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THE ASSOCIATION OF PROLACTIN RECEPTOR GENE POLYMORPHISM WITH RECURRENT MISCARRIAGE IN A SAMPLE OF IRAQI WOMEN

Hamsa A. Jassim*[1], Sanaa Jasim Kadhim[2], Header Alhabbobi[3]

[1,2,3] University of Baghdad/ institute of genetic engineering and biotechnology for post graduate studies/Iraq
dr.hamssa@ige.uobaghdad.edu.iq

ABSTRACT

Recurrent miscarriage (RM) is a common occurrence, about 15% of all clinically recognized pregnant women resulting in pregnancy failure. RM has been inconsistently defined. When defined as 3 consecutive pregnancy losses prior to 20 weeks from the last menstrual period, it affects about (1- 2) % of women. our study study whether the polymorphisms in *PRL-R* gene are correlated with the risk of RM. RM casas was (n=25) and apparently healthy control subject (n=15), were enrolled genotyping of *PRL-R* gene SNP (rs37389 G>T) polymorphism were determined by using Taqman genotyping assay. This study was carried out in the laboratory of institute of genetic angering and biotechnology for postgraduate study- university of Baghdad from November 2018 to February 2019, and the sample were collected from several laboratories in Baghdad. the distribution of genotypes and alleles frequencies results showed that at rs37389 SNP of *PRL-R* gene, as related with TT, TG and combined TG+GG genotypes, significant differences in frequency percentage were recorded between RM patients and apparently healthy subjects, the frequency of wild TT genotype was significantly lower in patients with RM than in apparently healthy subjects. In contrast, the frequency of heterozygous TG genotype and mutant genotype were significant higher in patients with RM compared with apparently healthy subjects. This study found an association between both heterozygous and homozygous mutants genotype at rs37389 of *PRL-R* gene with a risk for RM susceptibility.

Keywords: Healthy; Risk factors; pregnancy failure

INTRODUCTION

Recurrent Miscarriage (RM), is described as two or more repeated spontaneous miscarriages, in general the abortion in women occur before the week number twenty of gestation [1]. There are many different causes that associated with the RM, including the genetic factors, also different defects such as endocrine , defect in anatomical, have been assumed as being of RM causes, but the real causes of it still unclear [2,3]. Prolactin hormone is define depending on its function in the development of breast and lactation process, The functions of Prolactin by expression of prolactin receptor, initiating signaling cascades, primarily utilizing activator of transcription (JAK-STAT) and Janus kinase-signal transducer. for understanding of prolactin physiological functions, The disruption pathway has been concerned in the abnormalities in reproduction, diabetes, and tumorigenesis,. Prolactin secretion is in general from extrapituitary gland [4,5] . In the duration of pregnancy, the site of prolactin synthesis is decasualized endometrial stroma [6]. In women which not pregnant, the prolactin and Prolactin receptor expression in the endometrium during the secretory phase at mid-late was discussed by Jabbour and Jones [7,8]. An association between RM and prolactin and Prolactin receptor has been studied by Jin *et.al.* [9]. Garzia *et al.* [10] proved a low levels of expression of prolactin in RM women , also Bersinger *et al.* [11] was found that low-regulation for the Prolactin receptor in the women suffering with miscarriage ,in addition to that, Many researchers have testing and proving the genetic constituent which causing the RM. the *PRL-R* gene in human is located in chromosome number 5, the gene of *PRL-R* has been found and expressed in the liver , ovary and the breast [12,13,14]. Hanna *et al.* In 2010 tested the genetic polymorphisms concerned in the regulation of the hypothalamic-pituitary-ovarian axis (HPO) would be related with recurrent miscarriage [15] .In hanna study , the thirty-one single - nucleotide polymorphisms (SNPs) and the four short tandem repeat (STR) polymorphisms in twenty candidate genes, the distribution of genotype with reflect on to the *PRL-R* gene differed between the control and RM patients. The study aimed to estimate the genotypes and alleles frequency in SNP of *PRL-R* gene and study their association with RM risk in Iraqi women.

MATERIALS AND METHODS

Subjects and blood sample collection:

This study was conducted during the period November 2018 until June 2019, this study was carried out on 25 Iraqi women patients with RM, non-smokers and nonalcoholic, aged (25-35 year), The RM samples were taken from the Kamal Al-Samarraee infertility treatment Hospital in Baghdad, as well as 15 apparently healthy individuals females collected randomly from population living Baghdad as control, aged (25 - 35 year) which are non-smokers, non-alcoholic as control group. Two ml of peripheral blood from all select subjects were collected and placed into sterile plain

tube that contained EDTA. The blood was placed in a cool - box under aseptic conditions and transfer to the laboratory.

