype and T allele in GC cases in comparison to controls, indicating its possible association with the disease.

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Key Words

TGF β 1, cytokine, polymorphism, gastric cancer, C/T genotypes, allele.

INTRODUCTION

Gastric cancer is a disease in which malignant (cancer) cells form in the lining of the stomach. Gastric cancer, commonly known as stomach cancer, can develop in any part of the [stomach](#) and may spread throughout the stomach and to other organs, particularly the [oesophagus](#), [lungs](#), [lymph nodes](#), and the [liver](#). Age, diet, and stomach disease can affect the risk of developing gastric cancer. In Asia gastric cancer has high incidence in China, South Korea and Japan, and has low incidence in Pakistan India and Thailand. In India, there is a wide variation in the incidence of gastric carcinoma. The incidence rate of gastric cancer is four times higher in Southern India compared with Northern India. It is a disease with a high death rate (~800,000 per year) making it the second most common cause of cancer death worldwide after [lung cancer](#).

Gastric cancer is multi-factorial in nature and exact causes cannot be specified. However, certain factors increase the risk of gastric cancer, the disease is more common in men over the age of 55, a diet high in salt and nitrates and low in vitamins A and C increases the risk for stomach cancer. Helicobacter pylori (*H. pylori*) infection of the stomach has been discovered as a one of the important risk factor for gastric cancer. Smokers are more likely than non-smokers to develop this cancer. People who are obese may have an increased risk of cancer developing in the upper part of the stomach and this may lead to chronic gastritis, Pernicious anemia Intestinal metaplasia, and gastric polyps contribute to the risk of developing gastric cancer⁽¹⁾.

Cytokines are low-molecular weight regulatory proteins or glycoproteins secreted by white blood cells and various other cells in the body in response to a number of stimuli. These proteins assist in regulating the development of immune effector cells, and some cytokines possess direct effector functions of their own. There are basically two types of cytokines, interferons and interleukins. ILs can be functionally classified into those that enhance cellular immune responses, type 1 (IFN- γ , TGF- β , etc.), and type 2 (IL-4, IL-10, IL-13, etc.), which favour antibody responses⁽²⁾.

Transforming growth factor (TGF- β) is a cytokine produced by both immune and non immune cells. TGF- β controls the differentiation, proliferation and state of activation of all immune cells. Wound healing and angiogenesis is implicated in immune abnormalities linked to cancer, autoimmunity infections and fibrotic complication. TGF- β acts as a growth inhibitor in normal epithelium including the intestinal epithelium. It is involved in multi important cellular processes and plays a biphasic role in carcinogenesis. In early stages of cancer TGF- β plays role in cellular differentiation and apoptosis and in later stages of cancer, the role of TGF- β shifts to that of a tumor promoter by stimulating angiogenesis and cell motility, suppressing immune response and increasing the interaction of tumor cell with the extracellular matrix, which leads to progressive invasion and metastasis. This biphasic nature of TGF- β action has also been demonstrated in animal modeling in which the TGF- β pathway has been altered⁽³⁾.

In view of the above facts, the present study is aimed at evaluating the roles of TGF- β (-509c/t) gene polymorphism in the pathogenesis of gastric cancer.

MATERIALS AND METHODS

Samples

Patients

Fifty endoscopically and histopathologically confirmed gastric cancer patients of age group of 30-70 years, referred to Department of Gastroenterology, Osmania General Hospital Hyderabad, were considered for the present study.

Controls

Fifty age and sex matched healthy subjects with no family history of gastric ulcer or gastric cancer were selected as controls.

Each subject was interviewed using a structured questionnaire to obtain information on epidemiological factors such as dietary habits, smoking, alcohol consumption, family history etc. Informed consent was obtained from all the patients and controls before including for the study. All the control subjects were analyzed along with the patients for comparative analysis.

Five ml of venous blood was collected from each subject in EDTA vacutainers. All blood samples were used for the isolation of DNA, whenever necessary and remaining blood was stored at -4°C.

Isolation of genomic DNA (Salting out method):

DNA was isolated from whole blood samples of all patient and control subjects by the salting out procedure⁽⁴⁾.

