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Relationship between Promoter Hypermethylation of DNMT3A and DNMT3B genes and Endometrial Cancer

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A B S T R A C T

Aberrant DNA methylation is an epigenetic event that occurs by methyltransferases. DNMT3A and DNMT3B are responsible for *de novo* methylation that plays important roles in normal development and disease. A number of reports on methylation of various genes in endometrial cancer have been published, but most of these studies focused on tumor suppressor genes. In this study, we determined the promoter methylation pattern of *DNMT3A* and *DNMT3B* genes; also we analyzed correlations between methylation statuses with clinicopathological parameters. 28 patients and 22 healthy controls were studied. Isolation of genomic DNA from FFPE and peripheral blood was performed and Methylation-Specific PCR (MSP) was applied for analysis the promoter CpG methylation status of *DNMT3A* and *DNMT3B* genes in the studied population. A significant difference was found between the study groups and the presence of promoter CpG hypermethylation status in the *DNMT3A* (P=0.04) gene. Furthermore methylation status between tissue and blood samples of *the DNMT3A* gene was not significant (p=0.78). Our results indicated a correlation between age and menopausal state with *DNMT3B* promoter methylation, but there were no significant relationships between parameters such as tumor grade, type of tumor, amount of metastasis and myometrium invasion, furthermore diabetes (p = 0.01) and obesity (p = 0.027) were two important items in endometrial cancer incidence. In our study hypermethylation of *DNMT3A* gene was found as an important event in carcinogenesis of endometrial cancer. The inactivation epigenetic of methylation regulation genes is a common occurrence in many cancers, including endometrial cancer.

Introduction

Endometrial cancer (EC) is fourth cancer that has been reported among women and its manifestation is increasing with the risk of 3%. In opposition to breast cancer and prostate cancer that can be diagnosed by a population screening test, endometrial cancer often is seen during endometrium's biopsy in patients who have the symptoms (like bleeding after menopause) (Covens et al, 2003).

According to clinical manifestations differences, EC was classified into two main types: Type I which often happens among fat and postmenopausal women (sometimes before menopause), and it is dependent on Estrogen. In contrast, type II tumors happen among old menopause women, they are estrogen independent and often accompanied by surrounding endometrial, atrophy. Approximately 90% of endometrial cancers are sporadic (Kimberly et al, 2012). One of the most important risk factors for EC is exposure to estrogen without progesterone, which potentially causes continuous reproduction of anomaly and endometrial cancer. Also, other factors such as obesity, polycystic ovarian

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syndrome (PCOS), non-ovulation, nulipase, type II diabetes could increase the risk of endometrial cancer (Sweet et al, 2012; Colombo et al, 2013).

Like other cancers, endometrial cancer starts with genetic and epigenetic changes and environmental factors. Importance of genetic alternations consists of changes in DNA sequence has been widely studied in cancers incidence including EC (Yeramian et al, 2013). Recent researches showed that there is a relationship between the role of epigenetic factors in the regulation of gene expression and endometrial cancer. Epigenetic is defined as an inherited change in gene expression without alteration in the nucleotide sequence, in human neoplastics, which is a dynamic and reversible change (Tao and Freudenheim, 2010). The best and the most frequent epigenetic event is aberrant methylation of DNA. It is formed by changes in the activity of DNA methyltransferases (DNMTs) and the addition of methyl groups to cytosine nucleotides in the CpG position, which regulates chromosomal stability and gene expression. It also plays an important role in natural growth and physiological processes such as cellular differentiation, oncogenic transformation, and long-term memory formation. Among the family of DNMT enzymes, there is DNMT3 sub-branch with two important elements DNMT3A and DNMT3B which have the responsibility of de novo methylation in genomic DNA and increased expression of these DNMTs lead to hypermetylation of tumor suppressor genes and poor prognosis of cancer (Naghitorabi et al, 2013). Promoter hypermetylation affects tumor suppressor genes expression and genes involved in cell cycle, DNA repair, carcinogen metabolism, cell adherence, apoptosis, etc (Widschwendter and Jones, 2002). Therefore, in the present study, we assess the hypermylation promoter of two key genes which are involved in cell growth.

