Gene Polymorphism of Phosphodiesterase 8B and its association with some biochemical variables that contribute to thyroid disorders

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Abstract
The aim is to describe the polymorphism of PDE8B gene that some of which are related to TSH levels in blood. The study focusses on the polymorphism of phosphodiesterase 8B (PDE8B cAMP) gene and its correlation with some biochemical variable for hyperthyroid patients. The polymorphism of PDE8B gene, levels of Enzyme and the thyroid hormones of the serum have been studied for the hyperthyroid patients (thyrotoxicosis) in Iraq. The polymorphism of (PDE8B)rs(rs4704397) has been studied for 60 people who are suffers from hyperthyroidism and 30 of healthy people. The genotyping for all sample has been determined by using SNP genotyping methods. The levels of Thyroid hormones in blood have been measured by RIA radioimmunoassay. The levels of T3 and T4 for the patients group were higher than those with the healthy group with a decrease in the concentration levels of TSH. The genotyping and the allele frequencies for PDE8B gene between the patients group and the healthy group weren't so much different. Comparing to the healthy group there was no correlation among the percentage, the allele frequencies and the genotypes of (PDE8B) with the Patients. The concentrations of the hormones levels also have been studies as per the genetic structure of PDE8B (cAMP) for the mutated genotypes compared to the normal genotypes in comparison with the normal Allele. The data shows the spread of hyperthyroidism with women (especially in the cases of pregnancy or confinement) more than with men. The disruption and the metabolism of the thyroid hormones that caused hyperthyroidism by the effects of the enzymes that encode by PDE8B gene.
Keywords: biochemical variables; patience; thyroid disorders

Introduction

The thyroid is an endocrine gland which is the most important gland after the pituitary gland in human body. Thyroid disease are the endocrine gland most common diseases and it is usually more common in women than men.(Karaca and A kpak,2015). According to the statistics of World Health Organization "W.H.O." about three billion people all over world live in the countries that suffers from the lack of Iodine. Iodine is the key element in producing the thyroid hormone. Thus, the lack of this element will negatively affect the protein synthesis. Iodine is required to produce T4 hormone and T3 hormone (Gharib et al., 2004; Fatourechi,) 2009; Villar et al., 2007). T3 and T4 hormones are produced by the follicular cells of the thyroid gland and are regulated by thyroid-stimulating hormone secreted by the anterior pituitary gland. TRH hormone (Kopp,2005)The control of the thyroid function depends very much on the regular function of the of thyroid - pituitary gland - thyroid (through a negative feedback loop) T3 and T4 have inhibitory process to produce TRH and TSH (Annika et al.,2014) The thyrotropin has a High sensitivity against the small variables in the concentrations of the thyroid hormone (Santiseban,2005) the range of the thyrotropin is a sensitive indicator for the function of the thyroid. The high and the low concentrations of the TSH refer to hyperthyroidism and hypothyroidism respectively (Annika et al.,2014 ؛ Alves and Manoel ,2017). T3 the active form of thyroid hormone TH, The half-life of T4 is also longer compared to T3 (7 days versus 24 hours). When T4 loses an iodine atom, it becomes T3 (Woeber et al.,2005)

The thyroid hormones control the body's growth, developments metabolism, heart functions and much more. Thyroid dysfunction may cause hyperthyroidism or hypothyroidism (Pedro et al.,2011 Alves and Manoel,2017).

hyperthyroidism in Graves is marked by increasing the TH levels (T3 and T4 with increasing TSH hormone levels as well) thyroid hyperplasia and dermatopathy. The prognose is characterized by duality. The main autoimmune processed are reflecting in thyrotoxicosis, lymphocyte infiltration and thyroid hyperplasia which may be accompanied by about 15 - 25% dermatopathy and 0.5 - 4.5% Myxedema. The process of autoimmune are associated to the development of autoantibodies against different antigens, such as TSH receptors and Thyroid peroxidase enzyme TPO.

