Polymorphisms of CYP1B1 Gene and the Risk of Endometrial Cancer in Iraqi Women

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Abstract

This study was designed to study the polymorphisms of *CYP1B1* genes and the risk of endometrial cancer in Iraqi women. A total of Fourty patients with endometrial cancer, aged between 15-72 year were contacted after surgery in Baghdad, and sixty apparently healthy women as control. The blood samples were collected in EDTA tube for extraction DNA and PCR experiments.

The results showed that the frequency of *CYP1B1* homozygous wild type Leu /Leu were 14% and 68% in patients and control respectively, while the homozygous mutant Val /Val were 32% and 28% respectively. The heterozygous mutant allele Leu /Val was 54% in endometrial cancer cases compared to 4% in control. The statistical analyses showed increased risk of endometrial cancer with homozygous mutant Val /Val genotype with an OR of 5.55 (95% CI 2.578- 11.951) and with heterozygous Leu/Val with an OR of 56.5 (59% CI = 20.407-210.687).

When stratified the patients according to different stages, we found no association between the endometrial caner and stage IC, the risk increased to tow fold with stage IIB, while in stage IIIA and IVB the risk was highly increased to more than six fold with an OR (odd ratio) of 6.138 and 6.277 respectively .The risk of endometrial cancer increased to 2.5 fold in patient who had *CYP1B1* mutant allele Leu/Val.

Key word :Cyp1B1, polymorphisms, endometrial cancer

الخلاصة

صممت هذه الدراسة من اجل دراسة الأشكال المتعددة من جين CYP1B1 وعلاقته بمخاطر الاصابة بسرطان بطانة الرحم في النساء العراقيات حيث تم جمع عينات من نساء مصابات بسرطان بطانة الرحم وتتراوح أعمارهن بين 15– 72 عام وايضا تم جع عينات من 6 نساء سليمات كسيطرة. عينات الدم التي تم جمعها وضعت بانابيب حاوية على مادة مانعة للتخثر وحفضت لحين اجراء عملية استخلاص الدنا.

بينت النتائج في هذه الدراسة ان تردد متشابه الزيجة البري بالنسبة لل CYP1B1 هو Leu /Leu وبنسبة 14 بالمائة في النساء المرضى و 68 بالمائة في النساء السليمات. في حين متشابه الزيجة الطافر Val /Val اظهر النسب 32 بالمائة و 28 بالمائة على التوالي. ايضا اظهر متباين الزيجة ذو الاليل الطافر Leu /Val نسبة 54 بالمائه في حالات مرضى سرطان بطانة الرحم مقارنة بنسبة 4 بالمائه في الاصحاء

بين التحليل الاحصائي زيادة احتمالية الاصابة بسرطان بطانة الرحم ف المرضى الحاملين للنوع الطافر ومتشابه الزيجة Val/Val وبمعامل انحدار قدره 5.55 (95 CI 2.¬578– 11.951) %وللنوع متباين الزيجة Leu/Val وبمعامل انحدار قدره 56.5 (59% CI = 20.407–210.687).

عند تصنيف المرضى حسب المرحلة التي وصل اليها المرض فاننا لا نجد اي علاقة بين سرطان بطانة الرحم والمرحلة IC وان مخاطر الاصابة تزداد بمعدل مرتين في المرحلة IIB في حين في المرحلة IIIA والمرحلة IVB فان خطرة الاصابة قد ارتفعت لاكثر من ست اضعاف وبنسبة 1.38 و 6.277

ان نسبة الخطورة للاصابة بسرطان بطانة الرحم قد ازدادت بنسبة مرتين ونصف في المرضى الحاملن للاليل الطافر Leu/Valلجين CYP1B1

Introduction

Endometrial cancer is the most common gynaecological cancer and occurs mainly among postmenopausal women. The disease is classified mainly into two types. Type1 tumours are associated with endometrial hyperplasia and accounts for 80% of endometrial cancers, whereas type2 tumours generally develop from atrophic endometrial tissue. However According to the FIGO Surgical System, uterine cancer is classified in 4 main stages. Each main stage is then divided into two to three sub

stages. Both endogenous and exogenous Estrogens play a crucial role in the aetiology of endometrial cancer, particularly endometrial adenocarcinoma type1 tumours (Oehler & Brand, 2003). Risk factors include early menarche, late menopause, nulliparity, postmenopausal hormone use, obesity (Akhmedhanov *et.al*, 2001), and smoking (Terry *et.al*, 2002); Whereas the use of oral contraceptives (Weiderpass *et.al*, 1999), and physical activity (Graham & Clarke, 1997) seems to decrease risk.

