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Molecular Detection of MTHFR gene Polymorphisms in Transitional Urinary Bladder Cancer Patients

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Abstract

The results of the present study showed MTHFR A1298C & C677T polymorphisms was found to be one of the important tumor inhibitors, These two groups were found to be associated with the risk of bladder cancer. Blood samples were collected from 60 patients with bladder cancer from the center of the Imam Hussein for cancer tumors and blood diseases in Al Hussein educational hospital in the holy of Karbala. DNA was extracted from blood samples. The presence of polymorphisms A1298C & C677T in the MTHFR gene was detected by polymerase chain reaction PCR and PCR- RFLP. MTHFR A1298C, the AC genotype was the highest in the patients and control groups 34 (756%) and 22 (55%), while the CC genotype was the least visible among patients and control groups 2% (3.3%) and 1 (2.5%), while the genotype (AA) appeared in patients more than control group 24% (40%) And 17 (42.5%) in the control groups. The results of the study also showed the effect of MTHFR C677T polymorphisms on the disease. In the patient group, the genotype (CT) was found in patients and control groups 32 (53.3%) and 20 (50%) while the genetic model CC in patients was the highest 27 (35%) compared to the control groups, while genotype (TT) in the control groups 6 (15%), while in patients 1 (1.66%).

Key words: Transitional Carcinomas Cell (TCC), MTHFR A1298C& C677T Polymorphism , PCR,PCR-RFLP,SNPs.

INTRODUCTION

Bladder cancer is one of the most common types in the world and is associated with infection with many deaths in many countries of the world, which is the most common type of infection in Men Compared with the Women (1). The bladder cancer is the second largest number of cancers of the urinary system (2) .Bladder cancer was second in males after lung cancer in the rate of (9.26%). In women, it was the second most common cancer in Iraq (2.72%). The rate of women was low among the common cancers in Iraq by (2.72%), where the number of infected (1163) and the number of infected males (866) and the number of infected women (297) (3). Three types of bladder cancer are transitional Carcinomas Cell TCC, the most common type of cancer in the world, with more than 90% of the types of bladder tumors (4) and Squamous cell carcinoma (SCC) is less common than type 1 and Adenocarcinoma This type of cancer accounts for 2% of all bladder tumors (5). MTHFR plays an important role in the development of bladder cancer, where the folate gene, which causes deficiency, impedes the repair and construction of DNA and thus increases the risk of cancer. This gene has two polymorphisms (A1298C, C677T), which play a major role in bladder cancer. This gene encodes the methyltetenetrahydrofolateductase, which converts 5,10-methyletetrahydrofolate to 5-methyltetrahydrofolate, which is the common form of foliate in the body. 5-methyltetrahydrofolate deficiency is the basis for homocysteine and methionine replication, which helps in homocysteine metabolism as well as methionine Methyline and methionine metabolites play an important role in building and mimicking DNA, MTHFR and methionine synthase (MS) are important enzymes involved in foliate metabolism, and the polymorphism of this gene leads to a lack of familiarity with the methylation, which causes the accumulation of metabolic products, Therefore, any defect in the methylate results in mutations in the MTHFR gene and the disruption of essential functions in the metabolic pathway such as DNA building and repair, neurotransmitter construction, gene regulation, protein function, antioxidant formation, heavy metal toxicity, immune activation, homocysteine regulation, hormone stimulation, and homocysteine synthesis and low methionine (6).

Objective of the study: Study the effect of the presence of individual (SNPs) in the MTHFR gene polymorphism, and its role in bladder cancer.

MATERIALS AND METHODS

The study samples were collected from the patients of the Imam Hussein Center for Cancer and Hematology at Al Hussein Educational Hospital in the holy of Karbala. The study consisted of (100) samples. The samples were divided into (60) patients after their clinical diagnosis in addition to (40) individuals as control group and for the period from March 1 to August 30 of 2016. The primers used in MTHFR A1298C F:5′-AAGGAGGAGCTGCTGAAGATG-3′ R: 5′-CTTTGCCATGTCCACAGCATG-3′,while the primers used in MTHFR C677TF:5′-TGAAGGAGAAGGTGTCTGCGGGA-3′ R:5′- AGGACGGTGCGGTGAGAGTG-3′ (7).

