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Association between preterm birth risk and polymorphism and expression of the DNA repair genes *OGG1* and *APE1* in Saudi women

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Abstract: Genomic instability and mutations caused by increases in oxidative stress during pregnancy can damage the fetoplacental unit and can upshot preterm birth. Oxidative damage to DNA may possibly be involved in etiology of preterm birth (PTB) which can be repaired by DNA repair gene. In the present study, we assessed the association of base excision repair gene family by analyzing the association of single nucleotide polymorphisms and genes expression in 8-oxoguanine *glycosylase-1 (OGG1)* and apurinic-apyrimidinic endonuclease 1 *(APE1)* genes with risk of preterm birth in Saudi women. We analyzed genotypes of four single nucleotide polymorphisms (SNPs) (rs1052133, rs293795, rs2072668 and rs2075747) in *OGG1* gene and three SNPs (rs1130409, rs3136814, and rs3136817) in *APE1* gene using TaqMan Genotyping assay kits in 50 pairs of preterm cases and individually matched controls. Also, gene expression level was explored by RT-PCR in 10 pairs of preterm placental tissues and individually matched normal placental tissues. Two *OGG1* SNP, rs1052133 (OR=0.497; c2=1.11; *p*=0.292) and rs2072668 (OR=0.408; c2=1.90; *p*=0.167) and one APE1 SNP rs3136817 (OR=0.458; c2=0.40; *p*=0.527) showed non-significant protective effect against PTB development. The expression of both genes under study was found lower in the PTB patients. Genotype and allele frequencies of both gene SNPs did not show any association with the risk of preterm delivery in Saudi women (*P*>0.05). However, synthesis and release of *OGG1* and *APE1* proteins decreased in preterm placental tissues compared to term delivery reflects the probability of being one of the mechanisms leading to preterm birth.

Introduction

Oxidative stress plays a major role in various pregnancy complications including spontaneous preterm birth (PTB) or preterm premature rupture of the membrane. However, the molecular mechanisms behind are not clearly understood (Longini *et al*., 2007; Menon, 2014; Poston *et al*., 2011).

Oxidative stress can damage proteins, membrane lipids, and DNA, resulting in genomic instability and mutations, which are mainly repaired by cellular DNA repair mechanisms, such as base excision repair and nucleotide excision repair pathways (Kasai, 2002; Mitra *et al.,* 2001). Normally, biological systems reduce major part of oxygen to ATP but some amount is released as reactive oxygen species (ROS), such as superoxide anion (O^{2-}) and hydrogen peroxide (H_2O_2), which are needed in small amounts for cell division, cell signaling, immune functions and stress response. However their over-production may cause many chronic diseases and some pregnancy complications (Huang, 2018). During the course of pregnancy, oxidative stress is tightly controlled until labor and delivery process. Few studied on human populations have confirmed that oxidative stress can be the underlying cause of PTB and low birth weight (Agarwal *et al*., 2005; Myatt & Cui 2004; Kim et *al*., 2005). ROS are able to cause oxidative DNA damage, which is mainly repaired by base excision repair [BER] (Whitaker *et al*., 2017).

Oxidative stress also activates the BER gene family, involving 8-oxoguanine glycosylase 1 (OGG1) and apurinic-

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apyrimidinic endonuclease 1 (APE1*)* encoding genes (Aaron *et al.,* 2017). *OGG1* and *APE1* are located on chromosome 3p26.2 and 14q11.2-q12, respectively. *OGG1* is a major repair enzyme which can directly remove 8-oxo-guanine (8-oxoG) from the damaged DNA (Park *et al,* 2007). Also, 8-oxo-guanine is capable to pair with adenine during replication and is used as a biomarker for cellular oxidative stress. Human *APE1* is mainly involved in the repair of apurinic apyrimidic sites through its endonuclease and its phosphodiesterase activities (Karahalil *et al*., 2012). Genetic factors play a major role in etiology of PTB, which is a most serious problem worldwide and the main cause of morbidity and mortality in newborns.

