Role of Transforming Growth Factor-B1 (T29C) Gene Polymorphism in Hepatic Stellate Cell Activation and Invasion and Susceptibility to Hepatitis B, C Infection in an Iraqi Patients

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Abstract. The interindividual varieties in the limit of changing development factor-β1 (TGF-β1) generation have been credited to hereditary polymorphisms in TGF-β1 quality. Changing development factor-beta 1 (TGFβ1) is a powerful suppressive cytokine that adds to ceaseless hepatitis B (CHB) disease. Abberations in TGFβ1 generation among people have been ascribed to TGFβ1 hereditary polymorphisms. We analyzed whether three putative polymorphisms in TGFβ1 (−509 C/T (rs1800469), +869 C/T (rs1800470), and +11929 C/T (rs1800472)) square measure connected with CHB illness during a South-Eastern Iranian people.

Methods: A total of 203 subjects with hepatitis infection (94 patients with hepatitis B virus infection and 109 patients with hepatitis C virus infection) whom admitted to Margan hospital, Center of liver diseases and gastrointestinal system were enrolled in the study. Allele specific (AS)-PCR, methods were used for assessing polymorphism of IL-10. Patients included (130 males and 73 females), with an age range (HBV: 44.6 ± 8.2), (HCV: 45.3±13.3) and (Control: 49.2 ± 9.04) years. The practical side of this study was done during the period from October 2017 to March 2018.

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Keywords: TGF-B1 (T29C), Genetic variations, Polymorphism, HBV, HCV
1. Introduction

Hepatitis B infection contamination (HBV) is an overall issue and it’s heretofore the basic issue of making incessant HBV, cirrhosis, and hepatocarcinoma (HCC), significantly in making nations (AbdAlredhaet al. 2018 and Sonneveld et al. 2010). There are a unit around four hundred million bearers of HBV contamination worldwide and quite one million passings happen once a year as a results of sudden viscous disappointment, cirrhosis, and hepatocarcinoma (Kew et al. 2010 and Al-Marzoqi et al. 2015).

Changing development factor-β1 (TGF-β1) is a multifunctional cytokine that directs cell development, multiplication, and separation (Massague et al. 2008 and Ibraheem et al. 2018). It’s delivered by many cell writes, as well as monocytes, macrophages, epithelial tissue cells, associated tube-shaped structure sleek somatic cell and it’s likewise created from an assortment of liver cell populaces as well as HSCs, hepatocytes, and LSECs still platelets and penetrating mononucleate cells. TGF-β1 is enter particle in numerous physiological procedures in the liver since it actuates apoptosis and diminishes hepatocytes multiplication other than its fundamental part in hepatic fibrogenesis. Host hereditary components assume a basic part in creating fibrosis though numerous qualities are accounted for to be related with liver fibrosis and cirrhosis including TGF-β1 (Gewaltig et al. 2002).

What’s additional, TGF-β1 has potential effect on the resistant reaction since it effectsly affects its inhibitory impact on T-cells expansion by means of IL-2 down-control. TGF-β1 quality is situated on chromosome 19q13.1–13.3 with seven exons and half dozen introns. A few polymorphisms in both coding and non-coding locales of the TGF-β1 quality have been accounted for and found to influence TGF-β1 protein articulation. there’s a sensible single ester polymorphism (SNP) at the twenty ninth ester (T29C), 868 NT in relevancy the interpretation begin website, (rs1982073 converged into rs1800470) in deoxyribonucleic acid one with progress from T to C transportation regarding amino acid within the scene coding the flag grouping from Lucien to amino acid at the tenth amino corrosive (Kirshner et al. 2006).

This progress upsets the structure and results in expanded levels of TGF-β1 protein and mRNA in people with C allele with a 2.8-crease increment in TGF-β1 emission contrasted and T allele in vitro. What is more, the substitutions of amino corrosive buildup might influence the capability of the flag amide, potentially by impacting intracellular trafficking or fare effectiveness of the TGF-β1 protein (Le Marchand et al. 2004).

