Role of MTHFR C677T and MTRR A66G genes polymorphism with thyroid disorders

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Abstract. Thyroid disorders is the most common disease of the endocrine system. It is estimated that there are approximately 300,000 new cases of thyroid cancer worldwide. Overcome of these are females, the number of cases for thyroid disorders has been influx increasing. The aim of this study was to determine the correlation between MTHFR C677T and MTR A2756G polymorphisms involved in folic acid metabolism with thyroid disorders. We also wanted to investigate the relationship between these genetic variation and risk factors. The blood samples were collected from all cases in two different tubes: first for biochemical analysis, the second have EDTA used for DNA extraction. By Bio drop, DNA concentration and purity were measuring done, In this study be accomplished the correlation between the genetic variation of MTRR A66G gene and MTHFR C677T gene polymorphism with thyroid disorders by ARMS-PCR. In this study show increase the levels of T3 and T4 and decrease the level of TSH in patients with hyperthyroidism compare with control groups, also this table show decrease the levels of T3 and T4 but increase the level of TSH in patients with hypothyroidism compare with healthy peoples. As well as the result showed increase in levels of glucose, cholesterol and triglyceride in patients with hypothyroidism compare with healthy people. Concerning in hyperthyroidism we showed decrease in levels of glucose, cholesterol and triglyceride compare with healthy people. The association between MTHFR C677T and MTRR A66G polymorphisms and the risk of thyroid disorders showed. The 677TT genotype was associated with increased risk for both hypothyroidism (30 %) and hyperthyroidism (10 %) but in different ratio. The 677TC genotype be found in this study have same influence in hypothyroidism (60 %) and hyperthyroidism (60 %) and the 677CC genotype present in result but have different effect on study groups in hypothyroidism (10 %) and hyperthyroidism (30 %). The result also show the genotype ratio of MTRR A66G gene in cases. The GG genotype was associated with thyroid disorders for both hypothyroidism (60 %) and hyperthyroidism (40 %) but in different ratio. The AG genotype be found in this study have same influence in hypothyroidism (20 %) and hyperthyroidism (20 %) and the AA genotype present in result but have different effect on study groups in hypothyroidism (20 %) and hyperthyroidism (40 %). The study has found that the correlation and interaction between MTHFR and MTRR genes polymorphism with thyroid disorders. In addition, we found the genotype (TT) MTHFR C677T polymorphism and the genotype (GG) for MTRR A66G increased risk factors in thyroid disorders.

Key words. Thyroid disorders, ARMS-PCR, Gene polymorphism, Methylation, MTHFR and MTRR genes.
1. Introduction
Thyroid disorder is one of the most common diseases of the endocrine system. It is estimated that there are probably 300,000 new cases of thyroid cancer worldwide [1]. Overcome of these are females, the number of cases for thyroid disorder have been infix increasing [2]. There are numerous risk factors like hormones, family history of disease, smoking and alcohol consumption, obesity, poor diet in folic acid, and genetic variations all play important role to the development of thyroid disorders [3].

Research that examines a single nucleotide polymorphisms (SNPs) in folic metabolism have been done in many type of disease [4]. The research in thyroid disorders research, have poorly study in the folic acid pathways [5]. The genomic instability through DNA synthesis, methylation, and alterations to repair mechanisms all of these caused by low folic acid levels, therefore, the decrease level of folic acid can stimulate carcinogenesis [6]. The folic acid metabolism regulated by many enzyme like methylenetetrahydrofolate reductase (MTHFR) and methionine synthase (MTR) [7].

The MTHFR gene encoded MTHFR enzyme, these enzyme convert 5,10-methylenetetrahydrofolate to 5-methyltetrahydrofolate in folic acid pathway, that have a great role in DNA methylation, an important factor gene expression regulation, there for many cancer development associated with Alterations in DNA methylation due to genetic variation in the MTHFR gene [8, 9].