Reagents required

1. TKM₁ buffer (TRIS, Potassium chloride and Magnesium Chloride) (500ml) –pH 7.6

- Tris HCl - 0.778g/ml
- KCl - 0.3728g/ml
- MgCl₂ - 0.0160g/ml

2. TKM₂ buffer (100ml) – pH 7.6

- Tris HCl - 0.158g/ml
- KCl - 0.3728g/ml
- MgCl₂.6H₂O - 0.0160g/ml
- EDTA - 0.074g/ml
- NaCl - 2.337g/ml

3. 10% SDS: 1g of SDS is dissolved in 10 ml of autoclaved distilled water. Do not autoclave the solution.

4. 6M NaCl: 8.765g of NaCl was dissolved in 25 ml of distilled water.

5. TE buffer (10mM) pH 8.0

- Tris base - 0.080g
- EDTA - 0.009g

6. 70% Ethanol

7. 100% Ethanol

8. Tris borate EDTA (1x)

- Tris base - 5.389g
- Boric acid - 2.7515g
- EDTA - 0.37225g

9. Triton-x 10%: 1ml of Triton in 9ml of distilled water.

Procedure for isolation of DNA:

1. Whole blood was collected in a vacutainer tube (purple stoppered bottle) containing 100 µl of 50 mM EDTA.

2. 300 µl of blood was transferred into a 2 ml Ependorff tube and added with 900 µl of low salt buffer TKM1 and 20 µl of Triton-X100 to lyse the cells and mixed well by vortexing and centrifuging at 6000 rpm for 5 min at 5°C.

3. The supernatant was discarded and the nuclear pellet was collected. The above steps were repeated until the pellet became white.

4. The pellet was gently suspended in 300 µl of high salt buffer TKM2 and 100 µl of 10% SDS and then the whole suspension was mixed gently and incubated for 10 min at 55°C.

5. 200 µl of 6M NaCl was added in the tube and mixed well.

6. Centrifuged at 12,000 rpm for 5 min in micro centrifuge.

7. Supernatant was transferred to fresh 2 ml ependorff tube and 400 µL of 100% ice cold ethanol was added. The tube was inverted several times until the DNA gets precipitated and then centrifuged at 15000 rpm for 5 minute.

8. The supernatant was discarded and added with 400 µl of 70% ethanol to the precipitated DNA and centrifuged at 15000 rpm for 5 minute at 4°C.

9. The pellet was air dried, resuspended in 60 µL of TE buffer and incubated at 55°C for 10 minutes to dissolve DNA.

10. The concentration of DNA was measured by taking A₂₆₀ & A₂₈₀ and the quality of DNA was checked by Agarose gel electrophoresis.

Estimation of DNA:

The isolated DNA needs to be studied for its quality before using it in molecular biology experiments. It is of 2 types.

- Qualitative
- Quantitative

Nucleic acids (DNA & RNA) have maximum absorbance at 260nm. One O.D value corresponds to approximately 50mg/ml of double stranded DNA, 40mg/ml Of single stranded DNA, 20mg/ml of RNA.

The ratio between the readings at A_{260nm} & A_{280nm} provides an estimation of purity of the nucleic acids.

- 1) Pure preparation of DNA & RNA has a ratio of approximately 1.8 and 2.0 respectively.
- 2) If DNA is Contaminated with protein then the ratio will be lesser than 1.8
- 3) If DNA is contaminated with RNA then the ratio will be greater than 2.0

Polymerase chain reaction:

PCR is based on the enzymatic amplification of a fragment of DNA that is flanked by two 'primers', short oligonucleotides that hybridize to the opposite strands of the target sequence and then prime synthesis of the complementary DNA sequence by DNA polymerase (an enzyme).

Steps in Polymerase chain reaction

1. Denaturation
2. Annealing step
3. Extension/elongation step
4. Final elongation

ARMS PCR

The ARMS PCR technique is used to identify SNPs (single nucleotide polymorphisms) by using 4 primers. The outer pair is perfect match and gives a large product. These primers are positioned asymmetrically so that the position of the SNP is not in the middle. The middle primers face in towards one another and towards different outer primers and have their 3' end base pairing with one of the possible SNPs. As each faces one of the outer primers, their products of PCR can be held apart by running on an Agarose gel. It's suggested that the -2 base at the 3' primer end is a mismatch.