Many studies on different genes have been done to assess the role of single nucleotide polymorphisms (SNPs) as a risk factor for preterm labor, however, no published report on the association of *OGG1* and *APE1* SNP variants are available. In this study, mRNA expression of *OGG1* and *APE1* genes in preterm placental tissues with normal tissue were compared; also the role of four polymorphism in *OGG1* (rs1052133, rs293795, rs2072668 and rs2075747) three in *APE1* (rs1130409, rs3136814, and rs3136817) in development of PTB was carried out in a Saudi population.

Material and methods

Subject recruitment and data collection

This hospital-based study was approved by the Institutional Review Board of the Ethics Committee at King Khalid University Hospital (KKUH) in Riyadh, KSA. A total of 100 healthy subjects not suffering from any disease or infection in the period of pregnancy and during labor were recruited. Out of these, fifty were normal women with full-term birth and fifty were patients with PTB. Each enrolled subject was given a written informed consent and the clinical data on maternal age, BMI, number of children and age at first pregnancy, gestational age at delivery, the newborn birth weight, and cesarean delivery were recorded**.**

Nucleic acid isolation and genetic analysis

Blood collection and DNA preparation for genotyping

For genomic DNA extraction, five milliliters of venous blood were collected from all subjects recruited in the study. QI Aamp DNA Blood Mini Kit (QIAGEN, Germany) was used to isolate the DNA by strictly following the protocol provided by the company. The extracted DNA was quantified and stored at 4°C until used for genotyping. Genotyping of four SNPs of *OGG1* gene and three SNP's of *APE1* gene was carried out using a TaqMan assay. PCR reaction was carried out using 5.84 µl Tag man®Genotyping Master Mix (Applied Biosystems, USA), 0.28 µl Tag man®Genotyping assays(Applied Biosystems, USA), 3.02 µl water, making the volume 9.14 µL, 2 µL DNA sample was added in each well which gives 11.14 total volume. Samples were carried out according to manufacturer's instruction. The details of primers and change in nucleotide position of the studied genes are given in Tab. 3.

Tissue collection and RNA preparation for quantitative real-time PCR (QRTPCR)

For gene expression studies, twenty placentas tissues were obtained in the delivery room in King Khalid University Hospital after delivery. Out of them, ten were from normal women with full-term birth and ten from patients with PTB. Each placenta was dissected and placed in a solution (Ambion, Life Technologies, USA) to maintain the quality and quantity of RNA, and was stored at -80°C until use. Total RNA was extracted from the frozen placental tissue using RNeasy Mini Kit (QIAGEN, Germany) and was subsequently treated with DNase I (DNA-free; Ambion Inc., Austin, TX) to remove genomic DNA. cDNA synthesis was performed using Reverse Transcription kit (Applied Bio-Systems, USA). QRTPCR was performed using SYBR Green. GAPDH was used as the internal normalizer. The expression primers for *OGG1* were F-5'-CAGCTCCACTGCACTGTGTACC-3' and R-3'-TCGCACACCTTGGAATTTCTG-5' and for *APE1* were F-5'-CTGCTCTTGGAATGTGGATG-3' and R-5'-TTTGGTCTCTTGAAGGCACA-3'.

Statistical analysis

All the statistical analyses were carried out using IBM SPSS version 22 statistical software (IBM SPSS, Chicago, IL, USA) and Microsoft Excel®. The genotypes and allele frequencies of the three SNPs were calculated manually. Independent sample *t*-test was used to achieve values of Odds Ratio, 95% Confidence Intervals, Chi-square to determine the significance between the patients and controls. The level of significance of differences was fixed at $P<0.05$.

Results

Tabs. 1 and 2 summarize, respectively, the demographic and clinical data of the 100 subjects who were recruited for this study. Gestational age at delivery and the newborn weight were significantly lower among PTB mothers (Tab. 1). The incidence of gestational diabetes mellitus and cesarean delivery were significantly higher in PTB mothers, while survival of the newborn was significantly lower in PTB babies.

Genotype and allele frequencies

Hardy-Weinberg equilibrium was separatedly estimated for each polymorphism. The genotype and allele frequencies of all four SNPs of *OGG1* and the three SNPs of *APE1* under study are presented in Tab. 4 and Tab. 5, respectively. Two SNPs in *OGG1* gene (rs1052133) and (rs2072668) showed a non-significant protective effect (OR 0.497 and 0.408 respectively) while the other two SNPs (rs293795) and (rs2075747) did not show any association. Only one SNP (rs3136817) in APE1 gene showed non-significant protective effect with OR 0.520 and *P*-value>0.05, while the other two SNPs from the same gene (rs3136814) and (rs1130409) did not show any association.

