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Molecular Analysis of Prolactin Receptor Gene of Hyperprolactemic Iraqi Patients

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Abstract
Prolactin hormone is a polypeptide hormone of pituitary origin, whose production is controlled by dopamine. Hyperprolactinemia is characterized by increased production of prolactin hormone levels often leading to reproductive dysfunction. The aim of this study is to investigate if there is any genetic variation or changes in nucleotide sequences of Exon 1 of prolactin receptor gene behind infertile cases of Iraqi women patients suffering from hyperprolactinemia. For this study 15 samples from infertile hyperprolactinemic women were taken and genomic DNA was extracted, specific primers was designed to amplify specific portion of prolactin receptor gene (Exon 1) using PCR technique . The PCR products of both healthy and hyperprolactinemic infertile women were subjected for DNA sequencing by machine AB137 30KL, Applied Biosystem, Macrogene company, USA. Polymorphic variants were identified , data obtained which is heterozygous SNP and SNPs was found between individuals. The DNA sequencing results of receptor from patients was found to be compatible 99% and score 481 with the wild type sequence of gene bank. And the differences may be attributed to one transition mutation (G/A) at position 61 of exon 1. Its substitution mutation that leads to changes amino acid from Arginen to Lysin.
Keywords: Prolactin receptor gene, Hyperprolactinemia, Mutation

1. Introduction

Prolactin is the hormone that is secreted predominantly by lactotrophs in the anterior pituitary gland (1). Hyperprolactinemia is a condition of elevated serum prolactin, is characterized by increased production of prolactin, often leading to reproductive dysfunction and galactorrhoea (2). In humans the prolactin receptor gene is member of the large class-1-cytokine receptor super family(4). Only a single isoform of the prolactin receptor has been identified in humans (5). It's located on chromosome 5 and is approximately 180 kb in length and is originally having 10 exons of which (3-10) coding exon.(6),(7).
The effect of prolactin are mediated by interaction with specific cells surface high affinity prolactin receptor (PRLR)(3). The binding of prolactin is mediated with its receptor (PRLR), the binding of prolactin activates the pre-dimerized prolactin receptor and result in activation of prolactin receptor-associated signaling cascades such as Jak2/Stat5 result in transactivation of prolactin-responsive genes(8).
The aim of this study is to investigate if there is variation in PRLR gene of hyperprolactinemic infertile woman by taking a closer look of DNA sequencing of them, so a portion of the PRLR gene was amplified by PCR using specific primers for Exon1 of PRLR gene also amplified for samples of hyperprolactinmic infertile patients and healthy fertile women as control.

1.1 Materials and methods

Sample were collected after diagnose as infertile hyperprolactemic patients by consultants, its collected from (15), infertile women sueffering from hyperprolactinemia their ages ranged between(20-50) years.
A volume of 5 ml of peripheral blood was collected by vein puncture tubes. Genomic DNA was extracted from whole blood by using DNA extraction kit Promega. The primers that have been selected for this study to amplify portion of prolactin receptor gene was designed, and the details were illustrated in (table 1). The preparation of PCR reaction was carried out in 25ml by using green master mix and the amplification condition were illustrated in table(2), for all primers. Then after amplification, 15 of PCR product (10 as abnormal, 5 as control) were send for DNA sequencing, the sequence reaction were performed .

1.1.1. Results and discussion

In this study the extraction was accurate for all samples by showing clear bands for all samples of hyperprolactinemic patients and for healthy control samples, Three hundred and seven bp were amplified by using primer 1, three hundred and six bp were amplified by PCR using primer 2, three hundred eighty two bp

were obtained by using primer 3 and four hundred thirty six bp was amplified using primer 4 , and they are indicated by agarose gel electrophoresis for all samples as shown in figure 1(a, b, c) and 2.

1.1.2. **Detection of mutation in PRLR gene.**
The prolactin gene receptor was sequenced by sending PCR product of primer 2 (306 bp) which amplify the region from 5013 to 5318 and of primer 4 (436 bp), which amplify the region from 5020 to 5455. Homology searches were conducted between the sequences of standard gene blast program which is available at the national center for biotechnology information (NCBI), and Mega6 program. Results of sequencing of the coding regions of the amplified product (exon 1) for these samples were done for seeking of any mutations within these sequencing related to hyperprolactinemic patients. It's clear that the product of primer 2 have one mutation in sample 5 site (61),which is obvious in table (3) shown type of mutation and predicted effect in which it changes the codon from AGA to AAA, that change the a.a from Arg to Lys. The peaks is obvious in figure (3), while the blast of it in NCBI is clear in figure (4). In this study there is an association between the PRLR gene mutation (that happened in sample 5 of hyperprolactemic patient), and hyperprolactinemia , this result agrees with those of Newey *et al*., (2013) where shown that there were a heterozygous mutation in the prolactin receptor gene resulting in an amino acid change but histidine to arginine at codon 188, and this substitution disrupted the high affininty ligand-binding interface of the prolactin receptor resulting in a loss of downstream signaling by Janus Kinase2 (JAK2) and Signal transducer and activator of