Aim: To amplify the TGF- β and IFN- γ gene by using ARMS PCR.

Materials required:**Table 1:** Programming of polymerase chain reaction for TGF β

PCR condition	Temperature	Time of reaction
Step1. Initial denaturation	94°C	2 minutes
Step2. Denaturation	94°C	30seconds
Step3. Primer annealing	61°C	20seconds
Step4. Extension	72°C	20seconds
Step5. Go to step 2 for 29 cycles		
Step6. Final extension	72°C	3 minutes

Materials

- Micro pipettes
- Tips
- Eppendorff tubes
- PCR tubes
- UV laminar flow

Reagents used in PCR

- Autoclaved Millipore water
- Common primer
- Forward primer (20 pmol/ μ L)
- Reverse primer(20 pmol/ μ L)
- Deoxy Ribonucleotides (dATP, dGTP,dTTP,dCTP) - 10mM
- 10X Assay buffer with MgCl₂ (15mM)
- Taq polymerase 1U/ μ L
- DNA samples(controls and patients sample) (100ng/ μ L)

Allele specific PCR for TGF β :**Primer details-**

Common primer (CF) antisense

5'-CTACGGCGTGGAGTGCTGAG -3'

C- Primer (RC) Sense

5' -AAGGGGCAACAGGACACCTGGG -3'

T-Primer (RT) Sense

5' -AAGGGGCAACAGGACACCTGGA -3'

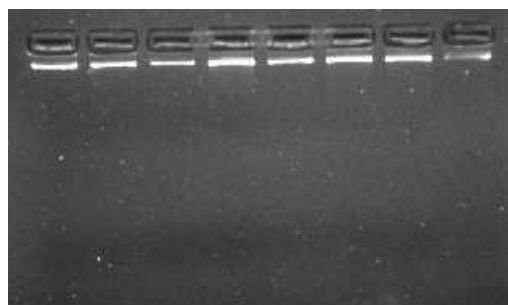
Product size: 349bp.

Step7.	4°C	10 minutes
Step8. Store	End programme	

Table 2: Working and Stock solution of TGF β gene PCR

Ingredients	Stock Conc.	Working Conc.	Amount for 1PCR tube		Amount for 10PCR tubes	
			C (μ l)	T (μ l)	C (μ l)	T (μ l)
Distilled water	-	-	12	12	120	120
10XTris buffer	10X	1X	1.5	1.5	15	15
dNTPs	100 mM	200 μ M each	0.2	0.2	8	8
Common forward(CF) Antisense primer	20 pmol/ μ l	0.5 pmol/ μ l	0.1	0.1	1	1
Reverse primer (C allele)	20 pmol/ μ l	5 pmol/ μ l	0.1	-	1	-
Reverse primer (T allele)	20 pmol/ μ l	5 pmol/ μ l	-	0.1	-	1
Taq polymerase	1U/ μ l	0.3U	0.2	0.2	2	2
DNA sample	100ng/ μ l	100ng	1	1	1 \times 10	1 \times 10

Electrophoresis was carried out immediately after PCR was over, on 2% agarose gel.

**Figure 1:** Gel picture showing genomic DNA

Analysis of Qualitative Variable:

Allele frequencies: individual allele frequencies for all the polymorphic markers were calculated from the frequency distribution of homozygous and heterozygous genotypes.

$P = \frac{\text{Frequency of homozygotes} + \frac{1}{2}(\text{frequency of heterozygotes})}{N}$

N

Where N= Total number of individuals studied.

RESULTS

Gastric cancer has a wide geographic variation. Countries in Asia with a high incidence include Japan, China, and South Korea; those with a low incidence include India, Pakistan, and Thailand. Until recently gastric cancer was the second most common cancer worldwide. Now it has moved to the third place, behind breast cancer. Incidence rates in men are twice than in women, in both low-risk and high-risk areas. The estimated number of new cancer cases in 1995 was 311,460 in men and 340,683 in women⁽⁵⁾. The number of new digestive tract cancer cases in 2001 was estimated to be approximately 145,000 in India. In men and women, the esophagus (24,925 in men and 18,608 in women) is the commonest site, followed by the stomach (23,785 in men; 11,890 in women). Gastric cancer occurs a decade earlier among South Indians compared with the North Indians.