TABLE 1
Demographic characteristics of studied patients

Characteristics	Controls (n=50)	PTB patients (n=50)	P value
Age (Yrs)	28.045±0.532	28.920±0.897	0.381
BMI (Kg/m^2)	29.938±0.593	28.444±0.862	0.159
Parity	2.642±0.200	3.200±0.352	0.144
No. of children	2.220±0.226	2.340±0.257	0.731
Age at first pregnancy	22.574±0.420	23.208±0.658	0.421
Gestational age (weeks)	38.800±0.279	29.816±0.761	0.0001
Weight (Kg) new born birth	3.224±0.054	1.496±0.111	0.0001

TABLE 2
Clinical data of studied patients

Characteristics	Controls (%)	PTB (%)	Fisher Exact Probability test
Previous PTB	0	86	-
Previous PROM	0	42	-
Infections	0	0	-
PCOS	4	12	0.0001
Gestational DM	2	14	0.002
Hypertension	5.4	10	0.200
Hypotension	8.6	2	0.09
Hypothyroidism	0	6	-
Mode of delivery (CS)	10	19	0.0001
Survival of the newborn	100	90	0.0001

TABLE 3
Details of SNP and change in nucleotide position of the studied genes

Gene	SNP ID	Gene location	Polymorphism
OGG1	rs1052133	Exonic region #7, Missense Ser to Cys	C/G, Transversion
	rs293795	Intronic region, UTR variant 3 primer	C/T, Transition
	rs2072668	Intronic region	C/G, Transversion
	rs2075747	Intronic region	A/G, Transition
APE1	rs1130409	Exonic region#5, Missense Asp to Glu	T/G, Transversion
	rs3136814	UTR 5 prime	A/C, Transversion
	rs3136817	Intronic region	C/T, Transition

Gene expression studies

Tab. 6 presents *OGG1* and *APE1* expression in preterm delivery (PTD) and control tissue. Placenta tissue of 10 PTD and 10 controls from Saudi women were analyzed for expression of *OGG1* and *APE1* using quantitative RT–PCR LightCycler® 480 Instrument II real-time PCR. GAPDH used as reference gene for the comparisons since it considered as housekeeping gene.

Discussion

Basal oxygen consumption increases in pregnancy resulting in alteration of many body systems in addition to energy substrate in the fetoplacental unit, however the fetus is protected from free radical generation and damage throughout pregnancy. Fetus growth starts with low oxygen environment but oxygen consumption peaks by the second trimester, to meet the embryo growth requirements resulting in high placental metabolism and oxidative stress (Torres-Cuevas *et al.,* 2017). Due to oxidative stress, DNA is constantly exposed to free radicals and reactive oxygen species (Dumont & Monari, 2015).

To counteract this risk, multiple systems have evolved to detect DNA damage and reconcile its repair. DNA glycosylase enzyme can recognize a damaged DNA base through the mechanism known as base-excision repair (BER), which can mediate base removal before nuclease, polymerase and ligase proteins complete the repair (David *et al,* 2007).

TABLE 4

Genotype and allele frequencies of four SNP under study in *OGG1* in PTB cases and control group