In contrast to the breast, the effects of oestrogen on endometrial cells is counteracted by progestin's, which have been shown to down-regulate oestrogen receptors and interfere with the transcription of oestrogen receptor–mediated genes (Reddy *et al*,1987). Hydroxylation of estrogens is performed by cytochrome P450 enzymes, such as cytochrome P450 1B1 (CYP1B1) and in human endometrium, both 2-hydroxylation and 4-hydroxylation of oestrogen have been shown (Hakkola *et.al*, 1997). CYP1B1 is expressed in human endometrial (Bo finger *et.al*, 2001) and the protein has been localized to the cytoplasm of epithelial glands (Hakkola *et.al*, 1997). Degradation of oestrogen by CYP1B1 locally in the endometrium may be of importance in the endometrial cancer aetiology, especially in the endometrial hyperplasia. In addition to the role of CYP1B1 as an oestrogen eliminator, CYP1B1 may also form potentially genotoxic catechol estrogens. The metabolite 4-hydroxyestrogen, which may be further oxidized to reactive intermediates, has been shown to induce tumours in animals (Liehr *et al*, 1986; Li&Li, 1987).

Biochemical investigations of these *CYP1B1* variants (*CYP1B1*.2, *CYP1B1*.3, and *CYP1B1*.4) by Shimada *et.al*. (1999) and Watanabe *et al*. (2001) indicate that the *CYP1B1*.3 variant has a higher activity toward 17ß-estradiol. However, other studies report no altered enzymatic activity (Aklillu *et.al*, 2002) or decreased activity of *CYP1B1*.3 (Hanna *et.al*, 2000).

Estrogen degradation has not been well- studied in relation to endometrial cancer. The expression of CYP1B1 in the uterus and the tissue-specific hydroxylation of E2 to reactive catechol estrogens underscore the genes' potential importance in endometrial carcinogenesis (Murray *et al* ,2001 ; Yager & Liehr ,1996;Mannisto*et al*,1992 and Hakkola *et al*,1997).

Materials And Methods

A total of Fourty patients women with endometrial cancer, aged between 15-72 years were contacted after surgery (differnet hospital). Also sixty normal women served as control. They were asked to fill-up questionnaire regarding their clinicopathological features, eating habits, marriage status and the number of deliveries, which have been implicated to be the risk factors in the causation of cancer. The data were analyzed statistically. The blood samples were collected in sterilized tube with EDTA, kept directly in -20^{o}C till used for DNA extraction.

PCR-RFLP analysis of CYP1B1 polymorphism

The polymorphism of the gene *CYP1B1*was studied according to protocol of Smith *et al. (*1992). Briefly genomic DNA was amplified using the primers and pre master mix (Bioneer, Korea) given in table 1, PCR was done using Excicycler 96 (Bioneer , Korea) and the condition of reaction was : Initial denaturation 94 ºC for 5 min, 30 cycles as follow : 94 ºC for 1 min, 59 ºC for 1 min and 72 ºC for 1 min then final extension 72 ºC for 10 min.

Table 1: Oligonucleotide primer sequences used for PCR amplification of *CYP1B1*` gene

Primers		Primer Sequences	Length	Tm	TA
	F	5'-CTGCCAACACCTCTGTCTTG–3'	20	65 °C	59 °C
CYP1B1	R	5'-CTGAAATCGCACTGTGAGC -3'	20	65 °C	59 °C

PCR products were detected by electrophoresing them in 2% agarose gel. *CYP1B1* PCR fragment was of 271 bp.

Digestion

PCR product was digested for 3 hrs at 37ºC with EcoR1(Biolab) restriction enzyme using 3µl of NE Buffer 2 , 0.5 µl (5 units) of enzyme and 10 µl of PCR product,0.3µl BSA. The digestion products were classified as homozygous wild type (105-166 bp), homozygous mutant type (271 bp), and Heterozygous (166-271 bp) alleles (Fig 1)

