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RESEARCH ARTICLE

GENE POLYMORPHISM OF PHOSPHODIESTERASE 8B (RS4704397) AND ITS ROLE IN SUDANESE EUTHYROID GOITER.

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Abstract

Background and objectives:- Thyroid enlargement and thyroid nodules are common in the general population. The currently to evaluate the relationship between phosphodiesterase 8B gene rs4704397 polymorphism and euthyroid goiter as well as to assess the relationship between this polymorphism and blood levels of TSH, free T4 and free T3 among Sudanese euthyroid goiter patients.

Materials and Methods:- In a case control study, thirty patients with euthyroid goiter and thirty five healthy controls were enrolled. The clinical data were obtained and thyroid hormones levels were measured using ELISA. The PDE8B rs4704397 alleles and genotypes were identified by PCR-RFLP analysis,

Results:- Analysis of allele frequency showed that, GG, GA and AA genotypes of PDE8B rs4704397 for patients were 13%, 63%, and 24% respectively and that for the control group were 33%, 37%, and 3%. Statistically significant difference was found ($p = 0.010$) (OR= 2.7). However, the gene polymorphism indicated no association with serum levels of the FT3, FT4 and TSH ($p = 0.900, 0.053$ & 0.070) when the normal allele (G) as compared with the mutant allele (A) among euthyroid goiter patients.

Conclusion:- The study concluded that PDE8B A allele is associated with euthyroid goiter where as no association observed with FT4, FT3 and TSH levels.

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Introduction:-

In Africa, goiter is endemic in several countries, notably Congo, Uganda, Kenya, and Sudan; the prevalence of goiter is as high as 81% in some parts of these countries⁽¹⁾. In Sudan, endemic goiter and iodine deficiency disorders are serious health problems in many areas. The incidence of goiter among schoolchildren was estimated to be 85% in the Darfur region in western Sudan, 74% in the Kosti area in the center of Sudan, 13.5% in Port Sudan in eastern Sudan, and 17% in the capital, Khartoum⁽²⁾ iodine deficiency as well as increases with advancing age⁽³⁾. The

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prevalence of thyroid nodules is elevated in women in areas of iodine deficiency and increases with advancing age⁽³⁾.

Little is known about the prevalence of goiter in other areas of Sudan. In the areas studied so far, iodine deficiency was known as the principal etiologic factor. Though, consumption of pearl millet, vitamin A deficiency, and protein-energy malnutrition were also suggested as instrumental factors in the etiology of endemic goiter in western Sudan^(4, 5).

WHO classification of goiter grade 0 – no goiter presence is found (the thyroid impalpable and invisible), grade 1 – neck thickening is present in result of enlarged thyroid, palpable, however, not visible in normal position of the neck; the thickened mass moves upwards during swallowing. Grade 1 include also nodular goiter if thyroid enlargement remains un seen and grade 2 neck swelling, visible when the neck is in normal position, corresponding to enlarged thyroid – found in palpation⁽⁶⁾.

TSH serum concentration also has genetic determinants, as proven in a number of populations. Recently, in a genome-wide association scan among more than 350 000 single nucleotide polymorphisms (SNP), the rs4704397 SNP in the phosphodiesterase 8B (PDE8B) gene has been shown to be associated with circulating TSH levels. This SNP, in the general population, accounted for 2.3% of the variance in TSH. phosphodiesterase 8B gene rs4704397 polymorphism is located in intron 1 of PDE8B, a gene that encodes a highaffinity cAMP-specific PDE expressed in the thyroid gland and, likely, regulating TSH signaling⁽⁷⁾. This study was essentially designed to detect the association of PDE8B rs4704397 polymorphism to the incidence of euthyroid goiter and the relationship to thyroid hormones in Sudan.

Materials and methods:-

In case control study, thirty patients with euthyroid goiter Sudanese women and thirty five healthy controls were enrolled in this study. Goiter was diagnosed according to World Health Organization criteria (8). Consent was obtained from each participant. All information regarding risk factors was explained to all participants under the study. The patients' mean (\pm SD) age was 29.8 \pm 9.2 and control was 30.6 \pm 12.7 years. Blood specimens was collected and transferred to the laboratory under the standard conditions.

Serological tests:-

Sera were separated from blood for serological tests which were done by enzyme linked immunosorbent assays (ELISA) Thyroid tests included TSH; free T4 (FT4), free triiodothyronine (FT3) using omega diagnostic kits. T4 were assayed by microplate competitive enzyme immunoassay and TSH was assayed by microplate immuno enzymatic assay

PCR:-

DNA extraction was done by phenol/chloroform/isoamyl alcohol method as described by Chomczynski and Sacchi⁽⁹⁾ The obtained DNA was stored at -20°C until used.

DNA samples were genotyped using polymerase chain reaction-restriction fragment length polymorphism (PCR–RFLP) using Maxime PCR premix kit (I-Taq) (Introgen Korea), positive control and negative controls, 2 μ l was added to PCR tube and the following solutions were placed in a total volume of 20 μ l; 10X Taq buffer, 2.5 mM 4dNTP stock (final concentration 200 μ mol), 10 pmol/ μ l primer F, 10 pmol/ μ l primer R, 100 ng of genomic DNA template, MgCl₂ (final concentration 1.5 μ m), H₂O (up to the total volume of 20 μ l) and 2.5uu Taq Polymerase. PCR amplification was performed using the following primers described by Anna *et al.*, (2012)⁽⁷⁾ as F: 5-GGCGCTACTCTAGGTTTGGA-3 and R: 5-GTCTGCTCCTTGCTTTTCC-3 which produces PCR product size 519 bp which cut by BslI restriction enzyme from NewEngland Biolab. The presence one fragment of 519 bp showed the presence of the PDE8B-A allele, to be AA (polymorphic homozygote) genotype, the presence of 318 and 201 pb fragments showed the GG (wild type) genotype; while the GA (polymorphic heterozygote) genotype was shown by three fragments of 519, 318 and 201 bp. Cycling conditions were as follows; initial denaturation at 94°C for 3 minutes, 35 cycles each at 95°C for 30 seconds, at 55°C for 30 seconds and 72°C for 30 seconds followed by at 5 minutes hold at 72°C. PCR and RFLP were examined on 2% agarose electrophoresis stained with Ethidium bromide.