transcription 5(STAT5), this resulted in a loss of function which lead to hyperprlactinemia(9). This result was coincidence with the study of Vaclavicek *et al*.,(2006)(10), when there are first screened the promoter regions and one SNP in each exon 5 and exon 6 which were supposed to change a.a , but the SNPs did not exist or they were too rare they were not considered. In this study, we choose the exon 1 as the PRLR gene region is not equally well characterized in the public database NCBI as PRL gene, also the exon 1 that exists in several variant forms and in non coding region(11). As a human PRLR is a single transmembrane spanning protein belonging to a superfamily of growth hormone/prolactin/cytokine receptors and alternative splicing result in different isoforms of PRLR (12) This isoforms of PRLR have different effect on intracellular signaling that may be stimulatory or inhibitory. These results suggested that the SNPs is located within non coding regions of PRLR genes based on location, the most likely mechanisms responsible for increased risk is through effect on transcriptional regulation (13). Also one other PRLR variant causing a change from Isoleucine to Leucine at codon 146 in the extracellular domain of the mature protein and resulting in an increased basal JAK2-STAT5 signaling invitro as Bogorad *et al*., 2008(14).

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Notes

Table 1. **Sequences of primers used to amplify human prolactin gene receptor:**

No.	Oligonucleotides	Oligosequance	Prod. Size (bp)	GC%	TM	Ref.
1	Forward primer Reverse primer	TCTTCGCAGGATTCCAGCTC CGCGAACGGTCGGTAAAATC	307	55.00 55.00	53.83 53.83	NCBI
2	Forward primer Reverse primer	GCCCTAATCATGCAAAACCGA GAAAGCCCAGCCCAGAAAAC	306	47.62 55.00	52.40 53.83	NCBI
3	Forward primer Reverse primer	GGCAGGCTCTGGACGTTTT TCCTCAGTGTTCGCCTCCAT	382	57.00 55.00	53.25 35.83	NCBI
4	Forward primer Reverse primer	TCATGCAAAACCGATCTGGG GTTTTGCCAGGGAGCAAAGT	436	50.00 50.00	51.78 51.78	NCBI

Table (2)

Program of PCR used for amplification of prolactin receptor gene using primer 1:

Thermal cycler protocol	No. of cycle	Temperature –time
Initial Denaturation	1 cycle	94 °C for 5 minutes
Denaturation	35cycle	94 °C for 1 min
Annealing		61 °C for 1 min
Extension		72 °C for 1 min
Final Extension	1 cycle	72 ° C for 10 mins.

Program of PCR used for amplification of prolactin receptor gene using primer 2:

Thermal cycler protocol	No. of cycle	Temperature –time
Initial Denaturation	1 cycle	94 °C for 5 minutes
Denaturation	35cycle	94 °C for 1 min
Annealing		57 °C for 1 min
Extension		72 °C for 1 min
Final Extension	1 cycle	72 ° C for 10 mins.