The MTR gene encoded the MTR enzyme and this enzyme convert the homocysteine remethylation to form methionine using vitamin B12 as cofactor and the increase level of homocysteine in the serum its caused by the mutation occur in MTR gene, accordingly result changes in the folic acid metabolism, and induced many disease like thyroid disorders [10]. Depending on many studies, the genetic variation on MTHFR C677T and MTR A2756G are able to change folic acid metabolism, which is important for DNA synthesis and methylation, as well as genomic stability [9, 11, 12].

The aim of these study was to determine the correlation between MTHFR C677T and MTR A66G polymorphisms involved in folic acid metabolism with thyroid disorders. It also wanted to investigate the relationship between the genotype with risk factors.

2. Materials and Methods

2.1. Samples
A total of 80 persons were evaluated in this study; 60 patients (30 with hyperthyroidism and 30 with hypothyroidism) and 20 healthy people without history of thyroid disorders were recruited between September 2018 and January 2019, and all cases have same age groups (35-55) years.

2.2. Blood collection
Peripheral blood samples have been gathered from the subjects utilizing EDTA (ethylenediamine tetraacetic acid)-containing tubes for DNA extraction, and used tube without EDTA for biochemical test.

2.3. DNA Extraction
The DNA was extracted from blood for all cases [13]. And measuring the concentration and purity of DNA by (Bio drop), and validate the DNA concentration on 25 ng/μic for ARMS-PCR.

2.4. Biochemical Test
Biochemical tests just as cholesterol, triglyceride, GGt, GOT, urea, uric acid and creatinine have been implemented by employing the Reflatrone, and T3, T4 and TSH measuring by veda lab regarding the way intended by the manufacturers.

2.5. Genotyping
MTHRF C677T gene polymorphism by ARMS-PCR:
100 ng of DNA was amplified using Gene Amp PCR system, The primers used are as follows: forward primer: 5-TGC TGT TGG AAG GTG CAA GAT-3, Revers wild primer : 5-GCG TGA TGA
TGA AAT CGG-3’ with PCR product 226-bp and Revers mutant primer : 5-GCG TGA TGA AAT CGA-3 PCR product 226-bp. PCR was carried out in a final volume of 20 μl, with master mix from biolaps [8]. And the PCR conditions used are as follows: Initial denaturation 95 C° for 5 min, 35 cycle include denaturation 95 C° for 45 second, Annealing 61 C° for 1 min, Extension 72 C° for 1 min. and Final extension 72 C° for 7 min [14].

MTRR A66G gene polymorphism by Tetra-ARMS-PCR:

For the MTRR A66G gene polymorphism 100 ng of DNA was amplified using Gene Amp PCR system and were used tetra-primer Amplification Refractory Mutation System-PCR (ARMS-PCR). And for each allele of MTRR A66G were used two PCR reaction. The primers used are as follows: forward wild type (5’-TCAAGCCCAAGTAGTTTCGAG– 3’) and reverse wild type (5’– TGTACCACAGCTTGCTACAT– 3’) with PCR product 367-bp wild-type allele (A). and for second PCR reaction were used primers as follows : forward mutant type (5’– TGTGCCTACAGGGTTGCACT–3’) and reverse mutant type (5’– TGTACCACAGCTTGCTACAC– 3’) with PCR product 401-bp mutant allele (G). [12] and the PCR conditions used are as follows: Initial denaturation 94 C° for 3 min, 35 cycle include denaturation 94 C° for 30 Sec, Annealing 60 C° for 30 second, Extension 72 C° for 30 Sec. and Final extension 72 C° for 5 min. [12].

