ZANCO Journal of Pure and Applied Sciences

The official scientific journal of Salahaddin University-Erbil
ZJPAS (2018), 30 (1); 85-94
http://dx.doi.org/10.21271/ZJPAS.30.1.9

MTHFR C677T polymorphism and thyroid cancer risk in Duhok, Kurdistan Region –Iraq

Soleen Sardar Zuhdi[1], Ahmed Mohammed Salih [2], Zeki Ali Mohammad [3], Intisar Salim Pity [4]

1- Reporter of Duhok Medical Research Center, assistant lecturer of cytogenetic, college of medicine, university of Duhok, Kurdistan Region, Iraq

2- Head, Duhok Medical Research Center, assistant professor of molecular biology & immunology, college of medicine, university of Duhok, Kurdistan Region, Iraq

3- Director of hematology - oncology center, Azadi teaching hospital, lecturer, department of internal medicine, college of medicine, university of Duhok, Kurdistan Region, Iraq

4- Professor of histopathology, department of pathology, college of medicine, university of Duhok, Kurdistan Region

A R T I C L E I N F O

Article History:
Received: 23/10/2017
Accepted: 30/1/2018
Published: 18/4/2018

Keywords:
MTHFR gene
MTHFR C677T Polymorphism
Thyroid cancer

***Corresponding Author:**
Ahmed Mohammed Salih

dr.ahmed@uod.ac

A B S T R A C T

Thyroid malignant tumors are common endocrine cancers that increased in incidence worldwide during the last decade. The disease recurrence is high, despite the death rate due to thyroid cancer is low. The etiology of thyroid cancer is still controversial, however, numerous genetic alterations in various thyroid tumors have been identified. Mutations in a gene encoding folic acid metabolizing enzyme, (Methyl Tetra Hydro Folate Reductase), named as (*MTHFR*), particularly the single nucleotide polymorphism *MTHFR* C677T has attracted our concern as a potential cause implicated in thyroid cancer. Formalin-fixed paraffin-embedded (FFPE) thyroid specimens with papillary carcinoma of 52 patients (18 male and 34 female) were donated kindly by Duhok histopathologic laboratories (Health Central laboratory). A group of 55 apparently healthy subjects were recruited (30 male and 25 female), after getting their formal consent. The DNA was isolated from both of the FFPE thyroid specimens of the patients and the venous blood samples of the healthy controls. The *MTHFR* polymorphism C677T was examined using the PCR-RFLP method using *Hinf*I restriction enzyme. The thyroid cancer group consisted of 18 (34.6%) males and 34 (65.4 %) females, their age mean was 42.41 years. The cases were 3 (5.8%) anaplastic carcinoma, 43 (82.7%) papillary carcinoma, 4 (7.7%) follicular carcinoma and 2 (3.8%) medullary carcinoma. The control group consisted of 30 (54.5%) males and 25 (45.5%) females, their age mean was 43.6 years. The frequency of *MTHFR* C677T (CC, CT) heterozygous and TT homozygous variants among 52 thyroid cancer patients were 59.6%, 15.4% and 25% respectively, and the CT +TT combined variants were 40.4%. There was statistical significant difference between the *MTHFR* CC wild genotype and *MTHFR*C677T (CT,TT) variants. Among the 55 healthy controls, the *MTHFR* C677T genotype variants frequency of CC, CT heterozygous and TT homozygous were 85.%, 3.6% and 10.9% respectively, and the frequency of combined CT heterozygous +TT homozygous variants among the healthy controls was 14.5%. In conclusion, our results did not support a statistical association of the *MTHFR* C677T gene polymorphism variants with the risk of developing thyroid cancer neither with gender nor age adjustment.

1. INTRODUCTION

Thyroid cancer is a common endocrine malignancy that has rapidly increased in global incidence in recent decades, it forms 1-10% of all cancers in women, 1-3% in men and about 1.4% in children (Jamil *et al*.,2011; Howlader *et al*.,2012). In Iraq, the prevalence of thyroid cancer is 1.01/100,000. In Kurdistan Region-Iraq, thyroid cancer crude rates reported during 2007, 2008 and 2009 periods ranged from 0.28-0.57/100,0005 (Othman *et al*., 2011; World health organization WHO,2004; Othman, 2011). The recurrence rate of the disease is relatively high, but the death rate among thyroid cancer patients is low (Tuttle *et al*.,2010). Several histopathological types with various cellular origins and characteristics with prognostic importance are defined to be associated with thyroid cancer. (DeLellis *et al*.,2004), those cells are of two types, follicular thyroid cells and parafollicular C cells, and thyroid cancer cells are derived from them. The majority of malignant thyroid tumors are derived from follicular thyroid cells which include - anaplastic thyroid cancer, papillary thyroid cancer, follicular thyroid cancer, and poorly differentiated thyroid cancer, where as parafollicular C cell-derived medullary thyroid cancer (MTC) has a small proportion among thyroid malignancies(Howlader *et al*.,2012).

