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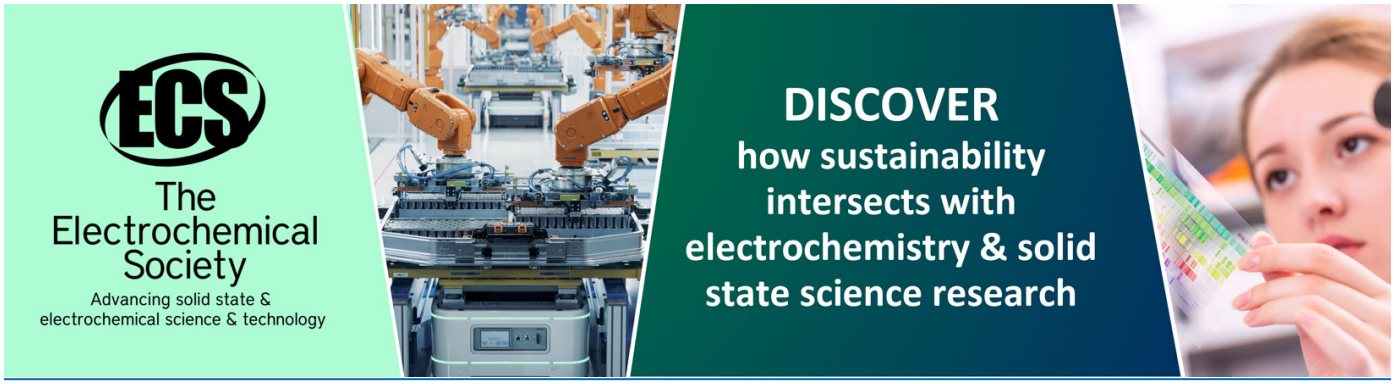

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Role of MTHFR C667T and MTRR A66G genes polymorphism with thyroid disorders

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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 infix 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 MTHRF 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.

Kew 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 createnine 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 TGA AAT CGA-3 PCR product 226-bp, PCR was carried out in a final volume of 20 µl, with master mix from bio-laps [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'– TGTACCACAGCTTGCTCACAT– 3') with PCR product 367-bp wild-type allele (A). and for second PCR reaction were used primers as follows : forward mutant type (5'– CTTGTCTACAGGGTTGCACT–3') and reverse mutant type (5'– TGTACCACAGCTTGCTCACAC– 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].

Table 1. The levels of thyroid hormones is all cases.

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].

Table 2. the levels of some biochemical parameters in all cases.

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].

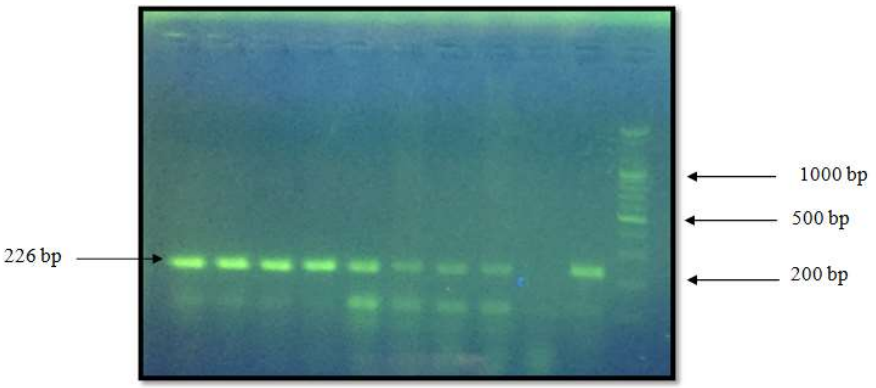

Figure 1. ARMS-PCR product 226 bp of wild allele of MTHRF C677T for study sample in 2% agarose gel electrophoresis