The cycling conditions were as the following:

Table 1 shows the program used in PCR Technique for the molecular detection of the formal polymorphisms A1298C and C677T in the MTHFR gene.

Steps	Temperature	Time	No. of cycles
Initial Denaturation	95C°	5min.	1
Denaturation	95C°	30sec.	35
Annealing	63 C°	30sec.	
Extension	72C°	1 min.	
Final Extension	72C°	5 min.	1
Final hold	4	-	

The amplified MTHFR gene PCR products underwent restriction enzyme digestion for 60 min at 37 °C. They were separated using 2% agarose gel electrophoresis and visualized with UV light. Amplification of the C677T region produced a PCR product of 198 bp, which was then digested with the restriction enzyme Hinf I (TaKaRa, Inc.) to produce either two bands of 175 and 23 bp (677TT) or one single undigested band of 198 bp (677CC). Amplification of the A1298C region produced a PCR product of 237 bp, which was then digested with the restriction enzyme MboII (TaKaRa, Inc.) to produce either three bands of 182, 28 and 27 bp (1298AA) or two bands of 210 and 27 bp (1298CC).

Molecular Detection Using Determination of Sequencing DNA Sequencing DNA Methods

After the PCR test, Molecular detection is carried out by DNA Sequencing. The samples were sent to Macrogen Inc., Geumchen, Seoul, South Korea, which specializes in sequence sequencing analysis For those samples. The PCR reaction products of the multiforme MTHFR gene (A1298C, C677T polymorphism).

The results of the sequences were compared with those of the DNA sequences mentioned previously and recorded globally, where the reference database (GenBank acc. AB082923.1, NC_000001.11, and NM_001126118.1) was extracted from the Genebank website (https: (www.ncbi.nlm.nih.gov), the products of the sequence of PCR sequence samples were analyzed, revised, lined up and analyzed alongside the NCBI samples by the BioEdit Sequence Alignment Editor Software Version 7.1 (DNASTAR, Madison, WI, USA).

RESULTS AND DISCUSSION

Molecular detection by PCR

The results of the molecular detection in Fig. 1 showing electrolysis of PCR products in the MTHFR polymorphism (A1298C, C677T) gene, were shown on the 2% agarose at 70 V for two hours. The first column represents the size marker 100-2000bp. Where the two columns (2,3) represent the MTHFR C677T polymorphism (198bp), while the columns (4,5) represent the MTHFR A1298C polymorphism (237 bp)

Vitamin B12 is a powerful antioxidant and taking high-volume foliate and B vitamins (B12, B6 and B2) reduces the risk of bladder cancer(8). MTHFR is the major enzyme in foliate metabolism, plays an important role in the foliate pathway (9), the results of studies(10) showed the PCR amplified amplitudes using the special polymorphisms in the polymorphism (A1298C) of the MTHFR gene, the size of these packets was 237bp, and the other C677T MTHFR size of 198bp after migration either on agarose. These results are consistent with current study results showing forms (A1298C, C677T) in the gene MTHFR the same size products in the two studies mentioned package.

Molecular detection with RFLP technology

The molecular diagnosis was performed using the RFLP technique to identify the genetic diversity in the MTHFRA 1298C and C677T polymorphism gene. Figure 2 shows the distribution of the genetic models in the MTHFRA 1298C polymorphism. The first column represents the size marker of the size(25-2000bp) The columns (2,4,5,6,7) represent the heterozygote (AC) genotype (182&201bp), while Column 3 shows the homozygote (AA) genotype (210 bp), Homozygote (CC) genotype is shown in the column 8 (182 bp). by MboII enzyme was used to digest PCR products and the products were electrophoresed on 2% agarose gel.

Figure 3 shows the electrophosis of the MTHFR C77T on agarose gel at 2% at 70 V for 2 hours, where the the PCR product of this gene is cut off by Hinf I. the first column represents the size marker, 25-2000bp. The columns (2,5,6,7) represent the heterozygote CT genotype (198&175 bp), and the homozygotes (CC) genotype shown in the columns (3,8,9,10) 198 bp, and column (3) represent the homezygote (TT) genotype (175 bp).