3. Results and Discussions

In this study be accomplished the correlation between the genetic variation of MTRR A66G gene and MTHRF C677T gene polymorphism with thyroid disorders, Table (1) show increase the levels of T3 (4.46 ± 0.21) and T4 (164 ± 15) and decrease the level of TSH (0.27 ± 0.003) in patients with hyperthyroidism compare with control groups, also this table show decrease the levels of T3 (0.86 ± 0.04) and T4 (12.6 ± 2.4) but increase the level of TSH (14.1 ± 3.2) in patients with hypothyroidism compare with healthy peoples. And the causes of this different between levels of thyroid hormones in study groups due to excitement in employment of thyroid gland in hypo and hyperthyroidism [15]. In hyperthyroidism the thyroid gland cell employment to gate large amount of Iodine but in hypothyroidism the thyroid gland cells shrink and cannot able to gate adequate amount of iodine [4].

| Groups        | TSH ± SE μl/mL | T4 ± SE nmol/L | T3 ± SE ng/mL |
|---------------|---------------|---------------|---------------|
| Control       | 3.6 ± 0.12    | 98 ± 13       | 1.6 ± 0.5     |
| Hyper thyroid | 0.27 ± 0.003  | 164 ± 15      | 4.46 ± 0.21   |
| Hypo thyroid  | 14.1 ± 3.2    | 12.6 ± 2.4    | 0.86 ± 0.04   |

In table (2) the result showed increase in glucose, cholesterol and triglyceride levels in patients with hypothyroidism compare with healthy people, and the reason for this increase due to the defect of metabolic pathway of this molecules in patients with hypothyroidism and depended on amino acids as source of energy [1, 16]. Concerning in hyperthyroidism we showed decrease in levels of glucose, cholesterol and triglyceride compare with healthy people, and the reason of this decrease duo to the patients depend on glucose the main source of energy and increase in consumption lipids compare with other cases [5, 17].

| Groups   | Glucose mg/dl | Cholesterol mg/dl | Triglyceride mg/dl | Uric acid mg/dl | Urea mg/dl | Bilirubin mg/dl |
|----------|---------------|-------------------|-------------------|----------------|------------|----------------|
| Control  | 115 ± 15.2    | 125 ± 13.3        | 110 ± 11.3        | 5.4 ± 0.64     | 101 ± 10.1 | 0.7 ± 0.02     |
| Hyper thyroid | 70 ± 11.2     | 120 ± 15.3        | 50 ± 8.3          | 3 ± 0.54       | 116 ± 12.1 | 0.5 ± 0.02     |
| Hypo thyroid | 145 ± 7.4    | 265 ± 17.9        | 160 ± 6.9         | 3.5 ± 0.34     | 120 ± 11.9 | 0.6 ± 0.04     |
The relationship between MTHFR C677T and MTRR A66G polymorphisms and the risk of thyroid disorders showed in table (3) and table (5). The 677TT genotype was associated with increased risk for both hypothyroidism (30%) and hyperthyroidism (10%) but in different ratio. The 677TC genotype be found in this study have same influence in hypothyroidism (60%) and hyperthyroidism (60%) and the 677CC genotype present in result but have different effect on study groups in hypothyroidism (10%) and hyperthyroidism (30%).

In this study, we estimate the correlation between MTHFR C677T and MTR A66G genes polymorphism with hypothyroidism and hyperthyroidism. The modify enzyme activities in the folate pathway due to some genes polymorphism [18]. It complicate in the methylation of DNA, purines and pyrimidine synthesis, instability of genome and motivate to increase susceptibility to cancer growth [19].

Table 3. The genotype ratio of MTHFR C677T gene in cases.

| Genotype | Control | Hypothyroidism | Hyperthyroidism |
|----------|---------|----------------|-----------------|
|          |         |                |                 |

The mutation in MTHFR gene decrease the enzymatic activity by prevent the chancing of 5,10 methylenetetrahydrofolate to 5- methylenetetrahydrofolate, which is required for DNA methylation reaction and conceder the main form of folate for this reaction. And it is causative to cancer development [20, 21].