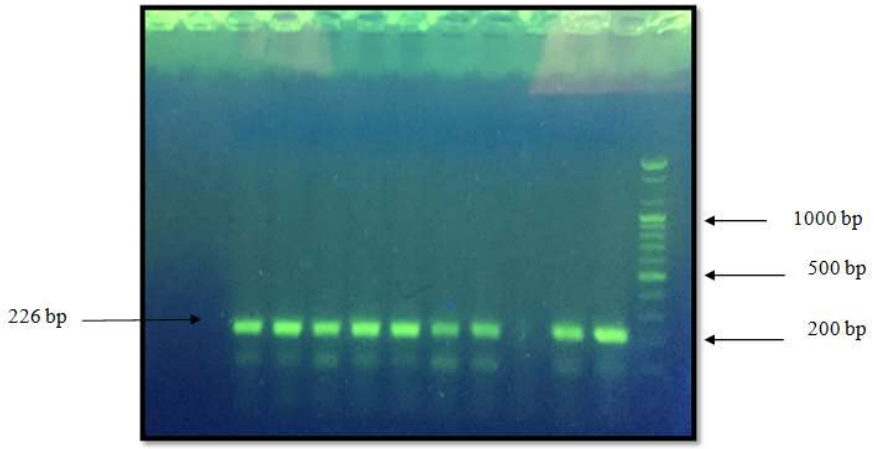

Figure 2. ARMS-PCR product 226 bp of mutant allele of MTHRF C677T for study sample in 2% agarose gel electrophoresis

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].

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

Genotype	Control	Hypothyroidism	Hyperthyroidism

	n=20	**n=30**	**n=30**
CC (wild homozygous)	16 (80 %)	3 (10 %)	9 (30 %)
CT (heterozygous allele)	2 (10 %)	18 (60 %)	18 (60 %)
TT (mutant homozygous)	2 (10 %)	9 (30 %)	3 (10%)

- n= number of cases

Table 4. The allele frequency of MTHFR C677T gene in cases.

Individual allele frequency				
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 χ^2 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 %).

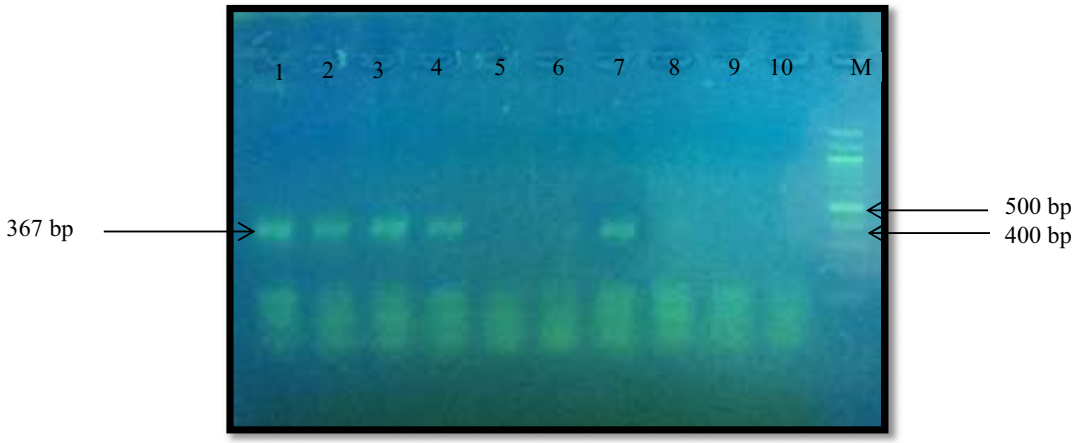

Figure 3. ARMS-PCR product 367 bp of wild allele of MTRR A66G (M: ladder, 1,2,3,4 and 7 have normal allele but 5,6,8,9 and 10 didn't have normal allele) for study sample in 2% agarose gel electrophoresis.