DNMT3A gene in human is located on 2P23 chromosomal position and consists of 26 exons and 25 introns that are 110 kb in length. It has an important role in creating static methylation pattern during development of primordial germ cell and primary embryo; furthermore, in some circumstances, it plays an oncogenic role in human cancers and in other situations, functions as a tumor suppressor gene. DNMT3A involved in de novo DNA methylation and its expressions during the development of primordial germ cell and blastocyst stage, has confirmed the role of DNA methyltransferase in mammalian growth. Furthermore, 98% amino acid sequence identity of its murine homolog indicated the protein conservations during evolution. Also, both human DNMT3A and mouse Dnmt3a contains similar structural domains and have de novo DNA methylation activity (Chen and Chan, 2014).

DNMT3A has 2 isoforms DNMT3A1, and DNMT3A2. DNMT3A2 expression level gradually decreases during differentiation in testicles, spleen, thymus and other somatic tissues. In contrast DNMT3A1 sustainably linked to the content of methylated chromatin in the CpG islands, in regions with duplicate DNA and in pericentromeric heterochromatin which can be distinguished by transcription silencing. It has been shown that DNMT3A1 acted as a transcription repressor whereas DNMT3A2 is linked to active transcription of euchromatin. DNMT3A is necessary for creating static methylation during gametogenesis and it is as a mediator between DNA methylation for regulating gene expression and survival of cell homeostasis (Kaneda et al, 2004).

DNTM3B is placed on 20 q 11.2 chromosome positions in human and has 23 exons. It involved in oncogenic and tumorigenic processes in the body by de novo methylation of specific genes. Down-regulation of DNTM3B has a positive effect in invasion procedures by inhibiting expression of adhering molecules including E-cadherin. DNMT3B can decrease EMT (mesenchymal transition epithelial) by siRNA or miRNA and causes cell invasion (Lee et al, 2005; Wang et al, 2007; Geiger and Peeper, 2009). It has an important role in aberrant methylation in cancers, methylation of centromeric satellite repetitive elements and suppression of transcription. Mutation in this gene causes immunodeficiency, chromosomal instability and facial disorders in the ICF syndrome. DNMT3B is not expressed in thyroid, bone marrow, and testicle tissues, but has high expression level in tumor tissues and involved in the cell proliferation process by methylation activates of CpG dinucleotides of precentric regions (Teneng et al,

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2015; Weisenberger et al, 2004; Esteller, 2007; Chédin, 2011; Cheng and Blumenthal, 2008).

The goal of the current research was to assess the possible relationship between promoter methylation of *DNMT3A* and *DNMT3B* genes in tumor tissues and blood samples of patients who suffer from endometrial cancer and making a comparison with normal endometrial tissue. In addition, we investigated the association between aberrant methylation of each gene with the pathologic and clinical condition of patients. Assessment and interpretation of altered methylation patterns in blood and tissue samples of endometrial cancer can be useful for early diagnosis and a better understanding of the disease mechanism.

Materials and methods:

1. Study population

In the current research, 28 endometrial cancer, 26 blood, and 22 control samples were available from the local Department of Gynecology and Obstetrics. All the patients visited gynecologist with abnormal bleeding and identified through vaginal ultrasound by physician obstetrics and gynecologist. Written consent was provided by all patients. Paraffin tissues were kept in the environmental temperature and all the patient's blood samples, after collecting in EDTA-containing tubes, were kept at – 20°C. The mean age of patients was 65 years old 17 (60.71%) of those aged less than 60 years old and 11(39.28%) of those aged 60 years or older.