Polymorphism	Control, n (%)	Preterm, n (%)	OR (95% CI)	X^2	p-value
rs1052133 C/G					
CC (wild)	16 (32%)	23 (46%)	Reference		
CG	27 (54%)	22 (44%)	0.567 [0.242-1.327]	1.72	0.189
GG	7 (14%)	5 (10%)	0.497 [0.134-1.847]	1.11	0.292
CG+GG	34 (68%)	27 (54%)	0.552 [0.245-1.247]	2.06	0.151
Alleles					
C	59 (59%)	68 (68%)	Reference		
G	41 (41%)	32 (32%)	0.677 [0.380-1.208]	1.75	0.186
rs293795 C/T					
CC (wild)	44 (88%)	39(78%)	Reference		
CT	6(12%)	8(16%)	1.504 [0.480-4.717]	0.49	0.482
TT	0	3(6%)	7.886 [0.395-157.449]	3.26	0.071
CT+TT	6(12%)	11(22%)	2.068 [0.700-6.116]	1.77	0.183
Alleles					
C	94 (94%)	86 (86%)	Reference		
T	6 (6%)	14 (14%)	2.550 [0.938-6.933]	3.56	0.059
rs2072668 C/G					
CC (wild)	15 (30%)	23(46%)	Reference		
CG	27(54%)	22(44%)	0.531 [0.225-1.256]	2.09	0.1479
GG	8(16%)	5 (10%)	0.408 [0.112-1.485]	1.90	0.167
CG+GG	35(70%)	27(54%)	0.503 [0.221-1.144]	2.72	0.099
Alleles					
C	57 (57%)	68 (68%)	Reference		
G	43 (43%)	32 (32%)	0.624 [0.350-1.111]	2.58	0.108
rs2075747 A/G					
AA (wild)	8 (16%)	5 (10%)	Reference		
AG	27 (54%)	22 (44%)	1.304[0.373-4.556]	0.17	0.677
GG	15 (30%)	23 (46%)	2.453[0.673-8.938]	1.90	0.167
AG+GG	42 (84%)	45 (90%)	1.714[0.520-5.657]	0.80	0.372
Alleles					
A	43 (44%)	32 (32%)	Reference		
G	57 (57%)	68 (68%)	1.603[0.900-2.855]	2.58	0.108

In BER pathway, numerous molecules together with *OGG1* and *APE1* encoded proteins (David *et al,* 2007) can repair the DNA damage caused by oxidative stress. *OGG1* removes 8-oxoguanine if incorporated during DNA replication from oxidation of dGTP by ROS, whereas APE1 bypasses the apurinic apyrimidic (AP) lyase activity of *OGG1* thus improving *OGG1* turnover and enhance the process of DNA repair (Zhang *et al*., 2012). Polymorphisms that influence DNA repair genes functions can be associated with the complex genetic disorders involved in PTD, because PTD show slow oxidative damage repair as compared to full term deliveries (Vande Loock *et al.,* 2012).

The clinical data revealed significant differences in gestational age and newborn weight between control and PTD patients, which are also reflected in the WHO classification of PTB (World Health Organization, 2016). Among the four *OGG1* SNPs, only rs1052133 and rs2072668 showed protective (OR=0.552 and 0.503 respectively) whereas rs293795 and rs2075747 were found to be non-significantly predisposing (OR=2.068 and 1.714 respectively) for the development of PTD in the Saudi population.

Comparison of genotype and allele frequencies for rs1052133 a C/G, transversion located on exonic with those reported by NCBI (dbSNP 2016) confirmed that C allele was the wild-type allele and CC genotype was the major genotype. The mutation C>G was polymorphic in the populations studied, including European, Asian and Sub-Saharan African ethnicities, where the homozygous genotype GG (mutant) was present in all populations as a minor genotype.

SNPs rs293795 and rs2075747 lead C/T transition and A/G transition, respectively, whereas rs2072668 C/G transversion were all located in the intron region. The mutant homozygous state TT of rs293795, and GG of rs2075747. with an OR 7.886 and 2.453, respectively, were all predisposing to

TABLE 5
Genotype and allele frequencies of 3 SNPs in APE1 gene association with PTD risk

Polymorphism	Control, n (%)	Preterm, n (%)	OR (95% CI)	X^2	P-value
rs1130409 T/G					
TT (wild)	8 (26%)	5 (10%)	Reference		
TG	24 (48%)	26 (52%)	1.733[0.498-6.035]	0.76	0.384
GG	18 (36%)	19 (38%)	1.689[0.465-6.135]	0.64	0.423
TG+GG	42 (84%)	45 (90%)	1.714[0.520-5.657]	0.80	0.372
Alleles					
T	40 (40%)	36 (36%)	Reference		
G	60 (60%)	64 (64%)	1.185[0.669-2.099]	0.34	0.56
rs3136814 A/C					
AA (wild)	47 (94%)	47 (94%)	Reference		
AC	3 (6%)	2 (4%)	0.667[0.106-4.174]	0.19	0.663
CC	0	1 (2%)	3.000 [0.119-75.519]	0.99	0.319
AC+CC	3 (6%)	3 (6%)	1.000[0.192-5.210]	0.00	1.000
Alleles					
A	97 (97%)	96 (96%)	Reference		
C	3 (3%)	4 (4%)	1.347[0.294-6.180]	0.15	1.000
rs3136817 C/T					
CC (wild)	1 (2%)	2 (4%)	Reference		
CT	24 (48%)	22 (44%)	0.458[0.039-5.414]	0.40	0.527
TT	25 (50%)	26 (52%)	0.520[0.044-6.101]	0.28	0.597
CT+TT	49 (98%)	48 (96%)	0.490[0.043-5.582]	0.34	0.557
Alleles					
C	26 (26%)	26 (26%)	Reference		
T	74 (74%)	74 (74%)	1.000[0.532-1.881]	0.00	1.000