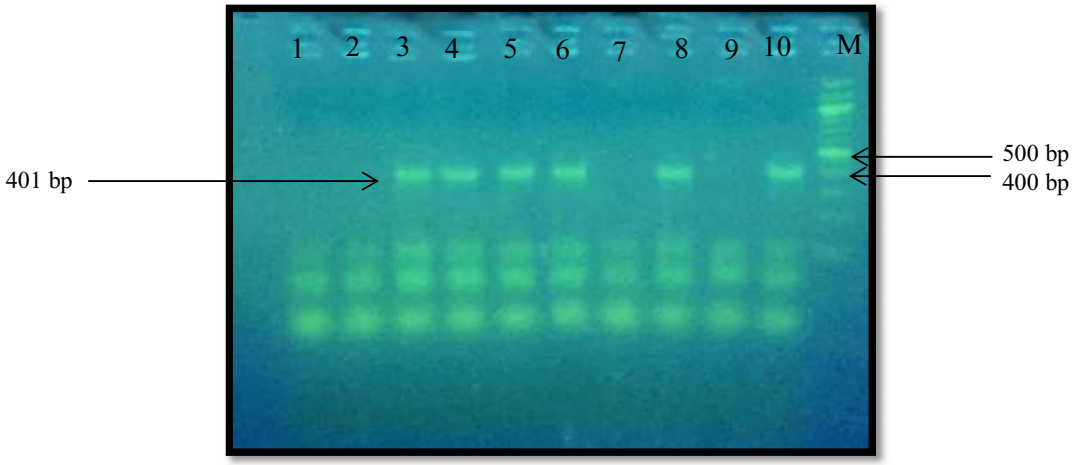

Figure 4. ARMS-PCR product 401 bp of wild allele of MTRR A66G (M: ladder, 3, 4, 5, 6, 8 and 10 have mutant allele but 1,2,7 and 9 didn't have mutant allele) for study sample in 2% agarose gel electrophoresis**.**

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 $\chi 2$ 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

homocysteine to methionine [10].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].

References

[1] Ahmad Poursade g h Zonouzi,1 Nader Chaparzadeh,1 Mehrdad Asghari Estiar. (2012) Mehtylene tetra hydro folate reductase C677T and A1298C mutation in women with recurrent spontaneous abortion in the north west of Iran.ISRNO bstetrics and Gynecology. 10. 5402 /2012 / 945486.

[2] Biondi B, Cooper DS (2010). Benefits of thyrotropin suppression versus the risks of adverse effects in differentiated thyroid cancer. Thyroid : official journal of the American Thyroid Association 20(2): 135-146.

[3] Boutin A, Allen MD, Geras-Raaka E, Huang W, Neumann S, Gershengorn MC (2011). Thyrotropin receptor stimulates internalization-independent persistent phosphoinositide signaling. Mol Pharmacol 80(2): 240-246.

[4] Carvalho Barbosa RdeC, Menezes DC, Freire TF, Sales DC, et al. (2012). Associations of polymorphisms of folate cycle enzymes and risk of breast cancer in a Brazilian population are age dependent. Mol. Biol. Rep. 39: 4899-4907.

[5] Cooper DS, Doherty GM, Haugen BR, Kloos RT, Lee SL, Mandel SJ, et al. (2009). Revised American Thyroid Association management guidelines for patients with thyroid nodules and differentiated thyroid cancer. Thyroid 19(11): 1167-1214.

[6] Davies L, Welch HG (2006). Increasing incidence of thyroid cancer in the United States, 1973-2002. Jama 295(18): 2164-2167.

[7] Durante C, Puxeddu E, Ferretti E, Morisi R, Moretti S, Bruno R, et al. (2007). BRAF mutations in papillary thyroid carcinomas inhibit genes involved in iodine metabolism. J Clin Endocrinol Metab 92(7): 2840-2843.

[8] Franco AT, Malaguarnera R, Refetoff S, Liao XH, Lundsmith E, Kimura S, et al. (2011). Thyrotrophin receptor signaling dependence of Braf-induced thyroid tumor initiation in mice. Proc Natl Acad Sci U S A 108(4): 1615-1620.

[9] Francesca Antonaros ,Giulia Olivucci , Elena Cicchini , Giuseppe Ramacieri1 ,Maria Chiara Pelleri and Lorenza Vitale1 (2019). MTHFR C677T polymorphism analysis: A simple, effective restriction enzyme based method improving previous protocols. J of Molecular genetics and Genomic Medicine . 7(5): e628.

[10] Greco A, Borrello MG, Miranda C, Degl'Innocenti D, Pierotti MA (2009). Molecular pathology of differentiated thyroid cancer. The quarterly journal of nuclear medicine and molecular imaging : official publication of the Italian Association of Nuclear Medicine 53(5):440-453.