The thyroid hormones play a vital role in the physiology of human body and affect on most of the Histology to control the growth and development, maintain the normal cognitive level, the heart functions and blood vessels, the bones health, metabolism and energy balance.

Recent developments have made better understating on how the function of the thyroid can

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be affected by the genes. This has also have led to a greater knowledge about the complexities of thyroid hormones system and the differences among the individuals and the resulted diseases (Panicker, 2011) even people who aren't associated with them share about 99.9% of their own genome.

The statistics show that 90% of the remaining discrepancies are due to the Nucleotide polymorphisms SNPs (approx. 10 million) and the major changes prevalent throughout the genome. This is, of course, is very important in the study of all the genes, the study of genotype since it is common the general population and this may cause change in the gene functions. The thyroid hormones are important organizations work through Nuclear thyroid hormone receptors TRS in approximately all the tissue during development and throughout the individual's lifetime Tetraiodothyronine (Thyroxine‹.L-5,3‹5‹3) T4 is a prohormone orbiting on high concentration in the peripheral blood in relation to active hormone L-TRI-3‹5‹3' the control in the concentrations of T3 and T4 can be made by the metabolism (Gharib et al., 2004; Fatourechi, 2009; Villar et al., 2007)

Hence, this study has been conducted to find out the polymorphism of PDE8B gene. Risks of thyroid disorders for those people whom suffer from this disease in Salahaddin Province, Iraq.

8B (PDE8B) is a kind of PDE8B in the human chromosome q14.1 in Antron 1 and refer to monophosphate adenosine (cAMP). PDE8B Gene is always mentioned as something related to the thyroid by recently it was discovered in other parts of human body; the placenta and the ovaries according to a new study at the genomic level. Six different kinds of the single-nucleotide polymorphisms SNP have been associated in PDE8B gene by increasing the concentrations of TSH in the blood serum (Granfors et al 2012). PDE8B was found in the chromosome 5 which encodes the protein that stimulate the hydrolysis and disables AMP.

Genome wide association studies (GWAS) has been conducted and A> G SNP (rs4704397) was found inside this gene to be associated with the concentrations of TSH. Each copy of A Allele enable and average increase mU/TSH. The strongest association of the increasing levels of TSH was reported (overthought it is within the normal range) for 1 SNP selected in PDE8B ‹rs 4704397 (Arnaud-Lopez et al., 2008);

This leads to a difference among the Homozygous major and minor topics from 0.25µL TSH and this SNP was associated to the hyperthyroidism subclinical acne (Shields et al., 2009). In SNP rs 4704397 of PDE8B, the adenine-nucleotide A is replaced by G.

The association among the polymorphism and the high levels of TSH and the decrease of free T4 levels which refer to the relative hyperthyroidism with the Homozygous females' transporters of A/A (Taylor et al., 2011).

Materials and Methods

The study has been conducted on 90 people, 60 of them have been diagnosed as hyperthyroidism and 30 of healthy people. All the risk related information has been explained to all the participants in this study along with their permission and approval accordingly. The average patient age was ranging from 20 - 40 years old. All the samples have been collected and transported to the lab as per the standard terms and conditions.

Measurements

The level of the serum for all the samples, Thyroid Stimulating Hormone TSH; Triiodothyronine (T3) and Thyroxine (T4), have been measured by the diagnostic kits to conduct the required experiments.

Testing

The serum was separated from blood to conduct the tests that was performed by the Enzyme-Linked Immunosorbent Assay (ELISA), TSH, T3 and T4 tests for the thyroid by using the diagnostic kits. T4 has been measured by Microplate enzyme immuno-assays and the test for TSH was by using Immuno-enzyme assays for the microplate.