TABLE 6
Expression of OGG1 **and** APE1 **in human placentas from PTB and controls mothers**

Genes	Group	Mean±S.D.	P value
OGG1	Control	1±0.144	0.11
	PTB	0.907±0.166	
APE1	Control	1±0.057	0.035
	PTB	0.66±0.202	

the development PTD. Coming to rs2072668, the presence of G mutant allele in the homozygous state GG decreased the OR to 0.408, suggesting that the mutant allele has a protective effect against PTD development in Saudi women, though the results were not statistically significant.

We further compared the results obtained from normal Saudi women with other populations (dbSNP 2016). The wild-type was the major genotype in other populations, but were found only exceptionally in this Asian population. Comparison of our results for the three SNPs showed that both genotype frequencies in the normal Saudi women compared with other populations were significantly different. However, the allele frequencies showed statistically significant differences between Saudis, Europeans, Asians, and Sub-Saharan Africans populations.

Among three SNP of *APE1* only rs3136817 a T/G, transversion located on exonic region#5, lead to missense Asp to Glu mutation and showed non-significant protective effects with OR 0.490, whereas rs3136814 and rs1130409 lead to A/C transversion and C/T transition (respectively) did not show any association (P>0.05).

Our results showed that the mutant allele G, in the homozygous state (GG) has OR 1.689, suggesting that the mutant allele was predisposing to PTD development in Saudi women. Comparison of genotype and allele frequencies for rs1130409 in APE1 gene in all listed populations confirmed that the T allele was the wild-type allele and TT genotype was the major genotype. The mutation T>G was polymorphic in the populations studied, including European, Asian and Sub-Saharan African ones, where the homozygous mutant

genotype GG was present in all populations as a minor genotype (dbSNP, 2016). rs3136814 showed the mutant genotype CC with an OR 3.000 was predisposing to the development PTD, though the result was not statistically significant. Comparison of results in normal Saudi women with the results obtained from other populations revealed that AA was the wild-type and major genotype in all populations. The mutation A>C was reported in several populations including, European, Asian and Sub-Saharan African ones. In the comparison of the genotype frequencies of Saudi women with other populations, it was observed significant similarities between Saudis and Sub-Saharan Africans in the genotype and allele frequencies (dbSNP 2016). rs3136817 showed the mutant genotype TT has a protective effect against PTD in Saudi women (OR of 0.520). The comparison with other populations confirmed the C allele as wild-type and the CC as major genotype. The mutation C>T is reported in all the populations (dbSNP, 2016). Similarities in genotype and allele frequencies were found between Saudis and Europeans, Asians and Sub-Saharan Africans. As there are differences in the prevalence of DNA repair polymorphisms across different populations, hence, it is important to keep in mind that a susceptibility factor in one population may not hold true for another. The reason for these contradictory findings in the allelic frequencies detected among these populations may be due to ethnic variation and heterogeneity. It is well recognized that ethnic background, due to different sociocultural traditions, may influence the susceptibility to suffer from certain diseases (Adcock *et al.,* 2003)

Both *OGG1* and *APE1* expression were decreased by 10% and 40% respectively in the preterm placental samples, as compared with controls. A lesser expression can dramatically affect the proteins synthesis in placental tissue, thus affecting the BER pathway, and can be linked to PTD (Kumar *et al*., 2012).

In conclusion, synthesis and release of *OGG1* and *APE1* was decreased in preterm placental tissues, as compared with term delivery, and that this may lead to PTB. Many genetic factors may be involved in PTD, and therefore genetically divergent populations may be affected differently.

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