The exact cause of thyroid malignancies is yet unknown, but ionizing radiation could be regarded as the most verified cause of thyroid cancer, specifically when radiation exposure happens at younger ages, also iodine deficiency in diet has been found to be linked to this type of pathology (Ron *et al*., 1995; Lund *et al*., 1999). Molecular genetic changes that have a crucial role in the tumor formation of different thyroid cancers have been reported. The T1799A transverse point mutation of *BRAF* gene is an example of a mutated gene that encodes BRAF-V600E mutant protein that leads to the activation of the serine/threonine kinase signaling pathway constitutively (Cohen *et al*., 2003;Fukushima *et al*.,2003). $BRAF^{V600E}$ mutation occurs in approximately 45% of PTCs (Xing M, 2005). Few other rare mutations in *BRAF* gene are reported in papillary thyroid cancer that affect nucleotides around codon 600, which activate the BRAF serine/threonine kinase pathway constitutively (Hou P, 2007; Trovisco *et al*., 2005). RAS mutations are the second in the prevalence related to *BRAF* mutations associated with thyroid cancer. The other genes that are identified to be implicated in thyroid tumor-genesis are β-catenin (*CTNNB1*) (Garcia-Rostan *et al*., 1999 ; Garcia- Rostan *et al*., 2001), *TP53(*Fagin *et al*., 1993*;* Donghi *et al*., 1993*)*, isocitrate dehydrogenase 1 (*IDH1*) (Murugan *et al*., 2010; Hemerly *et al*., 2010), anaplastic lymphoma kinase (*ALK*) (Murugan *et al*, 2011) and epidermal growth factor receptor (*EGFR*) (Murugan, Dong J *et al*., 2011).It has also been suggested that individuals possessing a modified ability to metabolize carcinogens are at increased risk of cancer (Laverdiere *et al*, 2002; Matsuo *et al*, 2004). A gene encoding folic acid metabolizing enzyme, methylenetetrahydrofolate reductase *MTHFR* C677T mutation has attracted our concern as a potential cause implicated in thyroid cancer. The balances the pool of folate coenzymes is controlled by the *MTHFR enzyme,* which is a fundamental enzyme in the DNA synthesis and methylation, both processes are found to be implicated in carcinogenesis of many types of cancers. Studies concerning the *MTHFR* gene polymorphisms have suggested that the low activity of folate metabolizing enzyme is regarded as risk factor in carcinogenesis (Laverdiere *et al*, 2002; Matsuo *et al*, 2004). The *MTHFR* gene is located on chromosome 1p36.3 and gathers a 2 Kbp coding site with

eleven exons (Langevin *et al*, 2009). It plays an important role in the regulation of cellular methylation by assisting the conversion of 5, 10- methylene tetrahydro folate to 5-methyltetrahydrofolate (Lee *et al*, 2004), the latter aids in the remethylation of homocysteine to de novo methionine (Macis *et al*, 2007), which serves as a precursor for the S-adenosylmethionine, a universal methyl donor for methylation reactions (Hosseini *et al*, 2011). Low dietary folate intake, decreased metabolism with no auxiliary folate intake might results in broken DNA molecules, increasing the mutagenesis rate and changes in the methylation profile of the DNA, in turn, affect the expression pathways of many genes (Kotsopoulos *et al*, 2008; Chou *et al*, 2006).So far, there is a controversy over the association between *MTHFR* C677T mutation with the PTC. Furthermore, very few works have studied this gene mutation in PTCs. In our study, we attempted to analyze the relationship between *MTHFR* C677T mutation and PTCs in Duhok city population.