Changing development factor-beta 1 (TGF-β1) is a pleiotropic cytokine that is ensnared in numerous organic capacities in the liver, as well as the deferral of hepatocyte multiplication, the balance of hepatocyte development issue tired, and therefore the cell death of HCC (Zhang et al. 2006 and Al-Marzoqi 2017). The articulation level of TGF-β1 is fundamentally expanded after liver damage, amid which hepatic stellate cells (HSCs) are thought to be the significant wellspring of emitted TGF-β1. As well, it has been accounted for that HBV replication actuates hepatocytes to mystery TGF-β (Martin-Vilchez et al. 2008 and Noor et al. 2016). Clinical examination likewise uncovered that plasma TGF-β1 is fundamentally hoisted in patients with perpetual hepatitis (CHB), cirrhosis, and HCC (Chou et al. 2007).

TGFβ1 polymorphisms have been assessed in various irresistible illnesses, as well as Mycobacterium tuberculosis (TB) illness (Bravo Chou et al. 2008, Al-Marzoqi 2018 and Hassan et al. 2016), brucellosis (Budak et al. 2008), hepatitis C infection (HCV) contamination, and CHB (Karaoglan et al. 2009); be that because it might, comes regarding are conflicting..

2. Materials & Methods

Study Subjects:

The practical side of this study was done during the period from October 2017 to March 2018. Two hundred and eighty five samples were collected. Two enrolled groups of subjects were involved in this study.
Patients:
This study included 203 patients with hepatitis infection, 94 patients with hepatitis B virus infection and 109 patients with hepatitis C virus infection admitted to Margan hospital, Center of liver diseases and gastrointestinal system. Patients included (130 males and 73 females), with an age range (HBV: 44.6 ± 8.2), (HCV: 45.3±13.3) and (Control: 49.2 ± 9.04) years, they were diagnosed by serological and molecular tests and selected in the current study. Blood and serum samples taken from every patient and control having thoroughly examined.

Healthy control group
Eighty two Actual healthy persons from various Iraqi populations were arbitrarily involved in this study.

Methods:

Blood Sampling:
About five milliliters of venous blood were collected from each patient in this study. The blood was divided into two parts: one part (about two milliliters) was collected into EDTA containing tubes for genetic part. The second part of the blood was placed in gel tube for thirty minutes, then transferred to plain tube and serum was obtained by centrifugation at 3000 rpm for 15 min; after that the serum collected and kept in the freezer (-20 ºC) until it was used for the immune and viral assay.

Isolation of genomic DNA:
Genomic DNA was used for molecular study by sequestered from the fresh blood, which collected in tubes of anti-coagulant EDTA and for frozen blood samples we recommended using protease K were applied using for DNA purification; Favor prep Blood genomic DNA purification kit. The isolation of DNA depended on the 5 stage procedure utilizing salting out techniques (Sambrook and Manianatis, 1989):

- Lysis of the RBCs in the Cell Lysis Solution.
- Lysis of the WBCs and their nuclei in the Nuclei Lysis Solution.
- A salt out precipitation step using the Protein Precipitation Solution then removed the cellular proteins.
- The genomic DNA was concentrated and desalted by Isopropanol precipitation.
- The genomic DNA was rehydrated using the DNA Rehydration Solution.

The Estimation of DNA Concentration and Purity:
The DNA concentration of samples was estimated by using the Nano drop by putting 2.5µl of the extracted DNA in the machine to detect concentration in ng/µL and the purity detected by noticing the ratio of optical density (OD) 260/280 nm to detect the contamination of samples with protein. The accepted 260/280 ration for purifying DNA was between 1.7-1.9 (Sambrook and Russell, 2001).

Electrophoresis of Agarose Gel:
Agarose gel electrophoresis was embraced to affirm the nearness and uprightness of the separated DNA after genomic DNA extraction (Sambrook and Maniatis, 1989 and Al-Marzoqi et al., 2018). Gel Electrophoresis Reagents:

- Powder of Agarose
- TBE Buffer with 1X concentration
- Loading dye
- Ethidium Bromide
- DNA Ladder Marker

Protocol of Gel Electrophoresis:
Tris Borate EDTA Buffer preparation (1X TBE)
This solution was prepared by adding 900 ml Distill water to 100 ml 10X TBE (Promega/ Germany), forming 1 liter of ( 1x) TBE buffer (Sambrook and Russel, 2001).