PCR

The genomic DNA has been extracted from the White Blood Cells (WBCs) by using Nacl/ chloroform/ Isoproponoal as mentioned in (Bartlett & White, 2003) for all hyperthyroid patients and those healthy people. The DNA samples were stored at 80°C. The genotype for the DNA samples was determined by using the polymerase chain reaction-restriction fragment length polymorphism (PCR– RFLP) using PCR premix kit (I-Taq). 2µm of Premix and 2µm of genomic DNA were added to the total of 20µm

The amplification was made by use the following: 5-GGCGCTACTCTAGGTTGGA-3 / R: 5-GTCTGCTCCTTGGCTTTTCC-3 that produced 519 PCR basis point that was cut by the BslI restriction enzyme BslI from New England revealed 1 part out of 519 points has PDE8B-G so the GG genotype is polymorph and homozygous, also it reveals 318 & 201 bp of AA Genotype (wild-type). Also the GA genetic makeup has been shown as polymorph and Heterozygous in three parts of 519,318 and 201 points. The below program has been adopted for the polymerase chain process:

(Table 1) : Polymerase chain process

Stage	Temperature	Time	number of cycles
Pre Deaturation	℃ 94	5 seconds	1
Deaturation	℃ 94	30 seconds	35
Anneling	55.57.60	30 seconds	
Elongation	℃ 72	30 seconds	
Final elangation	℃ 72	5 seconds	1

Analysis of statistical data

The date has been recorded and then analyzed by T-test and the independent chi-square by SPSS program. The value of P <0.05 was found as a statistically significant.

Results

The most participants of this study were women, the average age for the patient and the control group was 20-30, 31-50, 51-65; and the standard deviation for the level of T3 in the hyperthyroidism was 0.856 ± 0.258 against the control group which was 0.519 ± 0.143 at P (≤ 0.01).

The standard average and deviation for the levels of Thyroxine T4 in the hyperthyroidism was 26.074 ± 4.529 while the control group was 21.097 ± 3.967 at P (≤ 0.01).

The standard average and deviation for the levels of thyroid stimulating hormone TSH in the hyperthyroidism was 3.4804 ± 0.930 against the control group which was 4.0242 ± 0.684 at P(≤ 0.01) (figure 1). 25% of the patients have a family history for Thyroid disorders and 75% have no family history, as for the control group they don't have any family history related to this disease.

As stated in figure 1, the results show that people who have normal genetic structure GG and suffer from hyperthyroidism is 3 persons; 5% of the samples under study whereas the number of the healthy people was 4 persons %13.33. The number of patients who have AG genotype is 51 persons and those they constitute around 85% of the participants whereas the number of the healthy people who have AG genotype is 20 persons 66,67. As for those with AA genotype they were 6 people (10%) against the number of healthy people who have the same AA genotype which was 6 people (20%).

Also, it turned out that the allele frequencies for the mutation A is 63 (52.5%) and G for the healthy people is 57 (47.5%) and for the patients is 28 (23.33%) for the control group is 32 (26.67) respectively. There were no statistically significant differences among the patients and the viewers group in the allele frequency P=(0.915) (OR=0.967) as listed in Table 2.

There was a slight difference in the levels of T3 and T4 when comparing the normal allele against the variant allele P=(0.027) and P=(0.018) and a big difference in the value P=(≤0.01) in the concentration level of TSH when comparing the normal allele against the variant allele (Table 3)

(Table 2) Comparison among the Hormones concentrations for the Patients and the Healthy People

Hormone	Patients (60 individuals)	Healthy (30 individual)	pG value
	Mean ± SD	Mean ± SD	
T3 µg/ml	0.856 ± 0.258	0.519 ± 0.143	**≤ 0.01
T4 µg/dl	26.074 ± 4.529	21.097 ± 3.967	**≤ 0.01
TSH mIU/l	3.4804 ± 0.930	4.0242 ± 0.684	**≤ 0.01

G is the normal allele, while A is the variant allele.