[11] Hu J, Zhou GW, Wang N, Wang YJ. (2010) MTRR A66G polymorphism and breast cancer risk: a meta-analysis. Breast. Cancer Res Treat; 124:779-84.

[12] Iranpur, V. and Esmailizadeh, A. (2010) Rapid Extraction of High Quality DNA from Whole Blood Stored at 4ºC for Long Period. Department of Animal Science, Faculty of Agriculture, Shahrekord University, Shahrekord, Iran. *www. Protocol-online.org.*

[13] Jazdzewski K, Boguslawska J, Jendrzejewski J, Liyanarachchi S, Pachucki J, Wardyn KA, et al. (2011). Thyroid hormone receptor beta (THRB) is a major target gene for microRNAs deregulated in papillary thyroid carcinoma (PTC). J Clin Endocrinol Metab 96(3): E546-553.

[14] Jeddi-Tehrani, R. Torabi and A. H. Zarnani (2011). Analysis of plasminogen activator inhibitor-1, integrin beta3, beta fibrinogen, and methylenetetrahydrofolate reductase polymorphisms in Iranian women with recurrent pregnancy loss. Amer ican Jour nal of Re pro duct ive Immunolog y, vol. 66, no. 2, pp. 149–156.

[15] Jones AM, Howarth KM, Martin L, Gorman M, Mihai R, Moss L, et al. (2012). Thyroid cancer susceptibility polymorphisms: confirmation of loci on chromosomes 9q22 and 14q13, validation of a recessive 8q24 locus and failure to replicate a locus on 5q24. J Med Genet 49(3): 158-163.
[16] Kwak SY, Kim UK, Cho HJ. (2008) Methylenetetrahydrofolate reductase (MTHFR) and methionine synthase reductase (MTRR) gene polymorphisms as risk factors for hepatocellular carcinoma in a Korean population. Anticancer Res ; 28:2807-11.
[17] Laura Tafuri1, Edouard Servy and Yves Menezo (2018). The hazards of excessive folic acid intake in MTHFR gene mutation carriers: An obstetric and gynecological perspective. *Clin Obstet Gynecol Reprod Med.* Volume 4(2): 1-2.
[18] Mildred Gonzales , Pojui Yu and Pamela Shiao (2017). MTHFR Gene Polymorphism-Mutations and Air Pollution as Risk Factors for Breast Cancer. Nursing Research • Volume 66 • No. 2: 152-163.
[19] Samereh Ajabi, Farhad Mashayekhi and Elham Bidabadi (2017). A study of MTRR 66A>G gene polymorphism in patients with autism from northern Iran. Neurology Asia 2017; 22(1) : 59 – 64
[20] Stolzenberg S. , Solomon RZ and Qiao YL. (2003). Esophageal and gastric cardia cancer risk and folate- and vitamin B (12)-related polymorphisms in Linxian, China. Cancer Epidemiol Biomarkers Prev. 12:1222-6.
[21] Suzuki T, Matsuo K, Hasegawa Y. (2007). One-carbon metabolism-related gene polymorphisms and risk of head and neck squamous cell carcinoma: case-control study. Cancer Sci; 98:1439-46.
[22] Wan Lin, Yuhong Li, Zhengrong Zhang, Zuoli Sun, Yi He and Rena Li, (2018). Methylenetetrahydrofolate reductase and psychiatric diseases. Translational Psychiatry 8:242. 1-12.
[23] Weiner AS, Boyarskikh UA, Voronina EN, Selezneva IA, et al. (2012). Polymorphisms in the folate-metabolizing genes MTR, MTRR, and CBS and breast cancer risk. Cancer Epidemiol. 36: e95-e100.
[24] Xin Zhang , Chen Hou , Peng Liu , Li Chen , Yue Liu , Peng Tang and Rui Li (2019). Methylenetetrahydrofolate Reductase (MTHFR) C677T Polymorphism and Subacute Combined Degeneration: Revealing a Genetic Predisposition. Genetic Factors in Subacute Combined Degeneration. Volume 9 .Article 1162.1-8.