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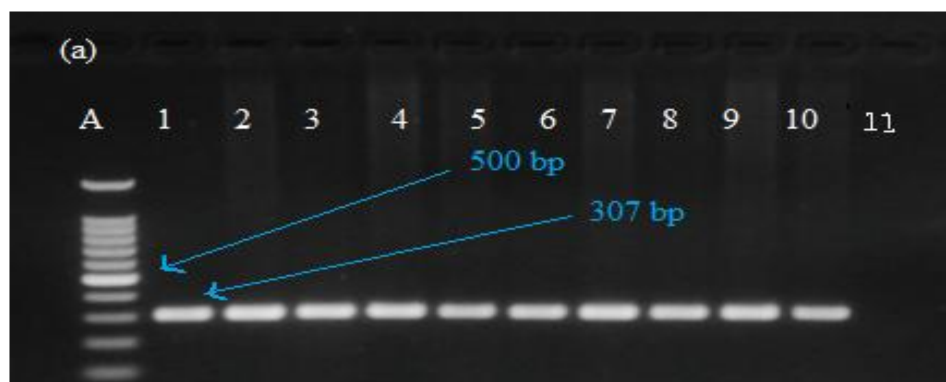
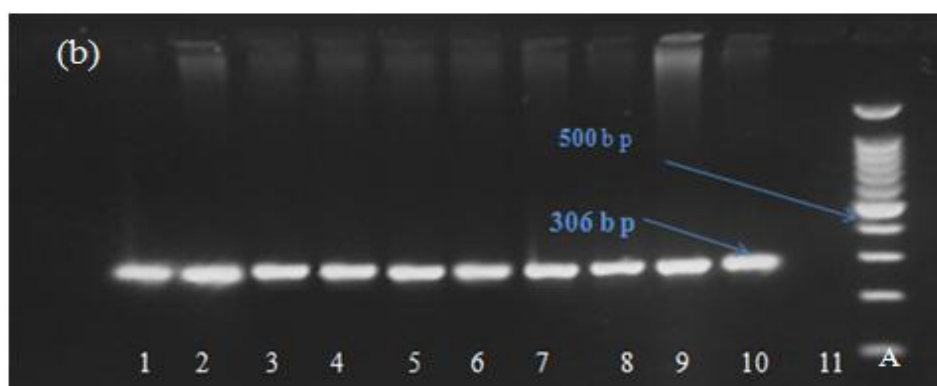
Program of PCR used for amplification of prolactin receptor gene using primer 3:

Thermal cycler protocol	No. of cycle	Temperature –time
Initial Denaturation	1 cycle	94 °C for 5 minutes
Denaturation	35cycle	94 °C for 1 min
Annealing		57 °C for 1 min
Extension		72 °C for 1 min
Final Extension	1 cycle	72 ° C for 10 mins.

Program of PCR used for amplification of prolactin receptor gene using primer 4:

Thermal cycler protocol	No. of cycle	Temperature –time
Initial Denaturation	1 cycle	94 °C for 5 minutes
Denaturation	35cycle	94 °C for 1 min
Annealing		57°C for 1 min
Extension		72 °C for 1 min
Final Extension	1 cycle	72 ° C for 10 mins.





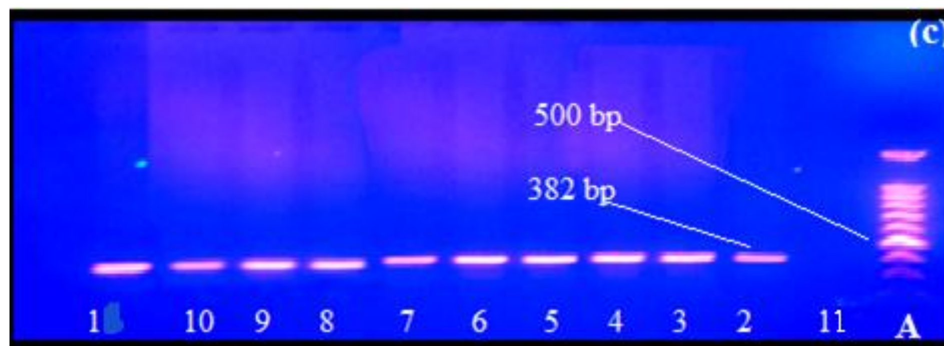


Figure (1; a, b and c): Gel electrophoresis of (a) PRLR 1, product size 307 bp. (b) PRLR 2 product size 306 bp.(c) PRLR3 product size 382 bp.Electrophoresis (1.5% agarose gel, at 80v/mAMP for 50min). Line A=100bp ladder, line (1-10), line (11) control negative.