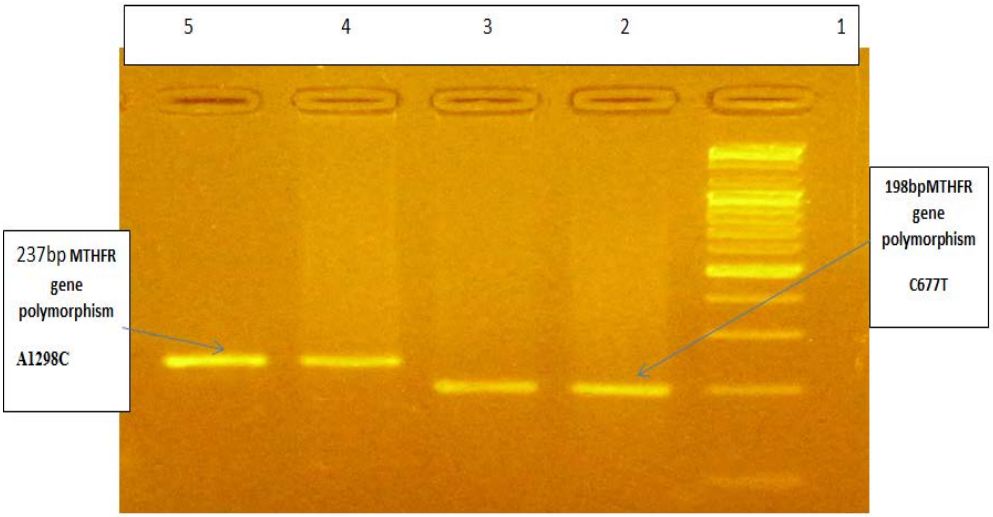

Fig. 1 : PCR amplifiction of MTHFR polymorphism (A1298C, C677T) gene sample(1) is size marker (100-2000bp),while the columns 2,3, is MTHFR gene polymorphism C677T (198bp), while the columns 4,5 is MTHFR gene polymorphism A1298C (237bp).