Preparation of agarose gel:

a) The amount of 1 X TBE (100 ml) was taken in a beaker
b) Agarose powder (1.5 gm) was added to the buffer
c) The solution was heated to boiling using a microwave oven for 2 min.
d) Ethidium Bromide (1 μl) of (10mg/ml) was added to the agarose solution.
e) The agarose was stirred in order to be mix and avoid making bubbles.
f) The solution was left to cool down at 50 – 60 C°.

**Casting of the horizontal agarose gel:**

Subsequent to settling the brush in 1 cm a long way from one edge, the agarose game plan was filled the gel plate. The agarose was allowed to bond at room temperature for 30 minutes. The modified brush was absolutely removed and the gel plate was set in the gel tank. The tank was stacked with 1 X TBE support until it accomplished 1-2 mm over the surface of the gel.

**DNA Loading & Electrophoresis:**

DNA (3 μl) was mixed with (2 μl) loading dye. The samples loaded carefully into the individual wells of the gel, and then electrical power was turned on at 70 volt for 1 hour, afterwards the DNA moved from cathode (-) to anode (+) poles. The Ethidium Bromide stained bands in the gel were visualized using UV. Trans illuminator at 350 nm and photographed.

**Technique:**

In this study two types of PCR were used include RFLP and conventional PCR, to detect mutation genes by using nine primers as shown in the following table (1). The primers were supplied by Ligo (USA) Organization as a lyophilized result of various picomols fixations. Lyophilized preliminary was disintegrated in a free DNase/RNase water to give a final concentration of 100 pmol/μl and kept as a stock in -20ºC, to prepare 10 M concentration as work primer suspended 10 pmol/μl in 90 μl of free DNase/RNase to reach a final concentration 10 M.

**PCR protocol**

A successful PCR program depends on the reaction conditions including reagents, temperature and the prevention of contamination. Previous study indicates that PCR is sensitive to reaction condition and that the optimization of these conditions is necessary to reach the highest specificity and product yield (Williams et al., 1990).

Standard amplification conditions were applied in PCR with primer sequences. The annealing temperature in which primers hybridize to complementary sequences on the template DNA is perhaps the most critical in PCR programming.

The annealing temperature for PCR primers is based on melting temperature (Tm) calculations. Tm is the temperature at which half of DNA strands are denatured. A Tm calculation for PCR primers is based on guanine and cytosine (G+C) content. The annealing temperature is usually below the Tm in 2-12 ºC (Newton and Graham, 1997).

The primers concentration represents the optimal concentration. A decrease in the primer concentration leads to weak PCR product while an increase could result in the formation of primer dimer artifact, leading to misinterpretation of results (Saiki et al., 1988). The PCR based techniques do not require highly purified DNA preparations as it works well with partially purified DNA samples (Edward et al., 1991 and McPherson and Moller, 2001). However,
DNA extraction may contain inhibitory compounds, like detergents used in cell lysis and protein denaturation in addition to other inhibitory compounds that could interfere with PCR leading to a reaction failure (McPherson and Moller, 2001). A master premix of Bioneer was used, with components in table (1).

### Table (1) Sequence of primers

| Gene          | Primer                                      | bp. | Reference          |
|--------------|---------------------------------------------|-----|--------------------|
| TGF-β1 T29C  | Forward1 5-CTCCGGGTGCAGGCAGCATGCTGCT-3       |     |                    |
|              | Forward2 5-CTCCGGGTGCAGGCAGCATGCC-3         | 346 | (Roba et al., 2013) |
|              | Reverse 5-GTTGTGGGTTCACATTCAG-3             |     |                    |

Detection of TGF-β1 T29C; was genotyped by single stranded polymorphism-polymerase chain reaction (SSP-PCR) (Welsh and Bunce, 1999). The primer sets manufactured by Ligo, USA. The primer sequences of TGF-β1 T29C:

- 5-CTCCGGGTGCAGGCAGCATGCTGCT-3
- 5-CTCCGGGTGCAGGCAGCATGCC-3
- 5-GTTGTGGGTTCACATTCAG-3

The gradient condition for TGF-β1 T29C are similar as shown in the following table (2). The PCR reaction mixture for gradient consisted of 5 µl template DNA, 5 µl master mix, 5 µl of each forward and reverse primer in 20 µl of total reaction volume.