2. MATERIALS AND METHODS

2.1. Subjects and methods

This case control study has been conducted in Duhok Medical Research Center (DMRC) at the college of medicine, university of Duhok, Kurdistan region-Iraq. The readily formalin-fixed paraffin-embedded thyroid specimens containing papillary carcinoma of 52 patients (18 male and 34 female)were donated kindly by Duhok histopathologic laboratories (Health Central laboratory). The specimens were belonging to thyroid cancer patients that were diagnosed between the period of May 2011 and August 2015, and they have received no prior therapy at the time of biopsy taking. All of the histopathological diagnoses and thyroid tumor staging was conducted previously by Pity et al(2015),briefly, a tissue section of four micron-thick was taken from each tumor specimen, stained with Hematoxylin and Eosin (H&E) for microscopic histopathologic diagnosis (Pity *et al*, 2015).DNA was extracted from the tissue sections using (Qiagen kit-USA) according to the manufacturer instructions. The DNA concentration and purity was measured with the use of nanodrop spectrophotometer. For the control group, a group of 55 apparently healthy subjects were recruited (30 male and 25 female), after getting their formal consent, a sample of 5 milliliter of venous blood was collected from each and the DNA was extracted from each sample. For the *MTHFR* gene polymorphism study, the *MTHFR* C677T was genotyped using the PCR-RFLP method according to Frosst *et al* (1995)with minor modifications. The primers targeting the *MTHFR* gene sequence flanking the regions close to C677T mutation prone point were designed with the help of NCBI software and provided by (Jena Bioscience, Germany), the primers were forward (5' GCCTCTCCTGACTGTCATCC3') and reverse 5'GGAGCTTATGGGCTCTCCTG3'). PCR of 14.4µl volume was conducted in 0.5 mL size PCR tubes consisting of 10 µl ready to use PCR master mix from Applied Bioscience, (containing *Taq* polymerase, dNTPs and PCR buffer), 0.8 µl each of the forward and reverse primers and 2.8 µl (40-60 ng) of genomic DNA from each sample. The PCR thermal cycling has been carried out in a thermal cycler (Applied bioscience) according to Abdul K. Siraj *et al*. 2008 with a few modifications, briefly the thermal conditions and cycling durations composed of: denaturation at 95°C for 5 minutes, followed by 40 cycles, denaturation at 95°C for 30 seconds, annealing at 66°C for 30 seconds, extension at 72°C for 30seconds, and a final extension at 72°C for 7 minutes(Abdul K. Siraj *et al*. 2008). The PCR products were digested

with *Hinf*I restriction enzyme in a total volume of 15.52µl consisted of: 14.4 µl PCR product, 1 µl restriction buffer (Promega, USA), 0.12 µl bovine serum albumin and 0.2 µl *Hinf*I restriction enzyme (Jena Bioscience, Germany). The digestion mix was incubated at 37 ^{o}C for 24 hours in a shaking water bath. The DNA fragments resulted from *Hinf*I enzymatic digestion were separated on a 2.0% agarose gel electrophoresis and observed by UV light.

2.2. Statistical analysis

Unpaired Student's *t* test was used to compare the differences of genotype and allelic frequencies between the cases and controls. The odds ratio and 95% confidence interval was depended to provide a measure for the strength of association. The SPSS statistical package (version 11.0)was used to calculate all of the statistical analyses at significant p value< 0.05 (Abdul K. Siraj *et al*. 2008).

3. RESULTS AND DISCUSSION

Study population characteristics are shown in Table 1. In this study, a total of 52 thyroid cancer cases and 55 healthy controls are involved. The thyroid cancer cases consisted of 18 (34.6%) males and 34 (65.4 %) females, their age mean was 42.41 years with age median of 41 years. The cases were 3 (5.8%) anaplastic carcinoma, 43 (82.7%) papillary carcinoma, 4 (7.7%) follicular carcinoma and 2 (3.8%) medullary carcinoma. The control group consisted of 30 (54.5%) males and 25 (45.5%) females, their age mean was 43.6 years. There was no significant difference (p value > 0.05) between the age means of both of the thyroid cancer group and the control group. There was statistically significant effect of gender on the rate of thyroid cancer cases, the female rate was significantly higher than the male thyroid cancer cases. The oligonucleotide primers used in the current study that target the *MTHFR* gene are producing a 254 base pair DNA band on agarose gel, for the RFLP, the *hinf*I restriction enzyme cuts the PCR products and yields two DNA bands on agarose gel with 147 and 107 bp size (figure 1).