2. DNA Extraction and Bisulfite treatment

In order to extract genomic DNA from paraffin blocks, pieces with 10-micrometer thickness were prepared and by the DNA extraction kit (Qiagene) genomic DNA was purified. DNA extraction from blood samples was carried out by a commercial DNA extraction kit (CinnaGen, Iran). 1-2 micrograms of extracted DNA was used for treatment with sodium bisulfite (Qiagene kit) according to the manufacturer's protocol. During treatment with sodium bisulfite, all the non-methylated cytosine's change to uracil and methylated cytosines remain unchanged. In this study Methylation Special PCR (MSP) method was used for assessment of DNA methylation.

Primers sequences that have been used for *DNMT3A* and *DNMT3B* genes were designed using meth primer software. 5' – TCGGTTTTAGAGAATTTGGTAATTC-3' (sense) and 5'-CCCTAACTAACACAAAACATAACGTA-3' (antisense) for $DNMT_{3A}$ methylated reaction (PCR product 123 bp), 5'-TGGTTTTAGAGAATTTGGTAATTTG-3 (sense) and 5'-CCCTAACTAACACAAAACATAACATA-3' (antisense) for $DNMT_{3A}$ unmethylated reaction (PCR product 121 bp). 5'-AAGTAGGATAGGTAGGGGTATC-3' (sense), 5'-AACGAAAAAAATAAAATCAAACGTC-3' (antisense) for *DNMT3B* methylated reaction (PCR product 89 bp) and 5'-AAAGTAGGATGATAGGTAGGGGTATT-3' (sense), 5'-AACAAAAAAAATAAAATCAAACATC-3' (antisense) for $DNMT_{3B}$ unmethylated reaction (PCR product 87 bp).

PCR reaction was carried out in a 20µl mixture containing 50 ng genomic DNA, 10 µl 2x Taq premix (Master Mix) (Iran, parstous) 0.5µM of each primers (Iran, gene fanavaran) and 7 µl deionized water. The cycling condition consisted of an initial denaturation at 95°C for 5 minutes, 34 cycles of denaturation at 95 °C for 45s, annealing at 55°C for 45 seconds and extension at 72°C for 45 seconds, followed by final extension for 5 min at 72°C. amplified products were separated by electrophoresis on 2.5% agarose gels and visualized under ultraviolet light.

3. Statistical analysis

All statistical analysis was performed with the SPSS20 software. The differences between gene methylation status and clinicopathologic characteristics were assessed using Pearson's chi-square ($\chi 2$) test. The association between hypermethylation of the genes and risk of EC was estimated by computing odds ratios (ORs) and 95% confidence intervals (CI) using the chi-square test and Fisher's exact test. The statistical level of significance was set to $P \leq 0.05$.

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Results

We examined 28 patients with endometrial cancer who had abnormal bleeding, ranging in age from 30 to 70 years old, 18 (64.2%) of the studied patients were less than 60 years old and grade G1 tumors and 10 of cases were G_2 or G_3 grades. IA stage and invasion of myomaterial was less than 50% and only 4 (14.3%) cases had a papillary serous type. The invasion of myomaterial was less than 50% in 15 patients and more than 50% in 10 patients. Among the present samples, only 9 (32.1%) patients had metastasis to other parts of the body. From the underlying disorders point of view, 19(67.8%) patients were obese, 11(39.2%) had diabetes, 8(28.5%) had high blood pressure and 4(14.3%) had low thyroid. The numbers of patients who had high blood lipids or used hormone were not significant.

Most of the cases were evident for two factors of obesity and diabetes. In this study, the CpG regions in the promoter of $DNMT_{3A}$ and $DNMT_{3B}$ genes were analyzed to detect the amount of hypermethylation. The obtained bands for $DNMT_{3A}$ and *DNMT3B* genes by methylated and non-methylated primers were 105 bp, 98 bp, 110 bp, and 123 bp respectively (fig 1).