Gene polymorphism on RM

DNA extraction

Genomic DNA was extracted from the peripheral blood with the Quick-gDNA Blood MiniPrep purification kit (Zymo,USA) [16]. Genotyping of *PRL-R* gene G/T genotypes polymorphisms was done, and the allelic genotyping was performed using the MGB- NFQ primer/probe TaqMan assay on the ABI Prism 7500 Real Time PCR System (Applied Biosystems, Foster City, CA). The concentration of DNA and purity were determined spectrophotometrically by measuring their absorbance at 260 (A260) and 280 (A280) by Nano spectrophotometer. The primers (They include PRL-R gene SNP (rs37389 G>T) polymorphism) were synthesized and designed by (Integrated DNA technologies /USA) were prepared according to William *et al*. (2004), and was stored lyophilized (20°C). Primer and probe sequence were matched by the bioinformatic programs (NCBI). The probe was prepared for the wild type and labeled with FAM at the 5′ end inaddtion to MGB at the 3′ end. While the mutant allele detecting probe (SNP) was labeled with HEX at the 5′ end with MGB at the 3′ end (the normal wild type). The Mutant genotype and the hetero-zygous genotype are shown in figure 1.

Figure 1-A Fam channel resulted curves

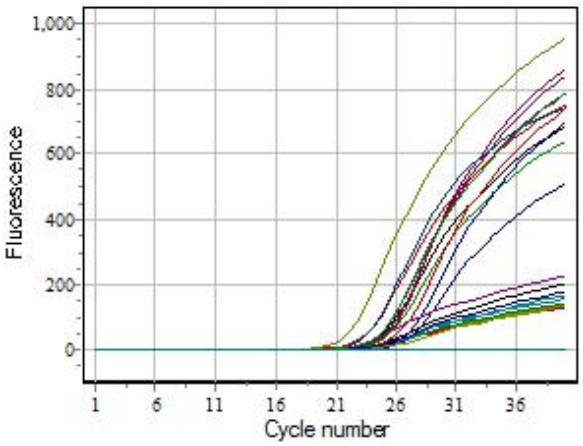
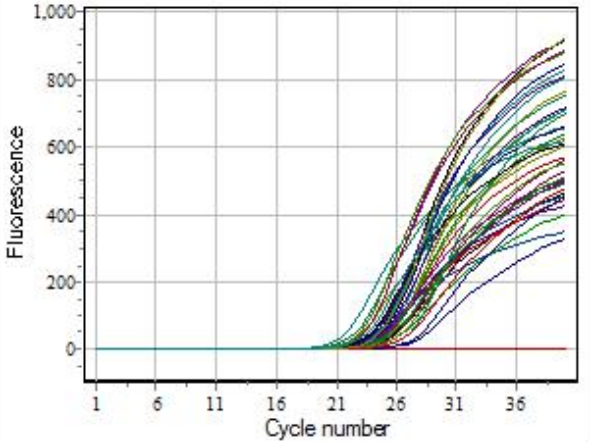


Figure 1-B HEX channel resulted curves



The amplification conditions of RT-PCR were: 95 °C, 10min; 95 °C, 15 sec, 60 °C, 60 sec in 40 cycles.

DATA ANALYSIS AND STATISTICS:

The data of this study was compiled into distribution and Statistical description (mean, SE), the computerized data file and frequency, were divided using Statistical software (SPSS). We used least significantly difference (LDS) test and statistical analysis of variance (ANOVA) test by probability of less than 0.01 ($p<0.01$) according to method which reported by ([17]).

RESULT AND DISCUSSION

Gene polymorphism study on RM patients:

This study examined the polymorphisms of at rs37389 of *PRL-R* gene among Iraqi women suffered from RM and the apparently healthy control and tested their association with RM. The distributions of genotype and the frequency in alleles were tested *PRL-R* gene polymorphism as shown in Tables 1.

Table 1: Frequency of genotypes and alleles at rs7389 SNP of *PRL-R* gene in Iraqi women with RM and control.

Genotypes	Patients[1](n=25)	Control[2](n=15)	OR[3] 95%CI	*P*
TT	1	0	1.9(0.08-45.18)	1.0
TG	15	8	1.31(0.37-4.60)	0.749
GG	9	7	0.64(0.18-2.28)	0.527
Allele		Frequency		
T	34	27	1.39(0.76-2.54)	0.357
G	66	73	0.72(0.39-1.31)	0.357

1. Patients with polycystic ovary syndrome. 2. Apparently healthy subjects. 3 Odd ratios.

The wild genotype frequency (TT) was significantly higher in patients with RM than in control subjects (OR= 1.9, p=1.0). The frequency of heterozygous TG genotype was significantly higher in patients with RM when compared with contorl subjects (OR=1.31, p=0.749). The frequency of homo mutant GG genotype was significantly higher in patients with RM when compared with apparently healthy subjects (OR=0.72, p=0.357). We found that the effect of heterozygous TG genotype of rs37389 of *PRL-R* gene on the susceptibility of the development of RM in Iraqi women could be better clarified with a large sample size.