The distribution of MTHFR C677T genotypes among the thyroid cancer patients and the healthy controls groups is shown in table 2. The frequency of MTHFR (C677T) CC, CT heterozygous and TT homozygous variants among thyroid cancer patients were 59.6%, 15.4% and 25% respectively, and the CT +TT combined variants were 40.4%. There was statistical significant difference between the MTHFR CC variants and MTHFRC677T (CT,TT) variants (P ‹ 0.005)

Table 2 also shows the distributions for MTHFR C677T genotype variants among healthy control group, the frequency of CC, CT heterozygous and TT homozygous variants was 85.%, 3.6% and 10.9% respectively, and the frequency of combined CT heterozygous +TT homozygous variants among the healthy controls was 14.5%. Using unpaired Student's *t* test, the odds ratio (Ors) and confidence intervals (CIs) to analyze the effect of CT genotype on the frequency of thyroid cancer frequency, it is shown that CT genotype has affected the frequency of the thyroid cancer cases among the case group, however the effected did not reach the statistical significance (OR = 0.9; 95% CI = 0.3-2.6; *t* test = 0.13; P = 0.89). Comparing the TT genotype variants between the disease group and the healthy control group, there was statistically no effect of the *MTHFR* C677T (TT genotype) mutation on the rate of the disease in the thyroid cancer group compare to the healthy group ((OR = 1.6; 95% CI = 0.6-4.45; *t* test = 1.02; P = 0.29). Comparison of the overall CT+TT combination genotype variants in thyroid cancer group with those in the healthy

control group, it is found that there was no significant effect of *MTHFR* C677T mutation on the frequency of thyroid cancer in the case group (OR = 1.3; 95% CI = 0.6-2.8; *t* test = 0.6; P = 0.533).

The frequency of the *MTHFR* C677T mutation (CT and TT genotypes) distribution regarding the gender in both the thyroid cancer and the healthy group is shown in table 3. In thyroid cancer group, the frequency of *MTHFR* C677T (CT,TT) in the females and males was 57.1% and 42.9% respectively, and in the healthy control group it was 47.4% in the females and 52.6% in the males. The unpaired Student's *t* test showed that there was no significant difference between both of the groups regarding gender, also there was no significant effect of gender on the frequency of *MTHFR* C677T in the same group.

The present case-control study that has been conducted on 52 thyroid cancer patients and 55 healthy controls, to the best of our knowledge, this the first study that examines the association between the thyroid cancer and *MTHFR* C677T polymorphism in Kurdistan region. Our findings demonstrate a significant effect of the gender on the rate of thyroid cancer among the patients group, there was a statistical significant difference between the males and female developed thyroid cancer, since the female rate of the disease was higher significantly among the disease group. Thyroid cancer is the most common malignancy of the endocrine system and the seventh most common malignancy in women (Ortega *et al*, 2004). Our results are consistent with the overall database of the incidence of thyroid cancer across different geographic and ethnic populations (Curado *et al*, 2007). The histologic subtypes of thyroid cancer are affected by gender variation. Anaplastic thyroid cancer and medullary thyroid cancer, which are the most aggressive types of thyroid cancer, are shown to have similar rates of incidence in men and women. Although, differentiated thyroid cancer such as follicular thyroid cancer and papillary thyroid cancer, are found to be more common in woman, since, the fluctuation of sex hormones during a woman's menstrual cycle and pregnancy has been hypothesized as the reason for the gender disparity in papillary thyroid cancer. In particular, the peak incidence of papillary thyroid cancer has been observed in women aged 40–49 years, this being the age group at which most women approach or enter menopause (Ortega J. *et al*, 2004; Kilfoy BA. *et al*, 2009).

. Other researchers have found that this disparity in gender is age-dependant, when women are found to have an earlier age of thyroid cancer onset but in men it is more aggressive at diagnosis. Moreover, in many studies, male gender is associated with a lower survival rate and higher mortality (Gilliland *et al*, 1997; Kilfoy *et al*,2009). Also in the present study, we found no significant contribution of *MTHFR* C677T (CT+TT variants) mutation in thyroid cancer in the disease group, since the *MTHFR* wild type CC genotype rate (59.6%) was significantly higher than the mutated CT, TT variants rate (40.4%). These results are in consistent with the results obtained by Abdul K. *et al* (2008) when they found that *MTHFR* polymorphism is of no importance to the thyroid cancer (Abdul K. Siraj *et al*. 2008). From the current results, it seems that subjects with *MTHFR* C677T single nucleotide mutation have about 1.5 times less risk getting thyroid cancer than those with the wild genotype of *MTHFR* (CC). Comparing our results with those obtained by others who investigated the association of *MTHFR* C677T polymorphism with other various tumors, they are consistent to an extent that no one strictly confirmed the association of the *MTHFR* C677T with thyroid cancer development. In an