Table 1- An example of a table

An example of a column heading	Column A (t)	Column B (t)
And an entry	1	2
And another entry	3	4
And another entry	5	6

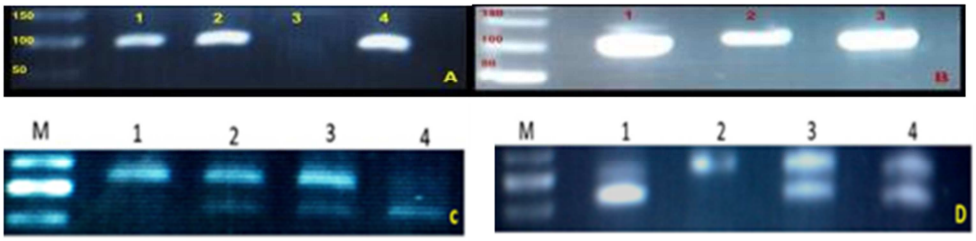

Figure 1- MPS Analysis of *DNMT3a* (A: blood, C: tissue) and *DNMT3b* (B: blood, D: tissue) genes methylation and unmetylation visualized under 2.5% agarose gel

(A) *DNMT3a:* The PCR products in the lanes 1 and 2 show the presence of methylated templates (105 bp), lane 3 negative controls and lane 4 unmethylated templates (98 bp).

(B) *DNMT3b*: The products in lane 1 indicate the presence of metylated templates (110 bp). Lanes 2 and 3 indicate the presence of unmetylated templates (123 bp).

(C) *DNMT3a:* The PCR products in the lanes 1 shows methylated template, lane 2 and 3 indicate the hemi methylated status and lane 4 shows the unmethylated PCR product, M: 50bp ladder.

(D) *DNMT3b*: lane 1 indicated the presence of unmetylated templates, lanes 2 is related to methylated PCR products and lanes 3 and 4 shows the hemi methylated status, M: 50bp ladder.

1. DNMT3A and DNMT3b genes methylation frequency:

Analysis of *DNMT3A* gene methylation frequency among healthy and patient samples showed that from overall 28 tumor tissue and 22 healthy samples 14%, 4.5% methylated, and 21.4%, 54% unmethylated respectively. Table 1 shows the results of studying the methylation frequency of this gene among healthy and patient samples at a significance level (p=0.0296). also simultaneous comparison of blood and tumor tissue samples, in 26 blood samples of patients and from 28 patients whose tissue samples were analyzed shows that 11%,14% methylation pattern, and 15%, 21% unmethylated pattern were affected and there were no significant differences between comparison of patient's tissue and blood samples (P=0.86). Therefore, it can be suggested that the blood samples can be used as a prognostic marker for rapid and invasive diagnosis.

Analysis of *the DNMT3B* gene's methylation's frequency among 22 healthy and 28 tumor patient's samples showed 13%, 10% methylation, and 50%, 78% unmethylation. According to table 2, the results do not show a significant difference

between methylation pattern of promoter *DNMT3B* gene in healthy and patient tissue (p=0.68). Simultaneous comparison of methylation pattern in tissue and blood sample of the patient showed 10%, 38% methylated, and 78%, 11% unmethylated pattern. Results suggested a significant difference between blood and tissue samples of patients (p = 0.016).

Table2- Comparison of the results of promoter hypermethylation *DNMT3A* and *DNMT3B* genes in normal tissue, patient's tumor tissue, and blood samples

Gene name	Sample(number)	Number of methlyted samples (%)	Number of hemi-methlyted Samples (%)	Number of non-methlyted Samples (%)	OR	95%CI	P-value
DNMT3A	Healthy tissue(22)	1(4.54%)	9(40.9%)	12(54.54%)	2.6	1.0990 to 6.1508	0.0296
DNMT3A	Patients tissue(28)	4(14.28%)	18(64.28%)	6(21.42%)			
DNMT3A	Patients' blood(26)	3(11.53%)	19(73%)	4(15.38%)	0.9360	0.4394 to 1.9937	0.8639
DNMT3B	Healthy tissue(22)	3(13.63%)	8(36.36%)	11(50%)	1.1905	0.5153 to 2.7504	0.6832
DNMT3B	Patients tissue(28)	3(10.71%)	14(50%)	11(39.28%)			
DNMT3B	Patients' blood(26)	10(38.46%)	13(50%)	3(11.53%)	0.3199	0.1458 to 0.7018	0.0045

2. Relationship of pathological and clinical characteristics with MNMTs promoter methylation:

The methylation results from the endometrioid carcinoma of endometrium specimens were compared against clinicopathological characteristics, including age; menopausal status tumor stage, tumor grade, histologic type, depth of myometrial invasion, metastasis, diabetes, High weight and High blood pressure (Table 2). No significant correlation between *DNMT3A* methylation and any of these parameters was observed for the patients with endometrioid carcinoma of the endometrium (p> 0.05).