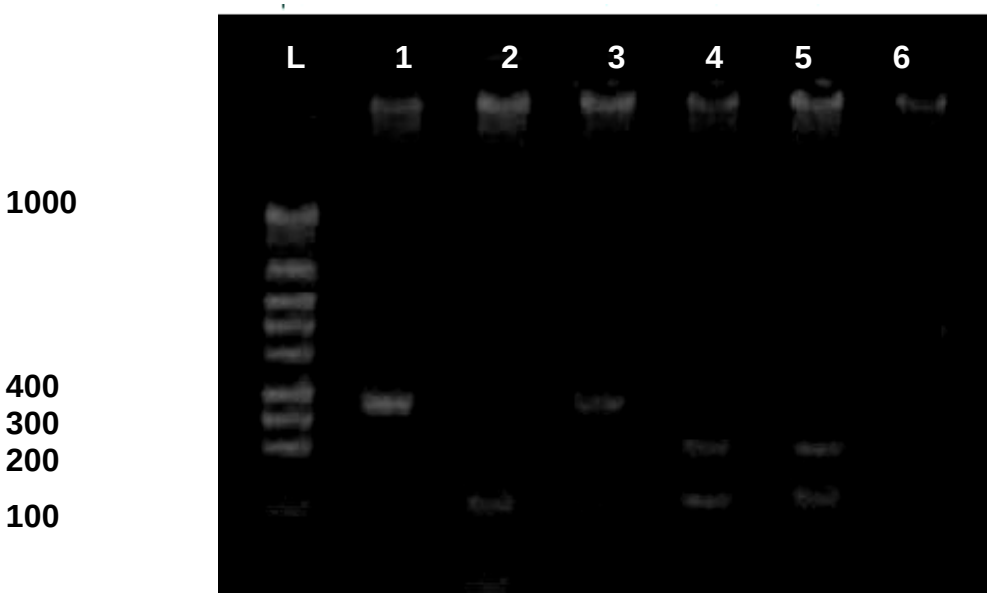

Figure 1: PCR product digested with Eco *R1* electrophoreses on agarose 2%
Lane L: DNA marker (100bp)
Lane 1 and 3: Homozygous mutant
Lane 2 : Homozygous wild type
Lane 4 and 5: heterozygous
Lane 6 : Negative control

Table 2: Distribution of polymorphisms of CYP1B1 gene among cases and control.

Genotype	Control	Cases	OR	(95%CI)	P.
Leu / Leu %	68	14	-	-	-
Val / Val %	28	32	5.55	2.578-11.951	0.03
Leu / Val %	4	54	65.5	20.407-210.687	0.018
Leu / Val+ Val / Val %	32	86	13.053	6.456-26.392	0.146

OR adjusted for age OR odds ratio 95%CI = 95% confidence interval.

When stratified the patient according to different stages of endometrial cancer ,we found no association between endometrial cancer endometrial cancer and stage IC

with OR of 0.111(95%CI=0.194-0.635) .The risk of cancer increased two fold with stage IIB stage IIB with an OR of 2.331(95%CI=0.18-29.03),while in stage IIIA and IVB the risk increased highly to more than six fold with an OR of 6.138(95% CI= 6.025 - 0.772)(Table 3)

Table 3: Relationship between *CYP1B1* genotype and stages of tumour in patient.

Genotype	Val / Val %	Leu / Val %	OR	(95%CI)	P.
Stage IB	2	12	1	-	-
Stage IC	12	8	0.111	0.194-0.635	0.0082
Stage IIB	0	14	2.331	0.18-29.03	0.002*
Stage III A	12	10	6.138	6.025-O.772	0.838*
Stage IV B	6	10	6.2778	0.045-1.6923	6.157*

* Refer to significant differences horizontally at (P≤0.05) within the group (patient status).

OR adjusted for age

OR odds ratio 95%CI = 95% confidence interval

When stratified according to different grades of endometrial cancer there was no association with homozygous mutant Val/Val and heterozygous Leu / Val genotype of CYP1B1 gene in grad II with an OR of 0.333(95%CI=0. 902-1.231) and grad III with an OR of 0.25(95%CI=0.698-0.895)(Table 4).

Table 4: Relationship between *CYP1B1* genotype and Grad of tumour in Patients.

Grad	Val/Val	Leu/Val	OR	(95%CI)	P.
G I%	4	18	1.0	-	-
GII%	12	18	0.333	0.902-1.231	0.273
G III%	16	18	0.25	0.698-0.895	0.731

OR adjusted for age OR odds ratio 95%CI = 95% confidence interval.

The results show that the risk of cancer increased in patient how had *CYP1B1* mutant allele Leu/Val 2.6 fold with an OR of 2.625(95%CI=0.838-8.22).

DISCUSSION

The ability to characterize polymorphic genes involved in the metabolism of oestrogen has opened up a new approach for the endometrial cancer risk assessment.

In the present study, the prevalence of genetic polymorphism in the CYP1B1 genes has been investigated to find out their association with the risk of endometrial cancer in the Iraqi population.