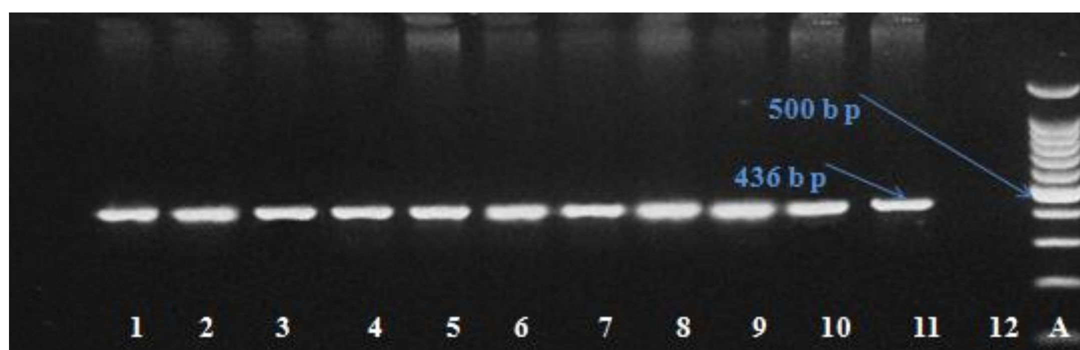


Figure (2): Gel electrophoresis of amplification of PRLR4 of hyperprolactinemic patients product size 436 bp. Electrophoresis was performed on 1.5% agarose gel and run with a 80v/mAMP current for 50min.Line A=100bp ladder, line (1-10) DNA isolated from blood samples of hyperprolactinemic patients, line (11) healthy and line (12) control negative.

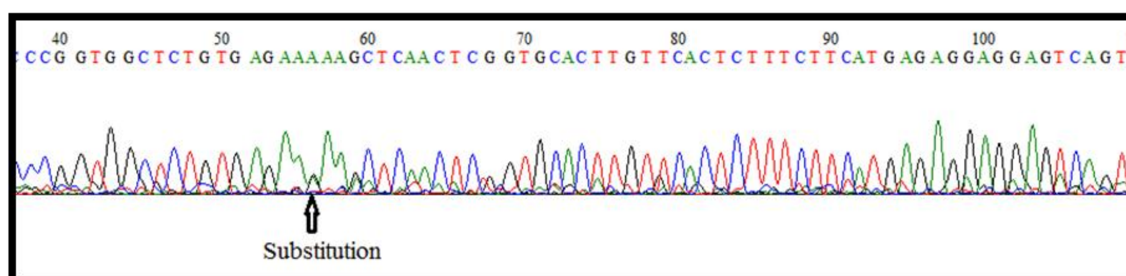


Figure (3): Peaks of sample 5, that indicate the substitution mutation.

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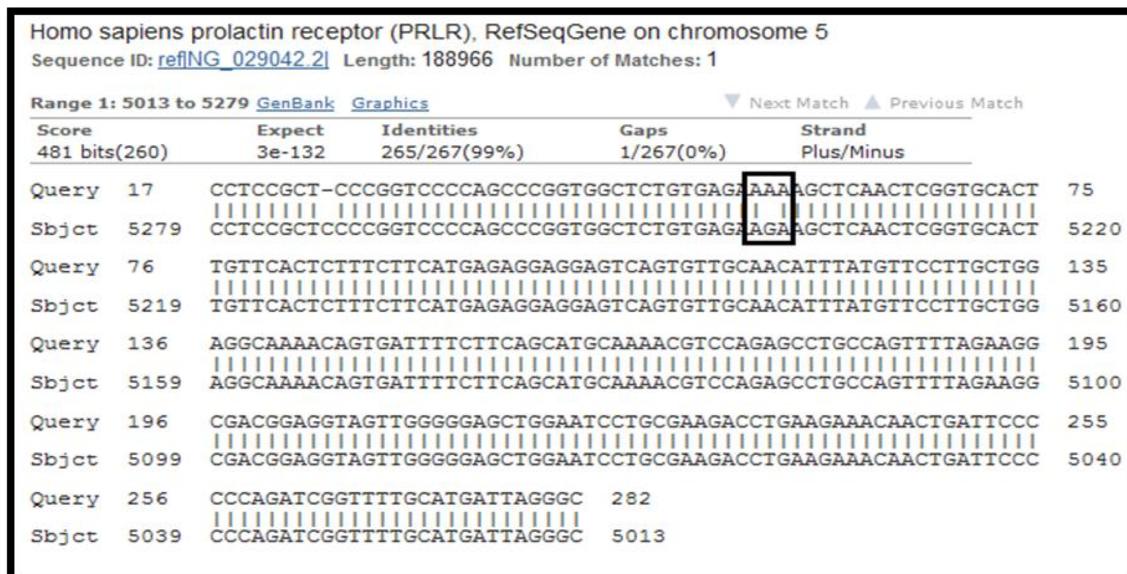

Figure (4): NCBI of hyperprlactemic patient (sample 5), of PRLR gene.

Table (3): Mutations of human PRLR gene of hyperprolactemic patients

No. of patient sample	Site on gene	Wild type	Mutant type	Change in a.a	Site on nucleic acid	Type of mutation
5	Exon 1	AGA	AAA	Arg/Lys	61	Substitution