Statistical analysis showed that hyper methylation of *DNMT3B* in blood is associated with endometrial cancer incidence after menopause.

Also, we can suggest that there is a significant relationship between age and the amount of $DNMT_{3B}$ methylation in the blood (p = 0.18), but there were no significant relationships between parameters such as tumor grade, type of tumor, amount of metastasis and myometrium invasion in patients and $DNMT_s$ promoter methylation.

After comparing the incidence of illnesses in premenopausal and postmenopausal women, it showed that 84.6% of cases have happened in postmenopausal women (table 2).

Our results confirmed that two factors, diabetes (p = 0.01) and obesity (p = 0.027) were important items in Endometrial cancer incidence (tables 3 and 4). Also, the study of hypermethylation of *DNMT3A* and *DNMT3B* genes with pathological conditions showed that methylation of *DNMT3B* gene in blood samples is associated with menopause inpatient and according to statistics it has nearly an acceptable significance level (p = 0.06).

Table 3- Relationship between *DNMT3A* and *DNMT3B* methylation and pathological condition

	BLOOD		TISSUE	
Parameters	DNMT3A	DNMT3B	DNMT3A	DNMT3B
Age				
<50(4)	3(40%)	2(40%)	2(50%)	2(50%)
>50(24)	11(52%)	15(71%)	11(84.5%)	9(37.5%)
	P=0.7	P=0.18	P=0.8	P=0.6
Menopausal status				
Premenopausal(4)	2(50%)	1(25%)	2(66.7%)	1(33.3%)
Postmenopausal(24)	11(50%)	16(72.7%)	12(48.0%)	9(36%)
	P=1.0	P=0.06	P=0.5	P=0.9
Tumor grade				
G1(18)	17(47.1%)	11(64.7%)	9(50%)	6(33.3%)
G2(5)	2(50%)	3(75%)	3(60%)	2(40%)

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G3(5)	3(60%)	3(60%)	2(40%)	2(40%)
	P=0.8	P=0.8	P=0.8	P=0.9
Tumor stage				
IA(17)	8(50%)	11(88.8%)	9(52.9%)	6(35.3%)
IB(2)	1(50%)	1(50.0%)	1(50%)	1(50%)
II(4)	1(33.3%)	2(66.7%)	1(25%)	1(25%)
IIIA(3)	2(66.7%)	2(66.7%)	2(66.7%)	2(66.7%)
IIIB(2)	1(50%)	1(50%)	1(50%)	1(50%)
	P=0.9	P=0.9	P=0.8	P=0.8
Histologic type				
Endometrioid type(25)	11(47.8%)	15(66.2%)	12(48.0%)	9(36%)
Nonendometrioid type(3)	2(66.7%)	2(66.7%)	2(66.7%)	2(66.7%)
	P=0.5	P=0.9	P=0.5	P=0.3
The depth of myometrial invasion				
Negative(3)	1(33.3%)	2(66.7%)	2(10.7%)	2(66.7%)
<50%(15)	7(50%)	10(71.4%)	7(53.6%)	5(33.3%)
>50%(10)	5(56.6%)	6(66.7%)	4(35.7%)	4(40%)
	P=0.8	P=0.9	P=0.7	P=0.5
Metastasis				
Negative(24)	9(50%)	12(66.7%)	10(52.6%)	8(42.1%)
Positive(4)	4(50%)	5(62.5%)	4(44.4%)	3(33.3%)
	P=1.0	P=0.8	P=0.6	P=0.6