The effect of a particular cytokine on a given cell depends on the cytokine, its extracellular abundance, the presence and abundance of the complementary receptor on the cell surface, and downstream signals activated by receptor binding. Transforming growth

factor-beta (TGF- β) is a multifunctional cytokine, which influences cell differentiation, proliferation, motility and apoptosis^{(6), (7)}. Among the TGF- β family, which comprises TGF- β 1, - β 2 and - β 3, TGF- β 1 is most abundantly expressed, especially in various pathological conditions including chronic inflammatory diseases and cancer⁽⁸⁾. Transforming growth factor β 1 has been shown to reduce the immune response⁽⁹⁾, stimulate angiogenesis^{(10), (11)}, increase synthesis of proteolytic enzymes^{(12), (13)} and stimulate extra cellular matrix (ECM) deposition⁽¹⁴⁾ in the tumor microenvironment. Several studies examined the role of TGF- β 1 in gastric cancer. Positive TGF- β 1 immunostaining was found to be related to invasion and metastasis of gastric cancer^{(15), (16)}.

Finally, gastric cancer patients showed strongly increased tissue TGF- β 1 levels and, unexpectedly, reduced serum TGF- β 1 levels⁽¹⁷⁾. Transforming growth factor- β 1 is synthesised as an inactive precursor, the large latent complex consisting of a TGF- β dimer, the latency-associated protein (LAP) and latent TGF- β binding protein (LTBP) for localisation and binding to the ECM⁽¹⁸⁾. Before TGF- β 1 can exert its biological effects, LAP and LTBP have to be dissociated. This can occur by conformational changes^{(19), (20)} like proteolytic cleavage, irradiation⁽²¹⁾ or by an acid environment⁽²²⁾. The complex release mechanism of TGF- β 1 might implicate that high total TGF- β 1 has no biological consequences without the presence of appropriate activation mechanisms in the tumor microenvironment. Therefore, measuring TGF- β 1 activity levels and the localisation in cancer could be more informative regarding the state of cancer progression.

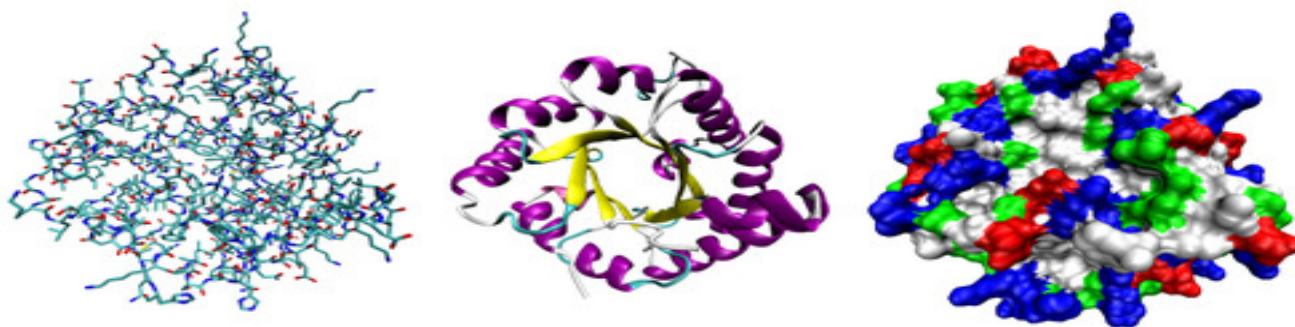
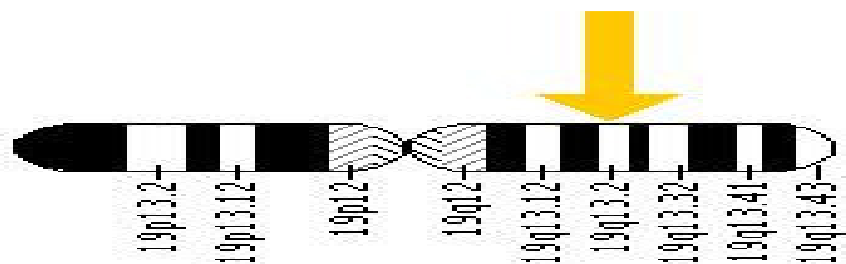