Figure 1. ARMS-PCR product 226 bp of wild allele of MTHRF C677T for study sample in 2% agarose gel electrophoresis

Figure 2. ARMS-PCR product 226 bp of mutant allele of MTHRF C677T for study sample in 2% agarose gel electrophoresis
| Allele                | Control (n=20) | Hypothyroidism n=30 | Hyperthyroidism n=30 | P value |
|----------------------|----------------|---------------------|----------------------|---------|
| C (normal allele)    | 34 (85 %)      | 24 (40 %)           | 36 (60 %)            | 0.01    |
| T (mutant allele)    | 6 (15 %)       | 36 (60 %)           | 24 (40 %)            | 0.01    |

Allele and genotype frequencies in cases and controls were compared using χ² test.

Regarding allelic frequencies, the mutant T allele has been significantly higher in hypothyroidism than in Hyperthyroidism (60 % vs. 40%), the opposite was noted with the wild type allele C, which has been significantly lower among in hypothyroidism and Hyperthyroidism (40% and 60%). Compare with healthy people (85%).

Figure 3. ARMS-PCR product 367 bp of wild allele of MTRR A66G (M: ladder, 1,2,3,4 and 7 have normal allele but 5,6,8,9 and 10 didn’t have normal allele) for study sample in 2% agarose gel electrophoresis.
Figure 4. ARMS-PCR product 401 bp of wild allele of MTRR A66G (M: ladder, 3, 4, 5, 6, 8 and 10 have mutant allele but 1,2,7 and 9 didn’t have mutant allele) for study sample in 2% agarose gel electrophoresis.

Table 5. The genotype ratio of MTRR A66G gene in cases.

| Genotype          | Control n=20 | Hypothyroidism n=30 | Hyperthyroidism n=30 |
|-------------------|--------------|---------------------|---------------------|
| AA (wild homozygous) | 14 (70 %)    | 6 (20 %)            | 12 (40 %)           |
| AG (heterozygous allele) | 2 (10 %)    | 6 (20 %)            | 6 (20 %)            |
| GG(mutant homozygous) | 4 (20 %)    | 18 (60 %)           | 12 (40%)            |

The result show in Table 5 the genotype ratio of MTRR A66G gene in cases. The GG genotype was associated with thyroid disorders for both hypothyroidism (60 %) and hyperthyroidism (40 %) but in different ratio. The AG genotype be found in this study have same influence in hypothyroidism (20 %) and hyperthyroidism (20 %) and the AA genotype present in result but have different effect on study groups in hypothyroidism (20 %) and hyperthyroidism (40 %).

Table 6. The allele frequency of MTRR A66G gene in cases.

| Individual allele frequency |
|-----------------------------|
| Allele | Control n=20 | Hypothyroidism n=30 | Hyperthyroidism n=30 | P value |
| A (normal allele) | 30 (75 %) | 18 (30 %) | 30 (50 %) | 0.01 |
| G (mutant allele) | 10 (25 %) | 42 (70%) | 30 (50 %) | 0.01 |

Allele and genotype frequencies in cases and controls were compared using χ2 test.

Concerning allelic frequencies, the result show in table 6 the mutant G allele has been significantly higher in hypothyroidism than in Hyperthyroidism (70 % vs. 50%) compare with healthy people the reverse was noted with the wild type allele A, which has been significantly lower among in hypothyroidism and Hyperthyroidism (30% and 50%). Compare with healthy people (75 %).

The results have showed there are association between this SNP and thyroid disorders for MTRR A66G polymorphism, some research found no association between polymorphism of MTRR A66G and thyroid disorders development (Cooper et al., 2009). However some study done in the Northeast region of Iranian population found an association of polymorphic allele (A66G) in some cancer [22]. Methionine synthase reductase (MTRR) is an enzyme that stimulation the remethylation of...
The polymorphism in MTRR gene associated with decrease level of MTRR enzyme and causes increase level of homocysteine and subsequently DNA hypomethylation [23]. The location of Human MTRR gene in chromosome 5 (5p15.2 – p15.3) [11]. MTRR enzyme have an important role in preserve cobalamin as active form and thus determine of plasma homocysteine concentration [12]. However, the A66G polymorphism in MTRR gene cause the change of isoleucine into a methionine residue and decrease the MTRR enzyme activity [24].

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