Indian Caucasian population study, it has been shown that individuals with 677 C>T substitution in the *MTHFR* gene have a 3.5 fold lower risk of breast cancer (OR = 3.41, 95%CI = 3.1–3.7, P<0.02) (Mir *et al*, 2008). Also Cui et al (2011) found that the co- existence of 677 TT homozygous with 677 CT heterozygous of *MTHFR* shown to confer a protection effect against the risk of squamous cell carcinoma (Cui *et al*, 2011). But in some other studies, the lack of association between *MTHFR* variants and the risk of breast cancer has been reported in south Indian populations (Kalyankumar *et al*, 2006; Alberg *et al*, 2000). Furthermore, Cui et al (2011) found that the *MTHFR* 677 CT and TT variants conferred a weak protection from lung cancer (Cui *et al*, 2011). Also, there are conflicting data regarding the role of *MTHFR* in cervical cancer susceptibility and presentation of cervical cancer have been reported by series of case-control studies (Prasad *et al*, 2011; Kohaar *et al*, 2010; Nandan *et al*, 2008; Zoodsma *et al*, 2005; Lambropoulos *et al*, 2003). In table 3, the gender adjusted differences between both the thyroid cancer group and the healthy control group, there was no significant difference regarding the *MTHFR* C677T genotypes between both genders in both groups, in contrast to the findings in the current study a meta analysis conducted by Yan *et al* (2014), they found that *MTHFR* C677T polymorphism is associated with thyroid cancer both in Caucasians and Asians (Yan *et al*, 2014), this could be due to the larger population size they compared in many studies as a meta analysis. Worldwide studies have emphasized that folate levels exerts a protective role in a different cancers. Based on the importance of *MTHFR* in maintaining the level of folate, the *MTHFR C677T* variants polymorphism has been explored in various types of cancer, included colorectal, thyroid, breast, ovarian, and cervical cancer (Prasad *et al*, 2011). Overall, our results did not support a statistical association of the *MTHFR* C677T polymorphism with the risk of developing thyroid cancer neither gender association with the gene variants, other researchers also stated that the *MTHFR* C677T is not a potential risk factor for developing thyroid cancer, also Parsad *et al* (2011) stated that the C677T mutation in the *MTHFR* gene plays as a risk factor for colorectal cancer but not the cervical, thyroid, and breast cancers and the variants are not gender-specific (Prasad *et al*, 2011). The small sample size investigated in this study is a limitation that could be affecting on the discrepancy of the results. Moreover, further studies with larger sample size of different populations are necessary to investigate the molecular contribution of *MTHFR* polymorphism in the thyroid cancer molecular pathology and its association with age and gender.

Table1. Characteristics of the study population

		Male	Female	Age range (year)	Age mean (Year)	Age median (Year)
Thyroid cancer cases (*n*=52)		18(34.6%)	34(65.4%)	19-72	42.41	41
Anaplastic carcinoma	3 (5.8%)	1 (33.3%)	2 (66.7%)			
Papillary carcinoma	43(82.7%)	14(26.9%)	29(55.8%)			
Follicular carcinoma	4 (7.7%)	1 (1.9%)	3 (5.8%)			
Medullary carcinoma	2 (3.8%)	2 (3.8%)	0 (0.0%)			
Healthy Controls (*n*=55)		30(54.5%)	25(45.5%)	20-70	43.6	43

Table2. MTHFR C677T distribution and their genotype (CT, TT and CT+TT) association with thyroid cancer

MTHFR C677T	Thyroid cancer *n* (%)	Control *n* (%)
CC (Wild type)	31 (59.6%)	36 (65.5%)
CT (Heterozygous)	8 (15.4%)	10 (18.2%)
TT (Homozygous)	13 (25%)	9 (16.3%)
CT+TT combination	21 (40.4%)	19 (34%)

Table3. Gender association with MTHFR C677T in thyroid cancer and controls

Group	MTHFR C677T (CT, TT genotypes) *n*(%)
Thyroid cancer group (*n*=52):	
- Females	12 (57.1%)
- Males	9 (42.9%)
Healthy control group (*n*=55):	
- Females	9 (47.4%)
- Males	10 (52.69)