Table 4- compared clinical features and methylation in *DNMT3A* and *DNMT3B* genes

	Blood, number (%)		Tissue, number (%)	
Parameters	DNMT3A	DNMT3B	DNMT3A	DNMT3B
Diabetes				
Negative(17)	7(43.8%)	11(68.8%)	9(52.9%)	7(41.3%)
Positive(11)	6(60%)	7(70%)	5(45.5%)	4(36.4%)
	P=0.4	P=0.9	P=0.6	P=0.7
High weight				
Negative(9)	5(55.6%)	7(77.8%)	4(44.4%)	2(22.2%)
Positive(19)	8(47.1%)	10(50.8%)	10(52.6%)	8(42.1%)
	P=0.6	P=0.3	P=0.6	P=0.3
High blood pressure				
Negative	10(52.6%)	12(63.2%)	10(50%)	8(40.0%)
Positive	3(42.9%)	5(71.4%)	3(37.5%)	3(37.5%)
	P=0.6	P=0.6	P=0.5	P=0.9

Table 5- The relationship between clinical characteristics of the study groups with endometrial cancer

	Diabetes		High blood pressure		High weight	
	Negative	Positive	Negative	Positive	Negative	Positive
Patients Normal	20	2	15	7	8	14
Patients Cancer	17	11	20	8	9	19
P-Value	P= 0.01		P= 0.8		P=0.027	

Discussion

Genetic and epigenetic alternations are common in the incidence and development of various types of human cancers. Abnormal expression of genes associated with DNA repair, cell cycle metabolism, and tumor suppressors are defects, which happen frequently in cancers. Aberrant methylation of DNA is one of the most important epigenetic factors in which directly involved in tumor genesis because DNA methylation can induce suppression of tumor suppressor genes or oncogenes activities (19-20). Gene silencing can be as the result of DNA's aberrant methylation which significantly causes neoplastic evolution, creation, and development of tumors (21).

DNA aberrant methylation can lead to cancer in many ways: 1) Methylation in CpG regions which facilitates the mutation of C to T in tumor suppressor genes. 2) Deamination of 5 methyl cytosine to thymine in oncogenes. 3) CpG sequence methylation increases the ability to attack by some of the biological and carcinogenic agents, such as benzopyrene dioxide epoxide. 4) Promoter hyper methylation of CpG Islands which cause tumor suppressor gene and DNA repairing genes' silencing (22). Evidence' collection shows that DNA methylation by DNMT proteins in promoter regions is related to gene silencing; therefore DNA methylation is associated with gene suppression (23-24).

DNMT3A and *DNMT3B* genes have multiple transcriptional start points (TSPs) that are separated on the chromosome and their expression controlled by multiple promoters. The *DNMT3A* gene has at least four TSPs and the expression is controlled by three different promoters. All three promoters lack typical TATA sequences adjacent to the TSPs. Two of them bear CpG-rich promoters and the other a CpG-poor promoter. The *DNMT3B* gene has at least two TSPs which exist in different exons and the expression is controlled by different promoters. Both promoter regions of the *DNMT3B* gene lack typical TATA sequences, where one promoter contains a CpG-rich area near the TSP, the other promoter is CpG-poor (25).

Previous researches showed that endometrial cancer's diagnosis is only possible by therapeutic surveys and in symptomatic patients, there is no effective screening test for its early diagnosis. The fatality rate of EC can be reduced in the early stages and before surgery (26). But tumor's early diagnosis needs new methods for describing and identifying specific cancer biomarkers and subsequently establishing reliable non- invasive methods for detecting these biomarkers in body fluids and advancing in molecular studies. Because of the role of aberrant methylation of DNA in uterus' endometrial cancer, the study of DNA's methylation pattern and progressing in this field can be used as a biomarker for diagnosis and prognosis of this illness (27).