### Table (2) Gradient condition for TGF-β1 T29C

| Step                | Temperature C° | Time/min. | Cycles |
|---------------------|----------------|-----------|--------|
| Initial denaturation| 95             | 5         | 1      |
| Denaturation         | 95             | 0.45      | 35     |
| Annealing Zones      | 49-51,53-55-57-59 | 0.45    | 35     |
| Extension            | 72             | 1         | 35     |
| Final extension      | 72             | 5         | 1      |
| Storage              | 4              | ∞         |        |

After the determination of optimum annealing temperature for TGF-β1 T29C and 26 genes by selecting the clearest band, which is 51 C°, PCR mixture was 5 µl DNA, 5 µl master mix, 5 µl forward and reverse primer, PCR conditions were performed as in the following table (3). PCR items were dissected on 2% agarose gel recolored with 1 µg/ml ethidium bromide. The product was 346 bp.

### Table (3) PCR condition for TGF-β1 T29C

| Step                | Temperature C° | Time/min. | Cycles |
|---------------------|----------------|-----------|--------|
| Initial denaturation| 95             | 5         | 1      |
| Denaturation         | 95             | 0.45      |        |
| Annealing            | 51             | 45 Sec.   | 35     |
| Extension            | 72             | 1         |        |
| Final extension      | 72             | 5         | 1      |
| Storage              | 4              | ∞         |        |

3. **Results & Discussion**

TGF-β1 T29C genetic polymorphism related to hepatitis B and C virus infection; in table (4)
which describe the Genotype frequency of polymorphisms of (TGF) gene in Hepatitis B, C and Control, it was revealed that TC allele was higher than others 56.10% in control, 54.26% in HBV and 55.96% in HCV respectively. Results of Allele frequency showed that T allele was higher than C (69.51% in control, 57.98% HBV and 61.01% HCV).

Table(4) Genotype frequency of polymorphisms of (TGF) gene in Hepatitis B, C and Control

| Genotype | Control (82) | Hepatitis B (94) | Hepatitis C (109) | P value |
|----------|--------------|------------------|------------------|---------|
|          | No | %         | No | %         | No | %         |        |
| TT       | 34 | 41.46     | 29 | 30.85     | 36 | 33.03     | 0.674  |
| TC       | 46 | 56.10     | 51 | 54.26     | 61 | 55.96     | 0.330  |
| CC       | 2  | 2.44      | 14 | 14.89     | 12 | 11.01     | 0.012  |
| TOTAL    | 82 | 100.00    | 94 | 100.00    | 109| 100.00    |        |

| Allele frequency | T | 114 | 69.51 | 109 | 57.98 | 133 | 61.01 | 0.259 |
|                 | C | 50  | 30.49 | 79  | 42.02 | 85  | 38.99 | 0.007 |

Changing development factor-β1 (TGF-β1) is a multi-practical cytokine that controls cell development, multiplication, and separation (Massague, 2008 and Al-Dahmoshiet al., 2018). It's created by a couple of cell composes, as well as monocytes, macrophages, epithelium cells, and tube-shaped structure sleek somatic cell (Border and Noble, 1994 and Liet al., 1999). And it's to boot created from an assortment of liver cells nevertheless platelets and penetrating mononucleate cells (Bedossaand Paradis, 1995 and Muraadet al., 2018). TGF-β1 is enter atom in numerous physiological procedures in the liver since it initiates apoptosis and diminishes hepatocytes expansion other than its fundamental part in hepatic fibrogenesis. Host hereditary components assume a basic part in creating fibrosis though numerous qualities are accounted for to be related with liver fibrosis and cirrhosis including TGF-β1 (Gewaltiget al., 2002 and Khamiset al., 2018). Moreover, TGF-β1 has potential effect on the safe reaction since it effectsly affects its inhibitory impact on T-cells multiplication by means of IL-2 down-direction (Kehrl et al., 1986). TGF-β1 quality is situated on chromosome 19q13.1– 13.3 with seven exons and six introns (Deryncket al., 1987). In the pathological process of liver pathology, changing development factor-β1 (TGF-β1) is accepted to be the most strong ace cytokine that advances hepatic fibrosis by fortifying the combination and restraint of debasement of an expansive range of extracellular grid proteins (Friedman, 2000 and Gressneret al., 2002). On these lines, components expanding the part of naturally dynamic TGF-β1 are conceivably associated with the tweak of movement of liver fibrosis. In accordance therewith theory, it has been accounted for that TGF-β1 detachment RNA is expanded in the liver of patients with interminable HCV disease (Castillaet al., 1991 and Fadhil et al., 2017).