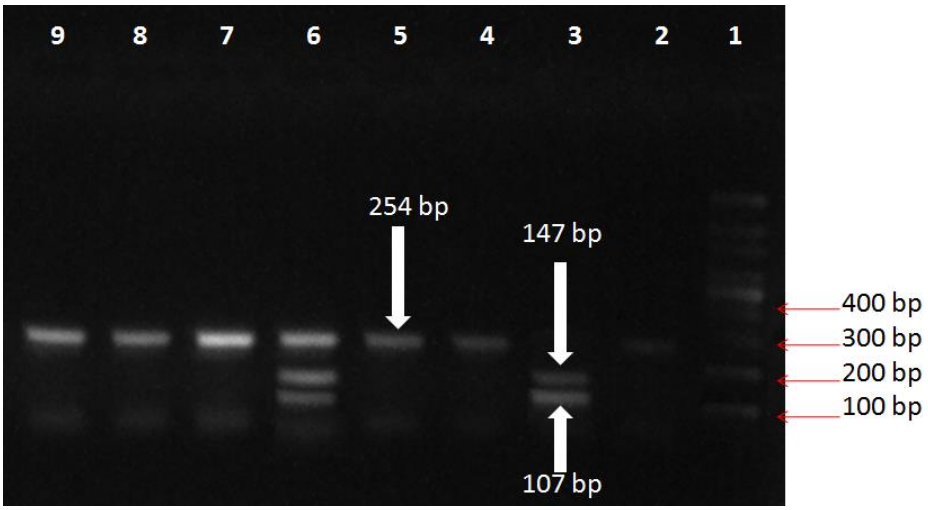

Fig.1: PCR- RFLP analysis: An agarose gel electrophoresis (2%) illustrating restricted fragments of *MTHFR* polymorphism (C677T). Lane1: 100 bp DNA ladder. Lane 2,4,5,7, 8 and 9 (254 bp fragment): *MTHFR* C/C wild genotype. Lane 3 (147 & 107 bp): *MTHFR* T/T homozygous genotype. Lane 6 (257, 147 & 107 bp): *MTHFR* C/T heterozygous genotype.

4. CONCLUSIONS

Our results in the present study did not support a statistical association of the *MTHFR* C677T gene polymorphism variants with the risk of developing thyroid cancer neither with gender nor age adjustment.

Conflict of Interest

Nothing to declare.

REFERENCES

ABDUL K SIRAJ, MUNA IBRAHIM, MAHA AL-RASHEED, JEHAD ABUBAKER, RONG BU, SHAKAIB U *et al*. 2008. Polymorphisms of selected Xenobiotic Genes contribute to the development of Papillary Thyroid Cancer susceptibility in Middle Eastern population. BMC Medical Genetics, 9,61.

ALBERG AJ, SELHUB J, SHAH KV, et al. 2000. The risk of cervical cancer in relation to serum concentrations of volate, vitamin B_{12}, and homocysteine. Cancer Epidemiol Biomarkers Prev, 9, 761–4.

CHOU YC, WU MH, YU JC, LEE MS, YANG T. 2006. Genetic polymorphisms of the methylenetetrahydrofolate reductase gene, plasma folate levels and breast cancer susceptibility: a case-control study in Taiwan. Carcinogenesis, 27, 295–2300.

COHEN Y, et al. 2003. BRAF mutation in papillary thyroid carcinoma. J Natl Cancer Inst, 95, 625–627.

CUI LIAN-HUA, MIN-HO SHIN, HEE NAM KIM, HYE-RIM SONG, JIN-MEI PIAO, SUN-SEOG KWEON, JIN-SU CHOI, WOO-JUN YUN, YOUNG-CHUL KIM, IN-JAE OH, KYU-SIK KIM CUI *et al*. 2011. Methylenetetrahydrofolate reductase C677T polymorphism in patients with lung cancer in a Korean population. Medical Genetics, 12:28.

CURADO MP, EDWARDS B, SHIN HR *et al.* 2007. (Eds). *Cancer Incidence in Five Continents.* IARC Scientific Publications, *4*, 160. IARC.

DELELLIS, RA.; LLOYD, RV.; HEITZ, PU.; ENG, C. 2004. World Health Organization Classification of Tumours. Pathology And Genetics Of Tumors Of Endocrine Organs. IARC Press.

DONGHI R, et al. 1993. Gene p53 mutations are restricted to poorly differentiated and undifferentiated carcinomas of the thyroid gland. J Clin Invest., 91, 1753.

FAGIN JA, et al. 1993. High prevalence of mutations of the p53 gene in poorly differentiated human thyroid carcinomas. J Clin Invest.,91, 179–184.