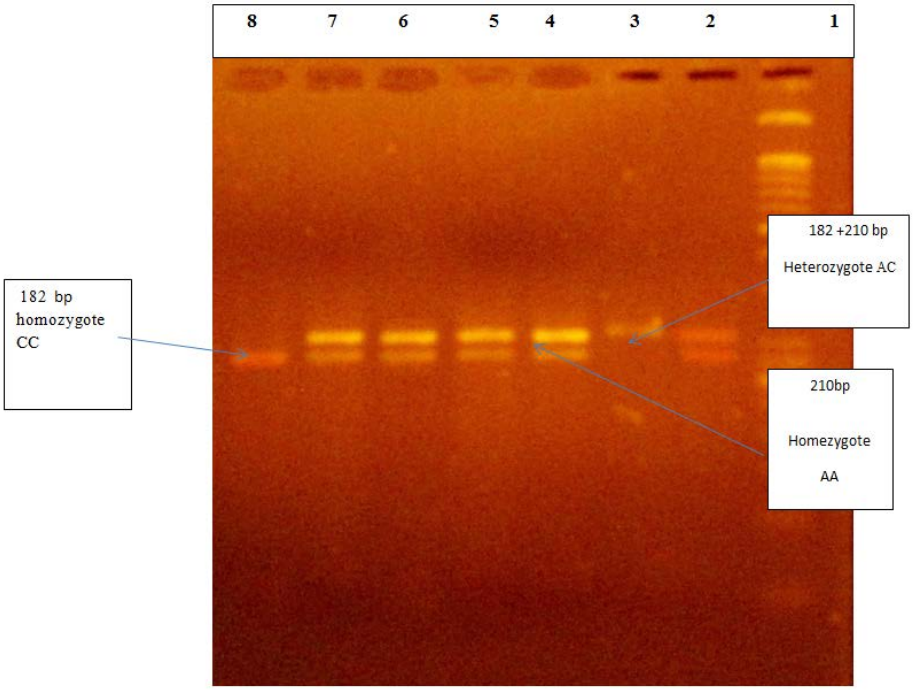

Figure 2 shows the analysis of the PCR product of the MTHFR A1298C gene using MboII enzyme, enzyme was used to digest PCR products and the products were electrophoresed on 2% agarosegel where The first column represents the size marker of the size(25-2000bp),while the columns(2,4,5,6,7) represent the heterozygote (AC) genotype (182&201bp),the column 3 represent the homozygote (AA) genotype (210 bp),while the column 8 (182 bp) is Homozygote (CC) genotype.

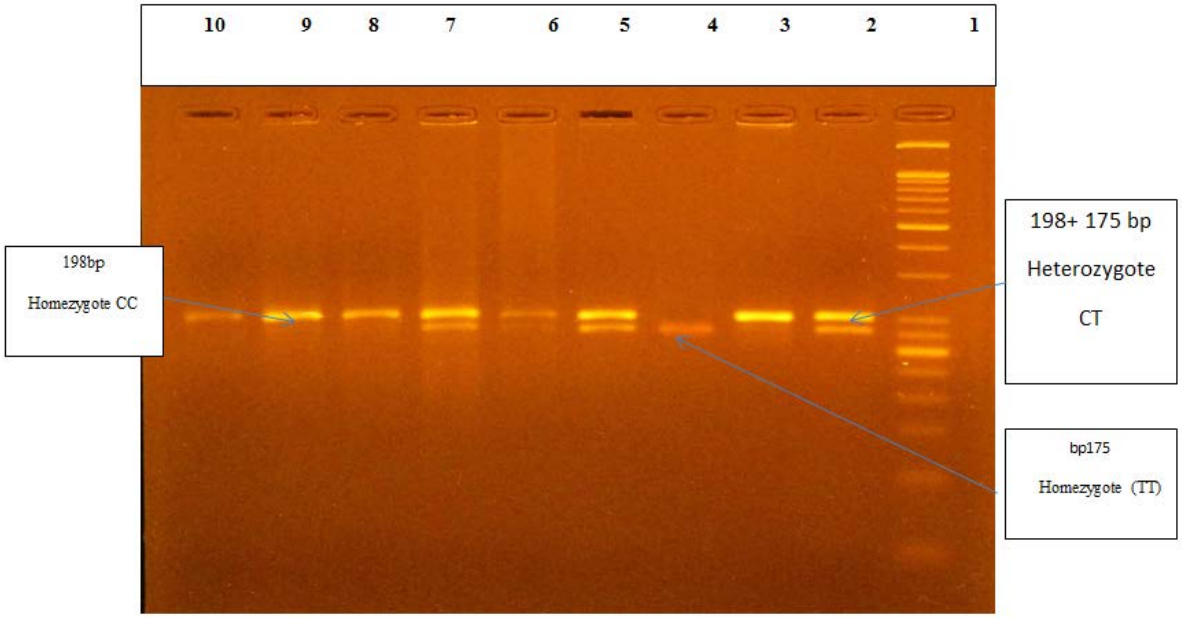

Figure (3) shows the analysis of the PCR product of the MTHFR C677T gene using the Hinf I enzyme was used to digest PCR products and the products were electrophoresed on 2% agarosegel . the first column represents the size marker, 25-2000bp,while the columns (2,5,6,7) represent the heterozygote CT genotype (198&175 bp). The homozygotes (CC) genotype shown in the columns (3,8,9,10) (198 bp), and column (3) represent the homezygote (TT) genotype (175 bp).