Figure 2: TGF- β 1 dimer in ball and stick model and structure

Cytogenetic Location: 19q13.2

Molecular Location on chromosome 19: base pairs 41,836,811 to 41,859,830

**Fig 3:** Gene location of TGF β 1

Role of TGF β receptors in tumorigenesis: In contrast to TGF β , components of its intracellular signaling have shown distinct tumor suppressor effects. Mutations of TGF β receptors and Smad proteins have been linked to acquiring of resistance to TGF β and, subsequently, to evasion from the TGF β -dependent control of cell proliferation and apoptosis. Inactivation of the TGF β signaling has been found in most of human cancers,

Table 3: Epidemiological variable of GC Patients

suggesting that its intactness is an important hinder which has to be disabled in the progression of carcinogenesis⁽²³⁾.

Fifty GC patients and fifty healthy controls were considered for the present study. The Table 3 and 4 gives details of epidemiological variables of GC and control subjects.

S. No.	Code	Age	Sex	Diet	Alcoholic	Smoking
1	D1	45	Male	Mix	No	Yes
2	D2	35	Male	Mix	Yes	Yes
3	D3	45	Male	Mix	Yes	Yes
4	D4	52	Male	Mix	Yes	Yes
5	D5	60	Male	Veg	Yes	Yes
6	D6	85	Female	Mix	No	Yes
7	D7	39	Male	Veg	No	No
8	D8	55	Male	Veg	No	No
9	D9	65	Male	Mix	No	No
10	D10	53	Male	Mix	Yes	Yes
11	D11	26	Female	Mix	No	No
12	D12	35	Male	Mix	Yes	Yes
13	D13	66	Male	Mix	No	Yes
14	D14	55	Male	Mix	Yes	Yes
15	D15	60	Female	Veg	No	Yes
16	D16	60	Female	Mix	No	Yes
17	D17	56	Male	Mix	Yes	Yes
18	D18	60	Male	Mix	Yes	Yes
19	D19	50	Female	Mix	No	No
20	D20	80	Male	Mix	No	No
21	D21	55	Male	Mix	Yes	Yes

22	D22	60	Male	Mix	Yes	Yes
23	D23	64	Female	Mix	No	No
24	D24	46	Female	Veg	Yes	No
25	D25	30	Male	Veg	Yes	Yes
26	D26	30	Female	Mix	No	No
27	D27	70	Male	Mix	No	No
28	D28	70	Male	Mix	Yes	Yes
29	D29	65	Male	Mix	No	2
30	D30	35	Female	Mix	No	No
31	D31	50	Male	Mix	No	No
32	D32	52	Male	Veg	Yes	Yes
33	D33	50	Male	Mix	Yes	Yes
34	D34	35	Female	Mix	No	No
35	D35	60	Male	Mix	Yes	Yes
36	D36	45	Female	Mix	No	No
37	D37	70	Male	Mix	No	No
38	D38	50	Female	Veg	Yes	Yes
39	D39	70	Male	Mix	Yes	Yes
40	D40	50	Male	Mix	Yes	Yes
41	D41	56	Female	Mix	Yes	Yes
42	D42	55	Male	Mix	No	No
43	D43	73	Male	Mix	Yes	Yes
44	D44	55	Male	Mix	Yes	No
45	D45	45	Female	Mix	No	Yes
46	D46	50	Female	Mix	Yes	Yes
47	D47	65	Male	Mix	No	Yes
48	D48	68	Male	Mix	No	Yes
49	D49	48	Male	Mix	Yes	Yes
50	D50	49	Female	Mix	No	No

Gender: out of 50 samples 34 i.e. 68% were males and 16 i.e. 32% females

Age: 8 were below the age of 40 i.e., 16% and 42 were above the age of 40 i.e., 84%.