The frequency of CYP1B1 homozygous wild type Leu/Leu for cases and control respectively were 14% and 68% and that of homozygous mutant Val/Val were 32% and 28% respectively. The heterozygous Leu/Val was 54% in endometrial cancer cases compared to 4% in control (table 1).

The statistical analysis shows no association between homozygous mutant Val/Val genotype and the risk of endometrial cancer with an OR of 5.55 (95% CI= 2.578-11.951), also with heterozygous Leu/Val with an OR of 65.5 (95% CI= 20.407-210.687) and with Leu/Val + Leu/Val genotype with an OR of 13.053 (95% CI = 6.456-26.392).

According to different stages of endometrial cancer the risk increased in stage IIB with homozygous mutant Val/Val and heterozygous Leu/Val genotype of CYP1B1 gene with OR of 2.331 (95%CI=0.18-29.03).

The risk of the cancer increased 6 fold with stage IIIA and IVB with OR of 6.138 (95% CI= 6.025- 0.772), increased risk was OR of 6.2778 (95%CI=0.045-1.692).

Oestrogen is known to be central in endometrial carcinogenesis , it induce proliferation but may also initiate cancer via metabolic activation to potentially catechol oestrogen metabolite (Yager and Liehr ,1996).The bioactivation of oestrogen and carcinogens via CYP1B1 (Williams et.al ,2000 ; Hayes et.al ,1996) have been associated with several tumours in various tissues , including uterus (Cavalieri et.al ,1997).

One of chemical structures responsible for tumorigenicity of CYPs is catechol estrogens, this form of oestrogen leads to the formation of semi–quinones, and quinones that are known to react with DNA (Thibodeau and Paquette, 1999).

The reports mentioned above suggest that oestrogen play a role in the endometrial carcinogenesis process; although the classic role of estrogens in cells are to bind to its receptors and produce their biological effects, they may also undergo hydroxylation, and the resulting catechol oestrogen that may play a role in endometrial carcinogenesis (Williams *et al* .,2000).

Hayes *et al*.(1996) refer that the conversion to catechol estrogens is due to cytochrome P450 (CYP) enzymes and includes CYP1B1 that have been shown to be expressed in human endometrial cells, as a consequence of their presence, these catechol estrogens and metabolites have been shown to induces adenocarcinoma as well as DNA single-strand breakage and mutation ,thus these findings show that catechol estrogens may play a causative role in the carcinogenesis process and may affect the endometrium .

CYP1B1 gene is a phase I enzyme that catalyzes the conversion of 17ß-estradiol (E2) to the catechol estrogens, 4-hydroxyestradiol (4-OH-E2) and 2-hydroxyestradiol (2-OH-E2) and is involved in the activation of polycyclic aromatic hydrocarbons (Sutter *et al.*, 1994).

Several single nucleotide polymorphisms have been identified in CYP1B1such as Leu432Val and Asn453Ser polymorphisms located in exon 3, which encodes the catalytically important hem-binding domain of the enzyme, were selected as candidate susceptibility alleles (Stoilov *et al*., 1998; Bailey *et al*., 1998). All CYP1B1 variants form 4-OH-E2 as their main product (Shimada et.al, 1999). Hanna *et al*. (2000) determined that inherited CYP1B1 variants displayed higher estradiol 2- and 4-hydroxylation activities compared with their wild type enzyme. Furthermore, the ratio of product formation of 4-OH-E2 to 2-OH-E2 was higher for CYP1B1 variants compared with their wild type counterpart, potentially contributing to higher tissue levels of 4-OH-E2 (Shimada *et.al* ,1999; Hanna *et al*., 2000),Hydroxylation of estrogens is performed by cytochrome P450 enzymes, such as cytochrome P450 1B1 (CYP1B1), in human endometrium, both 2-hydroxylation and 4-hydroxylation of oestrogen have been shown (Hakkola *et.al*,1997).

CYP1B1 gene is expressed in human endometrium and the protein has been localized to the cytoplasm of epithelial glands (Hakkola *et al*., 1997; Bofinger *et al.*, 2001).

The degradation of oestrogen by CYP1B1 locally in the endometrium may be important in the endometrial cancer aetiology, especially in endometrial hyperplasia; in addition to the role of CYP1B1 as an oestrogen eliminator, CYP1B1 may also form

potentially genotoxic catechol estrogens. The metabolite 4-hydroxyestrogen, which may be further oxidized to reactive intermediates, has been shown to induce tumours in animals (Liehr *etal.*, 1986; Li & Li, 1987).

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