Figure (1): The Electrophoresis Pattern of TGF-β1 (T29C) Polymorphisms. L lane contain the 100 bp DNA Ladder, 5 % NuSieve® 3:1 agarose gel in 1X TBE buffer containing 0.5µl ethidium bromide. (2, 3, 4, 5 and 6) Lanes positive results for CC genotype with 364 bp (9, 10, 11, 12 and 13) Lanes positive results for TC genotype with 364 bp (1 and 7) Lane was control negative of PCR product
Moreover, these patients had a larger amount of TGF-β1, both absolutely and organically dynamic structures contrasted with sound controls recommending that TGF-β1 undoubtedly advances hepatic fibrogenesis (Nelson et al., 1997). Additionally, corresponding work from numerous research facilities exhibited that abnormal articulation of TGF-β prompts hepatic fibrosis and aggravation in various test models. Especially, transgenic mice over communicating TGF-β1 are inclined to build up different tissue sores including hepatic fibrosis (Ueberham et al., 2003 and Hassan et al., 2016). A few polymorphisms in both coding and non-coding districts of the TGF-β1 quality have been accounted for and found to influence TGF-β1 protein articulation. there’s AN utilitarian single ester polymorphism (SNP) at the twenty ninth ester (T29C), 868 nongovernmental organization in relevancy the interpretation begin website, (rs1982073 converged into rs1800470) in deoxyribonucleic acid one with progress from T to C conveyance concerning amino amendment within the district coding the flag arrangement from essential amino acid to amino alkanoic acid at the tenth amino corrosive (Watanabe et al., 2002). This change upsets the structure (Cambien et al., 1996) and brings about expanded levels of TGF-β1 protein and mRNA in people with C allele with a 2.8-overlay increment in TGF-β1 emission contrasted and T allele in vitro (Dunning et al., 2003 and Kirshner et al., 2006). Moreover, the substitutions of amino corrosive deposit might influence the capability of the flag amide, perhaps by impacting intracellular trafficking or fare proficiency of the TGF-β1 protein (Dunning et al., 2003 and Al-Marzoqi et al., 2012). It was likewise revealed that C allele of 29T/C is related with expanded TGF-β1 serum levels, along these lines the T29C polymorphism possibly impact the improvement and seriousness of TGF-β1-related infections and it has been related with weakness to a few sicknesses (Dunning et al., 2003 and March and and Haiman, 2004). During this manner, this starter think about was custom-made to examine the part of TGF-β1 quality (T29C) in HBV contamination in Egyptians. No such examination has been directed to explore the relationship between SNP in TGF-β1 quality (T29C) and HBV disease in Egypt. Genotyping of TGF-β1 T29C demonstrated a huge abatement (P&lt; 0.01) within the conveyance of TT genotype in controls in distinction with HBV patients. Whereas CC genotype wasn't recognized within the management gathering whereas it showed up within the patient gathering with A level of fifteen.4%. If truth be told, the return of TC genotype was unimportantly extraordinary in typical controls contrasted and HBV patients. In 104 solid management subjects and in ninety patients with HBV contamination taken from China the hereditary polymorphisms weren't watched. Curiously, forty of 260 Caucasian subjects were watched its polymorphisms. The frequencies of TT, TC, and CC genotypes within the Chinese people among 194 subjects were fifty (25.8%), 87 (44.8%) and fifty seven (29.4%), on an individual basis. within the gathering of 260 Caucasians the watched esteems were 106 (40.8%), 127 (48.8%) and twenty seven (10.4%), once more showing that the 2 populaces square measure distinctive for these ester substitutions. In rundown, these outcomes affirm the theory that TGF-β1 polymorphisms are related with fibrosis movement in Caucasians incessantly tainted with hepatitis C (Haet al., 2005) which concurred with our outcomes.

4. Acknowledgment
Authors thank Hospital Staff of MERJAN for their help in specimen’s collection. This study was supported by Faculty of Science for women and Department of biology at Babylon University.

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