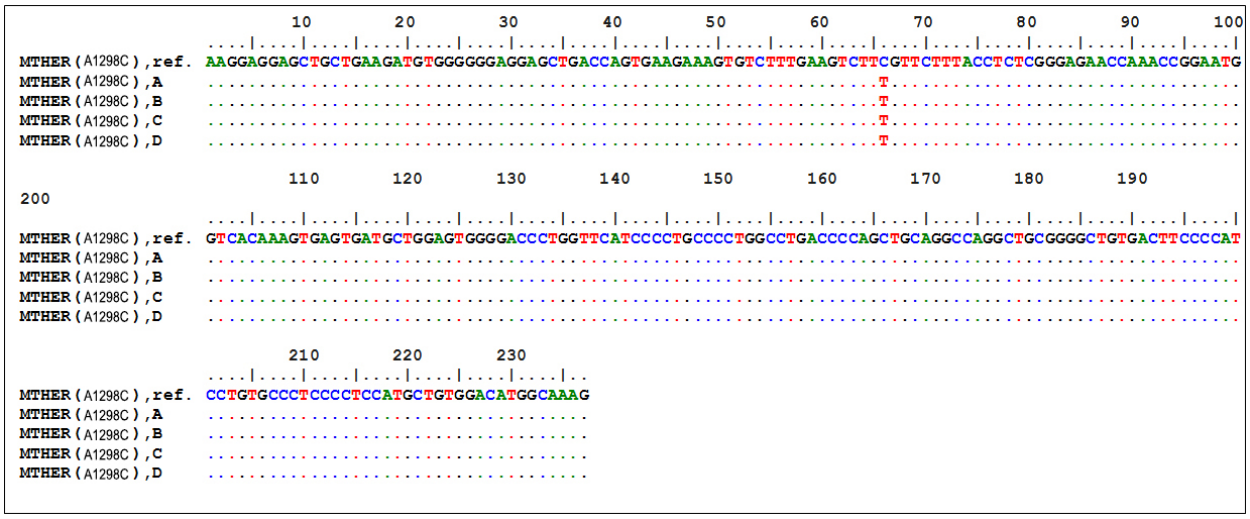

Figure (4) The sequence of multiple MTHFR A1298C reference sequences with the four samples A,B,C,D The results of the current study shown in Table 1 show that the new SNPs detected in the reference MTHFR A1298C sequences are non-functional, with no change in the resulting amino acids. When compared with a gene bank based on the site review (https://www.ncbi.nlm.nih.gov/projects/SNP/snp_ref.cgi?geneId=7157).

The MTHFR gene contains two common forms: C677T and A1298C. A study(11) indicated that these two polymorphisms are associated with bladder cancer. A study(12) showed that the polymorphism C677T is not associated with bladder cancer, while (11) MTHFR C677T polymorphism is one of the risk factors associated with bladder cancer, and the study showed that the genotype CT in polymorphism C677T is the most common in Asians of Europe and Africa and indicated the appearance of the other two genotypes with different percentages. When a study (13) indicated that the two gene models (MTHFR 677CT, TT) War protective against cancer,the results of the present study show a distribution of the AA, AC, and CC genes in polymorphism A1298C after digestion of the PCR product by the MboII, In this polymrophism to two packages or one package to show the three different genotypes and heterozygous pairing and these results correspond to the results(14).

Molecular Detection Using Determination of Sequencing DNA Sequencing

MTHFR A1298C polymorphism

Figure 4 shows the sequence of multiple sequences of the MTHFR A1298C reference gene with all four selected samples where samples A, B and C samples of bladder cancer patients and sample D represented the control sample. The results were analyzed using the Bio Edit Sequence Alignment Editor Software, the analysis result showed the presence of one SNP (C66T) in the four samples

Mutations in the multilevel MTHFR gene C677T and A1298C cause a decrease in the level of folate and an increase in homocysteine, which causes many diseases (15). Mutations in the MTHFR gene are genetic changes affecting the co-enzyme in homocysteine breakdown, raising homocysteine levels in blood or blood, and 33% of Americans having common mutations present in a single copy of the multilevel MTHFR gene C677T. Mutations in the C677T and A1298C are not found in the control group. Two copies of the polymorphism A1298C do not affect the risk of bladder cancer in the control group and that about 11% of Americans have mutations in the two versions of DNA with polymorphism C677T(16) and that these mutations contribute to the occurrence of heart disease . And that these mutations are inherited either in one copy of this gene or in two copies.