A range of molecular alterations, such as DNA mutations and methylation have been found in tumor cells, in which reflected that cell-free circulating DNA (circDNA) released from the tumor into the blood, thereby making circDNA an ideal candidate for the basis of a blood-based cancer diagnosis test(28). In the present study evaluation of methylation status of *DNMT3A* and *DNMT3B* genes in blood and tissue samples, showed that there is no significant difference between DNMT3A promoter methylation in tissue and blood samples (14.2 and 11.5% respectively), in contrast, promoter methylation analysis for DNMT3B gene, in tissue and blood samples demonstrated statically significant different between methylation status of tissue and blood samples (10.7 and 37.4% respectively), therefore it could be suggested that DNMT3A promoter hypermethylation in blood sample could potentially be employed as a biomarker for early and non-invasion diagnosis and as a predictor of treatment response in EC.

In the present research, we studied the methylation status of *DNMT3A* and *DNMT3B*'s genes in EC. The achieved results showed a significant relationship between patient and control group with hyper methylation in a specific region of CpG promoter of *DNMT3A* gene. These results confirmed that increasing the amount of methylation in patients' tissue elevate the risk of EC and can be considered as a biomarker for prognosis of illness. In a simultaneous study of blood and tumor tissue samples, there was not an acceptable difference between the amount of methylation in patient's blood and tissue. Based on these results blood samples can be used as a biomarker for quick and non- invasive diagnosis. Furthermore, in the study of *the* $DNMT_{3B}$ gene, no significant difference has been observed between the amount of methylation of healthy and patient tissues. But a significant difference was seen between the patient's tissue and blood. In this research, it was confirmed that 38% of analyzed blood samples were methylated and it was reported that the amount of methylation in both healthy and patient tissues were 6%. Also in the study of the amount of hyper methylation of *the DNMT3B* gene with the pathologic condition, it was observed that there was a significant relationship between menopause's incidence and hyper methylation. Also, it was concluded that there was a significant relationship between the

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incidence of hyper methylation of *DNMT3B* in blood and age factor.

In the previous studies, there was not a coherent study about the relationship of hypermethylation of *DNMT3A* and *DNMT3B* genes and EC. But a lot of studies have been done about polymorphism and genes expression on the other cancers. In the study performed by Irlandand and his colleagues, they observed overexpression of *DNMT3B* in HaCaT cells; modulate the expression of genes related to cancer. Down-regulation the expression of 151 genes with CpG islands including VAV3 via methylation of its promoter. They found statically significant overexpression of *the DNMT3B* gene in cervical cancer and various cancer cell lines (29). Also, J Devon Roll and his colleagues analyzed 12 breast cancer cell lines for detection of differential expression of 64 methylation-sensitive genes. They suggested that DNMT3b significantly contributes to total DNMT activity. Their results were in assent with those of other recent studies, in which aberrant *DNMT3b* overexpression reported to be involved with methylation abnormalities of breast cancer. (30). In 2007 yan wu and his colleagues studied expression of *DNMT1*, $DNMT_{3B}$ and $DNMT_{3A}$ in women with endometriosis and they observed that *DNMT1*, $DNMT_{3B}$, and $DNMT_{3A}$ genes were over-expressed in the ectopic endometrium as compared with normal control subjects or the eutopic endometrium of women with endometriosis, and their expression levels were correlated positively with each other (31).

Previous studies demonstrated that Over-expression of DNMTs in cancer may be related to hypo-methylation of the promoter region of their genes, subsequently increased expression of the DNMTs can result in hyper-methylation of specific tumor suppressor genes. Rajendran and coworkers reported that DNMT3B overexpression is accompanied by DNMT3B promoter hypo-methylation in glioma tumors (32). Also Naghitorabi and his colleagues with quantitative evaluation claimed that DNMT3B promoter might be hypo-methylated in breast cancer, they observed a significant correlation between the methylation status and the sample type, cancer type, and tumor size but no significant correlation were observed between the methylation status with clinical stage and metastasis, nodal involvement and age (7). Also, our results confirmed that *DMNT3B* promoter is nearly 40% hypomethylated in tumor tissues and a significant difference was observed between normal and tumor tissue. Similar to our studies, M.Naghitorabi and his colleagues did not observe any association between methylation status of *DNMT3B* gene with metastasis, invasion and tumor stage, In contrast, Zhu and colleagues showed low methylation level at the promoter region of *DNMT3B* in both normal and neoplastic pituitary samples (33).