FROSST P, BLOM HJ, MILOS R, GOYETTE P, SHEPPARD CA, MATTHEWS RG, BOERS GJ, DEN HEIJER M, KLUIJTMANS LA, VAN DEN HEUVEL LP, et al. 1995. A candidate genetic risk factor for vascular disease: a common mutation in methylenetetrahydrofolate reductase. Nature genetics, 10(1), 111-113.

FUKUSHIMA T, et al. 2003. BRAF mutations in papillary carcinomas of the thyroid. Oncogene., ;22, 6455–6457.

GARCIA-ROSTAN G, et al. 1999. Frequent mutation and nuclear localization of β-catenin in anaplastic thyroid carcinoma. Cancer Res, 59, 1811–1815.

GARCIA-ROSTAN G, et al. 2001. β-catenin dysregulation in thyroid neoplasms: down-regulation, aberrant nuclear expression, and CTNNB1 exon 3 mutations are markers for aggressive tumor phenotypes and poor prognosis. Am J Pathol., 158, 987–996.

GILLILAND FD, HUNT WC, MORRIS DM, KEY CR. 1997. Prognostic factors for thyroid carcinoma. A population-based study of 15,698 cases from the Surveillance, Epidemiology and End Results (SEER) program 1973–1991. Cancer, 79, 564–573.

HEMERLY JP, BASTOS AU, CERUTTI JM. 2010. Identification of several novel non-p. R132 IDH1 variants in thyroid carcinomas. Eur J Endocrinol,163, 747–755.

HOSSEINI M, HOUSHMAND M, EBRAHIMI A. 2011. MTHFR polymorphisms and breast cancer risk. Arch Med Sci, 7, 134–137.

HOU P, LIU D, XING M. 2007. Functional characterization of the T1799-1801del and A1799-1816ins BRAF mutations in papillary thyroid cancer. Cell Cycle, 6:377–379.

HOWLADER, N., et al. 2007. SEER Cancer Statistics Review 1975–2009. National Cancer Institute. 2012. [online], http://seer.cancer.gov/csr/1975_2009_pops09

IRAQI CANCER REGISTRY. 2004. Results of Iraqi cancer registry 1976-2004. World health organization, Ministry of Health (WHO), Baghdad, lraq.

JAMIL A, et al. 2011. Global cancer statistics. CA Cancer J Clin, 61,69–90.

KALYANKUMAR C, JAMIL K. 2006. Methylenetetrahydrofolate Reductase (MTHFR) C677T and A1298C Polymorphisms and Breast Cancer in South Indian Population. International Journal of Cancer Research, 2, 143–151.

KILFOY BA, DEVESA SS, WARD MH, et al. 2009. Gender is an age-specific effect modifier for papillary cancers of the thyroid gland. Cancer Epidemiol. Biomarkers Prev,18,1092–1100. [PubMed]

KOHAAR I, KUMAR J, THAKUR N, HUSSAIN S, NIYAZ MK, et al. 2010. Homocysteine levels are associated with cervical cancer independent of methylene tetrahydrofolate reductase gene (MTHFR) polymorphisms in Indian population. Biomarkers.,15, 61–68.

Kotsopoulos J, Zhang WW, Zhang S, McCready D, Trudeau M, Zhang P, et al. .2008. Polymorphisms in folate metabolizing enzymes and transport proteins and the risk of breast cancer. Breast Cancer Res Treat; 112, 585–593.

LAMBROPOULOS AF, AGORASTOS T, FOKA ZJ, CHRISAFI S, CONSTANTINIDIS TC, *et al*. 2003. Methylenetetrahydrofolate reductase polymorphism C677T is not associated to the risk of cervical dysplasia. Cancer Letters, 191, 187–191 .

LANGEVIN SM, LIN D, MATSUO K, GAO CM, TAKEZAKI T, STOLZENBERG-SOLOMOM RZ, et al. 2009. Review and pooled analysis of studies on MTHFR C677T polymorphism and esophageal cancer. Toxicol Lett, 184, 73–80.

LAVERDIERE C, CHIASSON S, COSTEA I, MOGHRABI A, KRAJINOVIC M. 2002. Polymorphism G80A in the reduced folate carrier gene and its relationship to methotrexate plasma levels and outcome of childhood acute lymphoblastic leukemia. Blood, 100,3832–4.

LEE SA, KANG D, NISHIO H, LEE MJ, KIM DH, HAN W, et al. Methylenetetrahydrofolate reductase polymorphism, diet, and breast cancer in Korean women. Exp Mol Med, 36, 116–121.