MTHFR C677T polymorphism

Figure (5) shows the results of the multithreaded MTHFR C677T gene sequencing study with all four samples where samples (C, B, A) showed samples of bladder cancer patients. Sample D was the control sample. The results were analyzed using BioEdit Sequence Alignment Editor Software, and the results of the analysis did not show any SNP in all samples examined compared to the reference sequence

Table 2 shows the (SNPs) detected in MTHFR A1298C polymorphsm sequences

No.	Native	Allele	A variant	B variant	C variant	D variant	Position in the PCR fragment	Position in the reference genome	Amino acid change
1	C	T	YES	YES	YES	YES	66	11794343	NO CHANGE

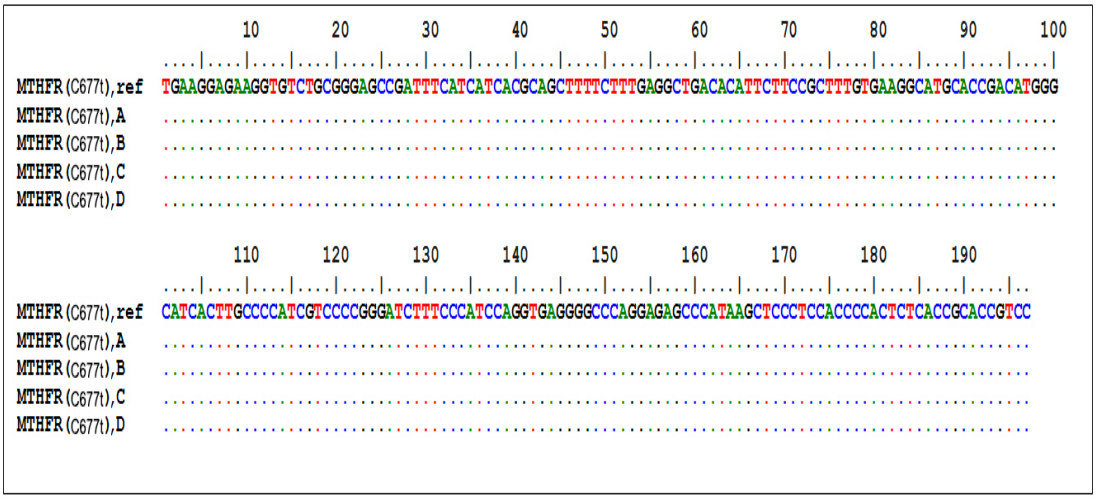

Figure (5) represents the sequence of multiple sequences of MTHFR C677T reference gene with all four samples A,B,C,D

methylenetetrahydrofolate reductase isoform 2 (MTHFR) [Homo sapiens]

MVNEARGNSSLNPCLEGSASSGSESSKDSSRCSTPGLDPERHERLREKMRRRLESGDKWFSLEFFPPRTAEGAV
NLISRFDRMAAGGPLYIDVTWHPAGDPGSDKETSSMMIASTAVNYCGLETILHMTCCRQRLEEITGHLHKAKQ
LGLKNIMALRGDPIGDQWEEEEGGFNYAVDLVKHIRSEFGDYFDICVAGYPKGHPEAGSFEADLKHLKEKVS
AGADFIITQLFFEADTFFRFVKACTDMGITCPIVPGIFPIQGYHSLRQLVKLSKLEVPQEIKDVIEPIKDNDAAIRN
YGIELAVSLCQELLASGLVPGLHFYTLNREMATTEVLKRLGMWTEDPRRPLPWALSAHPKRREEDVRPIFWA
SRPKSYIYRTQEWDEFPNGRWGNSSSPAFGELKDYYLFYLKSKSPKEELLKMWGEELTSEESVFEVFVLYLSG
EPNRNGHKVTCLPWNDEPLAAETSLLKEELLRVNRQGILTINSQPNINGKPSSDPIVGWGPSGGYVFQKAYLEF
FTSRETAEALLQVLKKYELRVNYHLVNVKGENITNAPELQPNAVTWGIFPGREIIQPTVVDPVSFMFWKDEAF

Figure (6) Sequence of complete amino acids for the sequence of the protein produced by the reference MTHFR gene

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