(Table 3) The Percentage, the Allele Frequencies and the Genotypes of (PDE8B phosphodiesterase) 8B for (rs4704397) for the Patients and the Control Group

Genotype	Patients (60 individuals)		Control Group (30 individual)		p value	OR	95% CI
	No.	No.	(%)	(%)			
GG	3	4	13.33	5	Ref.		-
GA	51	20	66.67	85	0.129	3.400	0.697 - 16.569
AA	6	6	20	10	0.763	1.333	0.204 - 8.708
Allele	No.	No.	(%)	(%)	p value	OR	95% CI
G	57	28	23.33	47.5	0.915	Ref.	-
A	63	32	26.67	52.5		0.967	0.519 - 1.799

(Table 4) The results of hormones' level saccording to the genotype pde8b for (rs4704397) for the patients

variant	GG (No. 3)	GA (No. 51)	AA (No. 6)	p value
	Mean ± SD	Mean ± SD	Mean ± SD	
T 3 (ng/ml)	0.629±0.139	0.861±0.382	0.925±0.419	0.027 *
T 4 (mµ/ml)	24.575±0.268	26.59±3.638	22.424±0.373	0.018 *
TSH (mµ/ml)	5.390±0.454	3.372±0.601	3.443±0.470	≤ 0.01**

P ≥ 0.05: Non-significant; *: Significant at p ≤ 0.05; **: Highly significant at p ≤ 0.01

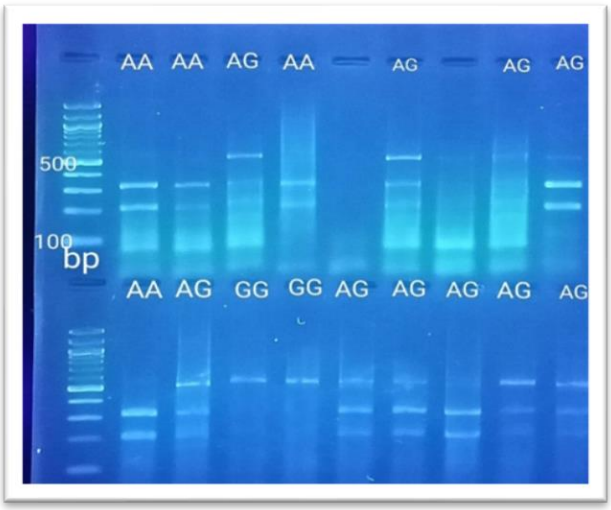

Figure 1 shows the electrophoresis for the results of PCR_RFLP for PDE8B gene on Agarose Gel with a concentration of 2%.

M: represents 100 DNA Ladder bp
AA: represents the mutant Homozygous genotype
AG: represents the heterozygous genotype
TT: represents the normal Homozygous genotype
The results of the PCR with the restriction enzyme have shown three kinds of genotypes; GG Normal Homozygous and is represented by the group of the particle size 519, the second type is AG Heterozygous and this is represented by (519,318,201) and the last type is AA Mutant Homozygous which is represented by two small groups 318,201 (figure 1)

Discussion

The interactions between the genetic and environmental factors determine the appearance of hyperthyroidism. The study aimed to investigate the polymorphism of (phosphodiesterase 8B and its correlation with hyperthyroidism. The results revealed a significant decrease in the average of TSH of hyperthyroidism compared to the control group with a value of P=≤ (0.01), on the contrary, it's been noticed that there is an increase at the comparison for the concentrations of FT3 and FT4 of hyperthyroidism and the control group with a value of P=≤(0.01, ≤ 0.01) respectively, such results, of course, may lead to hyperthyroidis.
This study complies with the earlier study for Tug et al. The current study shows an evidence that the polymorphism (phosphodiesterase 8B) isn't correlated to hyperthyroidism with the value P=(0.915) and OR(0.967). The studies of Anna Grandone et al didn't show any correlation among SNP and T3 & T4. The proposed results of this study found out differences in the levels of FT3 and FT4 respectively when comparing the G normal allele and the A mutant allele among patients. These results don't comply with Michaela Granfors and others who advised that there is a correlation between the polymorphism and the high levels of TSH and T4. It refers to the relative hyperthyroidism, and it has been found in the Homozygous genotypes for A/A; Anna Grandone et al and Arnaud et al found that there is an increase in the level of TSH while in the current study this level has been decreased to be P= (≤ 0.01).

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