Alcoholics: 25 out of the 50 were alcoholics, i.e. 50% and 25 were non alcoholic i.e., 50%

Smokers: 31 out of the 50 were smokers, i.e. 62% and 19 were nonsmokers i.e., 38%.

Table 4: Epidemiological variable of Control Patients

S. No.	Code	Age	Sex	Diet	Smoking	Alcohol
1	C-1	24	Male	Mix	No	No
2	C-2	19	Male	Mix	No	No
3	C-3	20	Female	Veg	No	No
4	C-4	36	Male	Mix	Yes	No
5	C-5	28	Male	Mix	Yes	No

6	C-6	30	Male	Mix	No	No
7	C-7	28	Male	Mix	Yes	No
8	C-8	33	Male	Mix	No	No
9	C-9	42	Female	Veg	No	No
10	C-10	53	Male	Veg	Yes	No
11	C-11	36	Male	Mix	No	No
12	C-12	34	Male	Mix	No	No
13	C-13	28	Male	Mix	No	No
14	C-14	45	Female	Mix	No	No
15	C-15	27	Female	Veg	No	No
16	C-16	60	Female	Mix	No	No
17	C-17	45	Male	Mix	No	No
18	C-18	38	Male	Mix	No	No
19	C-19	32	Male	Mix	No	No
20	C-20	40	Male	Mix	No	Yes
21	C-21	36	Female	Mix	No	No
22	C-22	32	Male	Veg	No	No
23	C-23	25	Female	Veg	No	No
24	C-24	25	Female	Mix	No	No
25	C-25	45	Male	Mix	No	No
26	C-26	26	Female	Mix	No	No
27	C-27	50	Male	Mix	No	No
28	C-28	32	Male	Mix	No	No
29	C-29	32	Male	Mix	No	No
30	C-30	34	Male	Mix	No	No
31	C-31	36	Male	Veg	No	No
32	C-32	31	Male	Mix	No	No
33	C-33	31	Male	Mix	No	No
34	C-34	40	Male	Mix	No	No
35	C-35	38	Male	Mix	Yes	No
36	C-36	29	Male	Mix	No	No
37	C-37	32	Male	Mix	No	No
38	C-38	39	Male	Mix	Yes	No
39	C-39	28	Male	Mix	No	Yes
40	C-40	25	Male	Mix	No	No
41	C-41	39	Male	Mix	No	No
42	C-42	56	Male	Mix	Yes	No
43	C-43	62	Female	Mix	No	No
44	C-44	58	Male	Mix	No	No
45	C-45	59	Female	Mix	No	No
46	C-46	42	Male	Mix	Yes	No
47	C-47	48	Male	Mix	No	No
48	C-48	49	Male	Mix	No	No

49	C-49	60	Female	Mix	No	No
50	C-50	54	Male	Mix	No	No

Gender: out of 50 samples 38 i.e., 76% are males and 12 are females i.e. 24 %.

Age: 12 were above the age of 40 i.e 24% and 38 were below that age of 40.i.e 76%.

Alcoholics: 2 out of the 50 were alcoholics, i.e. 4% and 48 i.e. 96% were nonalcoholic.

Smokers: 10 out of the 50 were smokers, i.e. 20% and 40 were non smokers i.e. 80%.

Table 5: Statistical Analysis of Diseased & Control Samples

Variables		GC		C	
		N	%	N	%
Mean Age \pm SD		54 \pm 12.946		38 \pm 11.33	
Sex	Male	34	68	38	76
	Female	16	32	12	24
Age	\leq 40	8	16	38	76
	>40	42	84	12	24
Diet	Mix	42	84	43	86
	Veg	8	16	7	14
Smoking	Smoker	31	62	10	20
	Non-Smoker	19	38	40	80
Alcohol	Alcoholic	25	50	2	4
	Non-Alcoholic	25	50	48	96

Figure 4: Gel picture showing TGF β 1 -509 C/T genotypes along with 100 bp ladder