In Conclusion according to the results of the current study, it can be suggested that analysis of CpG Island's hyper methylation in the promoter region as the main factor in endometrial cancer's incidence. Based on achieved results in this research and existence of a significant relationship between promoter methylation pattern in $DNMT_{3a}$ gene in patient and healthy tissues it can be suggested as an early diagnosis cancer marker, but there was no significant difference between tissues and blood samples in $DNMT_{3A}$ gene. In addition, it is realized the relationship between two factors of diabetes and obesity and endometrial cancer's incidence, so it may be able to prevent the disease incidence by controlling diabetes and overweight of women who are at high risk. Also by studying the relationship between hyper methylation of $DNMT_{3B}$ gene in blood with pathologic status, it was found that there is a significant relationship between hyper methylation of this gene, menopausal age, and endometrial cancer's incidence. But there was not a significant relationship between hyper methylation of both genes in patients' blood and tissue with other pathological factors such as tumor's grade and type also tumor's grade and clinical status.

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بررسی ارتباط بین هایپر متیلاسیون پروموتور ژنهای DNMT3A و DNMT3B با سرطان آندومتر

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چکیده

متیلاسیون نابجای DNA یک رویداد اپی ژنتیکی است که توسط متیل ترانسفرازها ایجاد می گردد. DNMT3A و DNMT3B مسئول متیلاسیون *de novo* هستند که نقش مهمی در تمایز طبیعی و یا بیماری زائی ایفا می نماید. گزارشات متعددی از متیلاسیون ژنها در ارتباط با سرطان اندومتر صورت گرفته است اما اغلب این مطالعات بر ژن های سرکوبگر تومر تمرکز داشته اند. در این مطالعه الگوی متیلاسیون ژن های *DNMT3A* و *DNMT3B* مورد بررسی قرار گرفته است. همچنین ارتباطات بین متیلاسیون با پارامترهای پاتولوژیک و بالینی مشخص گردید. در این مطالعه ۲۸ فرد بیمار و ۲۲ فرد سالم مورد مطالعه قرار گرفت. جداسازی DNA ژنومی از بلوک پارافینی وخون محیطی انجام شد و PCR اختصاصی متیلاسیون (MSP) به منظور تجزیه و تحلیل وضعیت متیلاسیون CpG پروموتور از ژنهای *DNMT3A* و *DNMT3B* در جامعه مورد مطالعه استفاده شد. تفاوت معنی داری بین دو گروه مورد مطالعه و وضعیت هایپر متیلاسیون ناحیه CpG پروموتور ژن *DNMT3A* (P=0/04) مشاهده گردید. همچنین وضعیت متیلاسیون بین نمونه های بافت و خون ژن *DNMT3A* معنی دار نبود (P= 0/78). یافته ها نشان داد که بین سن و وضعیت یائسگی با متیلاسیون پروموتر *DNMT3B* همبستگی وجود دارد. اما ارتباط مشخصی با سایر پارامترها از جمله درجه و نوع تومر، میزان متاستاز و تهاجم به میومتریوم یافت نگردید. بعلاوه دیابت(P=0/01) و چاقی(P=0/027) بعنوان دو فاکتور حائز اهمیت در شیوع سرطان اندومتر می باشند. در مطالعه حاضر، هایپر متیلاسیون ژن *DNMT3A* یکی از مهمترین رخدادهای سرطان زایی در سرطان آندومتر است. غیر فعال شدن اپی ژنتیکی ژن های تنظیم کننده متیلاسیون یک رخداد رایج در بسیاری از سرطان ها، از جمله سرطان آندومتر می باشد.

واژگان کلیدی: سرطان اندومتر، هایپرمتیلاسیون، MSP، DNMT3a، DNMT3b.

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