In this study, we found an association between the *PRL-R* gene polymorphism and RM. In general the human *PRL-R* gene have multiple promoters, that allow tissue-specific transcriptional regulation the biologic role of the *PRL-R* gene polymorphism didn't get its real attention ,an association was reported between recurrent miscarriage and genetic variation in this gene. However, according to our data, the distribution of *PRL-R* gene polymorphism in patients with RM differ from that found in controls, this finding suggest that it has a major role in the development of RM in Iraqi women. Several study showed that Prolactin and PRL-R were found to have a role in implantation and placentation, and PRL-R expression is up-regulated in the mid-to-late secretory phase end ometrium and continues to early pregnancy [10,11.22.19.20]. In addition to that , regulation of the *PRL-R* gene was found to be associated with miscarriage, and an animal study has been found that mice carrying a germ-line null mutation in *PRL-R* gene also have failure of embryonic implantation or defective pre implantation embryonic development [10] In

another study, Bersinger *et al.* (2008) compared gene expression in the endometrium from women with different in vitro fertilization (IVF) outcomes, and found down-regulation of the PRL-R in women with miscarriage abortion compared with those with ongoing pregnancies, suggesting that there is an import biological role of the PRL-R in normal pregnancy.[11]

Addition investigation were study if there was genetic component for the risk of recurrent pregnancy loss. In addition Jin Ju Kim *et al* in 2018 investigate whether genetic polymorphisms was involve in the regulation of hypothalamic-pituitary-ovarian axis would be associated with recurrent miscarriage. Among 35 polymorphisms in different genes, the genotype distribution with regard to the prolactin receptor gene intron C/T polymorphism (rs37389) differed between women with recurrent miscarriage and control subject. This study reporting the candidate association between the prolactin receptor gene and recurrent miscarriage. The genotype distribution of the prolactin receptor gene C/T polymorphism in the recurrent miscarriage group did not differ from that in the control group. Our result is disagree with the result of Jin Ju Kim's study. [9]

In addition to that, Hanna *et al.* (2010) study the association of 31 SNPs and 4 STR polymorphisms in 20 candidate genes involved in regulating the HPO axis in 227 women with RM and 130 controls. This study involved genetic variation within the oestrogen receptor b gene, the *PRL-R* gene, the glucocorticoid receptor gene and the active in receptor type 1 gene were associated with RM. Since the publication of the first report on candidate association between *PRL-R* gene G/T polymorphism and recurrent miscarriage. [15]

The genotype distribution of the G/T polymorphism in the current study was remarkably differed from that of Hanna *et al*. (2010) results. In Caucasian women , the frequency of the GG genotype was approximately 80%, and it was about 50% in Korean women, and the frequency of the GT genotype was significantly different between patients and controls in Hanna *et al*.'s study, was approximately 12–15%.[15] This finding highlights the importance of ethnicity and population background on the examination of *PRL-R* gene polymorphism. Seven isoforms have been recognized in humans, which may have different biological activities. Peripheral blood polymorphism status may be different from the local one, and we suppose that *PRL-R* gene intron G/T polymorphism alone cannot reflect the tissue-specific or direct effect on local tissue. Our study was not coupled with endometrial or decadal tissue analysis of prolactin receptor expression. Second, we focused on a single SNP; the studies cannot exclude the possibility that other polymorphisms of the *PRL-R* gene, which were not tested in this study, could be associated with RM. Furthermore, considering interaction between multiple genes in human genome or disease risk, analysing joint effect of multiple risk alleles might be import-ant. Hanna *et al*. (2010) reported that genetic variation with in the oestrogen receptor b gene, the *PRL-R* gene, the glucocorticoid receptor gene and the activin receptor type 1 gene were associated with RM.[15]

Synergistic effect of combinations of SNPs epigenetic modification of the genome can change gene activity, in addition to that both ethnic background and environmental exposures might alter epigenetic changes [21,23]. The effect of gene-environmental interactions and epigenetic modification was difficult to address in the current study. Although we could not evaluate whether PRL patients and controls shared similar environmental exposures or not, but subjects were all iraqi and shared common ethnic background. Our study has merits at this point. Finally, although the sample size of the present study play an important affect in the final result, somewhat smaller than that of Hanna *et al*.'s previous study, negative results may originate from insufficient power to detect the differences. In addition, power calculation was not planed in advance, but it was performed based the enrolled subjects at the investigation. In conclusion, in our study we observe significant differences in the genotype distributions of the *PRL-R* gene polymorphism between patients with RM and controls. Our study may be useful in that it is the first replication study since the initial report of the association of this polymorphism with RM.

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