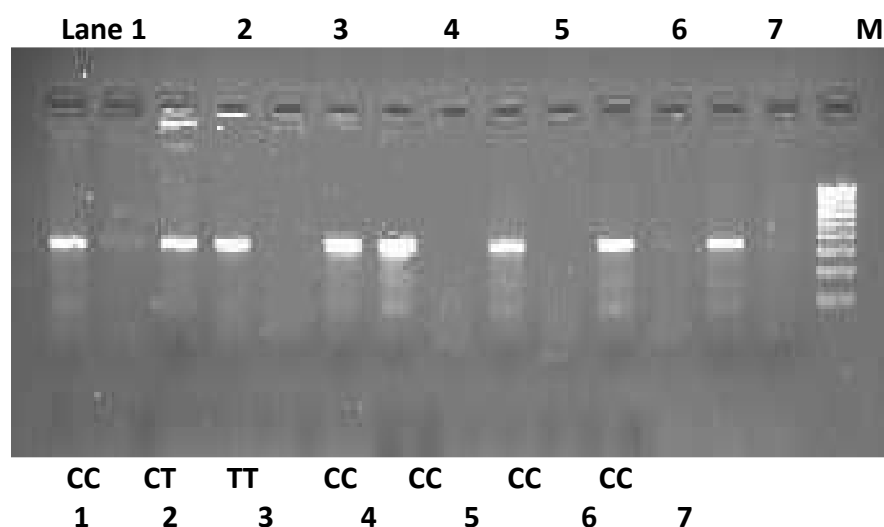


Table 6: Genotypic Distribution and Allelic frequencies of TGF- β – 509 (C/T) in Diseased & Control

Genotype	Gastric cancer		Control	
	N=50	%	N=50	%
CC	18	36	22	44
CT	25	50	23	46
TT	7	14	5	10
Alleles	Allelic Frequency		Allelic Frequency	
C	61	0.61	67	0.67
T	39	0.39	33	0.33

We analyzed 50 gastric cancer cases and an equal number of controls for the TGF β 1 -509C/T gene polymorphism. Table 6 gives the comparative view of these parameters in gastric cancer cases and controls. Distribution of genotypes was CC 36%, CT 50% and TT 14% in GC cases and CC 44%, CT 46% and TT 10% in controls. Allelic frequencies of C and T alleles were 0.61 and 0.39 in GC cases and 0.67 and 0.33 in controls respectively. We observed higher frequency of TT genotype and T allele in GC cases in comparison to controls, indicating its possible association with the disease.

DISCUSSION

Gastric cancer is a disease in which malignant (cancer) cells form in the lining of the stomach. Gastric cancer, commonly known as stomach cancer, can develop in any part of the [stomach](#) and may spread throughout the stomach and to other organs. In India, incidence rate of Gastric Cancer is about 6.5/10⁵, and 4 times higher in southern India compared with northern India. Gastric Cancer has a multi-factorial, multi-gene, multistep, etiology. Several factors are implicated in the development of Gastric Cancer including diet, *Helicobacter pylori* infection; chronic atrophic gastritis etc.

Cytokines are small molecules secreted by cells in response to specific stimuli that alter the behaviour of the same or other cells. It acts on target cells, generally within the haematopoietic system, by binding to specific receptors and initiating signal transduction and second messenger pathways within the target cell. This can result in gene activation, leading to mitotic division, growth and differentiation, migration, or apoptosis.

Genetic variation in regulatory region of cytokine genes has been associated with susceptibility to several diseases including cancer.

The results revealed T allele association and an increased risk of TT and CT genotypes to gastric cancer while CC genotype and C allele may confer protection from carcinogenesis. Studies have shown that the -509 T allele was significantly associated with higher levels of TGF β 1 than the -509 c allele ⁽²⁴⁾. The -509 C/T polymorphism is located within a yin yang 1(YY1) consensus binding site. One study demonstrated that transfection with construct containing the T allele enhances YY1 binding and increased promoter activity compared to the C allele, thus this promoter variant may alter TGF β 1 transcription activity and the binding of the YY1 resulting in increased level of TGF β 1 ⁽²⁵⁾. A Chinese study of 167 GC cases and 193 healthy controls showed increased frequency of T allele and -509 CT and -509 TT genotypes ⁽²⁶⁾. Our results indicated that T allele of TGF β 1 -509 C/T polymorphism may be an important factor in the development of gastric cancer.

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