LUND E, GALANTI MR. 1999. Incidence of thyroid cancer in Scandinavia following fallout from atomic bomb testing: an analysis of birth cohorts. Cancer Causes Control, 10:181–7.

MACIS D, MAISONNEUVE P, JOHANSSON H, BONANNI B, BOTTERI E, IODICE S, et al. 2007. Methylenetetrahydrofolate reductase (MTHFR) and breast cancer risk: a nested-case-control study and a pooled meta-analysis. Breast Cancer Res Treat, 106,263–271.

MATSUO K, HAMAJIMA N, SUZUKI R, OGURA M, KAGAMI Y, TAJI H, YASUE T, MUELLER NE, NAKAMURA S, SETO M, MORISHIMA Y, TAJIMA K. 2004. Methylenetetrahydrofolate reductase gene (MTHFR) polymorphisms and reduced risk of malignant lymphoma. Am J Hematol,77,351–7

MIR MM, DAR JA, DAR NA, DAR MS, SALAM I. 2008. Combined impact of polymorphism of folate metabolism genes; glutamate carboxypeptidase, methylene tetrahydrofolate reductase and methionine synthase reductase on breast cancer susceptibility in Kashmiri women. Int J Health Sci,; 2, 3–14.

MURUGAN AK, BOJDANI E, XING M. 2010. Identification and functional characterization of isocitrate dehydrogenase 1 (IDH1) mutations in thyroid cancer. Biochem Biophys Res Commun, 393, 555–559.

MURUGAN AK, DONG J, XIE J, XING M. 2011. Uncommon GNAQ, MMP8, AKT3, EGFR, and PIK3R1 mutations in thyroid cancers. Endocr Pathol, 22, 97–102.

MURUGAN AK, XING M. 2011. Anaplastic thyroid cancers harbor novel oncogenic mutations of the *ALK* gene. Cancer Res. 71, 4403–4411.

NANDAN NK, WAJID S, BISWAS S, JUNEJA SS, RIZVI M, *et al*. 2008. Allelic variations in 5, 10-methylenetetrahydrofolate reductase gene and susceptibility to cervical cancer in Indian women. Drug Metab Lett., 2, 18–22 [PubMed]

ORTEGA J, SALA C, FLOR B, LLEDO S. 2004. Efficacy and cost–effectiveness of the UltraCision harmonic scalpel in thyroid surgery: an analysis of 200 cases in a randomized trial. J. Laparoendosc. Adv. Surg. Tech. A, 14, 9–12. [PubMed]

OTHMAN RT, ABDULLJABAR R, SAEED A, KITTANI SS, SULAIMAN HM, MOHAMMED SA, et al. 2011. Cancer Incidence Rates in the Kurdistan Region/Iraq from 2007-2009. APJCP, 12, 1261-64.

PITY IS, SALIH AM, HASSAN N. 2015. $BRAF^{V600}$gene mutation in thyroid cancer in Duhok-Iraq. DMJ, 1, 30-6.

PRASAD VVTS, WILKHOO H. Association of the functional polymorphism C677T in the methylenetetrahydrofolate reductase gene with colorectal, thyroid, breast, ovarian, and cervical cancers. Onkologie, 34, 422–426

RON E, LUBIN JH, SHORE RE, MABUCHI K, MODAN B, POTTERN LM, SCHNEIDER AB, TUCKER MA, BOICE JD., JR. 1995. Thyroid cancer after exposure to external radiation: a pooled analysis of seven studies. Radiat Res, 141, 259–77.

TROVISCO V, et al. 2005. Type and prevalence of BRAF mutations are closely associated with papillary thyroid carcinoma histotype and patients' age but not with tumour aggressiveness. Virchows Arch, 446, 589–595.

TUTTLE RM, et al. 2010. Thyroid carcinoma. J Natl Compr Canc Netw, 8, 1228–1274.

XING M. 2005.BRAF mutation in thyroid cancer. Endocr Relat Cancer, 1, :245–262.

YAN Y, HAN F, FU H, XIA W, QIN X. 2014. Association between MTHFR C677T polymorphism and thyroid cancer risk: a meta-analysis. Tumour Biol., ;35(8), 7707-12

ZOODSMA M, NOLTE IM, SCHIPPER M, OOSTEROM E, VAN DER STEEGE G, *et al*. 2005. Methylenetetrahydrofolate reductase (MTHFR) and susceptibility for (pre)neoplastic cervical disease. Hum Genet, 116, 247–254