Ethical consent:-

Permission of this study was obtained from the local authorities in the area of the study. The objectives of the study were explained to the local authorities in the area of the study (in White Nile State) and to all individual in the study. A written consent was obtained from each participates in this study.

Statistical data analysis:-

Data were recorded and then were analyzed using independent *t*- test and chi-square test by SPSS software, version 20.0. All tests were two-tailed, and a *P*- value of <0.05 was considered statistically significant.

Results:-

All participants in this study patients and controls are females. The mean (\pm SD) age of patients was 29.8 ± 9.2 and for control was 30.6 ± 12.7 . The mean and standard deviation of serum free T3 levels in euthyroid goiter and control group were (2.4 ± 0.7 pg/ml) versus (2.4 ± 0.7 pg/ml) ($P=0.800$), The mean and standard deviation of serum free T4 levels in euthyroid goiter and control group were (10.1 ± 1.8 pg/ml) versus (11.4 ± 2.1 pg/ml) ($P=0.140$) and the mean and standard deviation of TSH levels in euthyroid goiter and control group were (1.4 ± 0.7 pg/ml) versus (1.4 ± 0.7 pg/ml) ($P=0.010$) (Table 1). 20(66.7%) of patients had family history of thyroid disorders and 10 (33.3%) with no family history, all controls had not family history of thyroid disorders. Duration of goiter in patients ranges from 0 to 22 years.

13.3% ($n=4$) of patients were homozygous for the wild-type allele (G/G), 23.4% ($n=7$) were (G/A) and 63.3% ($n=19$) were (A/A). 34.3% ($n=12$) of control were homozygous for the wild-type allele (G/G), 25.7% ($n=9$) were (G/A) and 40% ($n=14$) were (A/A) While the allele frequency for the mutant A and the normal G were found to be 45 (75%), 15 (25%) for patients and 37 (52.9%), 33 (47.1%) for controls respectively. Statistically significant difference was found between patients and control group in allele frequency ($p = 0.010$) (OR= 2.7) (Table2). There was insignificant differences in FT3, FT4 and TSH levels when compared the normal allele with the mutant one ($p = 0.900$, 0.053 & 0.070) respectively (Table 3).

Table 1:- Free T3, free T4& TSH means, standard deviation (SD) and *P*.value of the Euthyroid goiter and controls.

Parameter	Patients($n=30$) (mean \pm SD)	Controls($n=35$) (mean \pm SD)	<i>p</i> -value
FT3	2.40 ± 0.7 pg/ml	2.30 ± 0.7 pg/ml	0.800
FT4	10.1 ± 1.8 pg/ml	11.4 ± 2.1 pg/ml	0.140
TSH	1.0 ± 0.70 pg/ml	1.4 ± 0.70 pg/ml	0.010*

$p < 0.05$, statistical significant followed with*

Table 2:- The association of the phosphodiesterase 8B Gene (rs4704397) genotypes and allelic distribution in patients and controls.

Case study	Genotype distribution			Allele Frequency		<i>p</i> -value/OR
	G/G (n)%	A/A (n)%	G/A (n)%	G (n)%	A (n)%	
Controls	(12)34.3	(14)40	(9)25.7	(33)47.1	(37)52.9	0.010* 2.7
Euthyroid goiter	(4)13.3	(19)63.3	(7)23.4	(15) 25	(45)75	

GG: wild type genotype; AA: homozygous genotype; GA: heterozygous genotype. $p < 0.05$, statistical significant followed with* and OR (Odd Ratio)

Table 3:- The effect of the phosphodiesterase 8B Gene Polymorphism on serum TSH levels and thyroid hormones FT4 and FT3 among euthyroid goiter patients.

Parameter	Alleles of phosphodiesterase 8B Gene (G/A)		<i>p</i> -value
	G Main \pm SD	A Main \pm SD	
FT3	2.40 ± 0.8	2.40 ± 0.7	0.900
FT4	12.4 ± 2.6	10.4 ± 1.7	0.053
TSH	1.60 ± 0.8	1.0 ± 0.6	0.070

Discussion:-

Interactions between individual genetic and environmental factors determine the onset of the Euthyroid goiter, as for the genetic factors there are only a few studies. The present study aimed to screen the phosphodiesterase 8B gene polymorphism and its relation to euthyroid goiter. The results of *t*. test analysis found significant decrease of mean TSH level of euthyroid goiter in comparison with control group with *p*. value 0.010, while insignificant differences were observed when compare the means of FT3 and FT4 concentrations of euthyroid goiter and control group with *p*. value 0.800 and 0.140 respectively these results may lead to subclinical hyperthyroidism. This finding is in agreement with previous reported study of Tug *et al*⁽¹⁰⁾. The current, study provide evidence that phosphodiesterase 8B gene polymorphism is associated with euthyroid goiter with *p*. value 0.010 and OR 2.7 This is the first published study of the phosphodiesterase 8B gene polymorphism in Sudanese patients with euthyroid goiter. Anna Grandone *et al*, Shields *et al* and Panicker *et al* studies have not shown any association of this SNP with free T4 or T3^(7, 11, 12) these results assumed with this study which found insignificant differences in FT3 and FT4 levels *p*. value 0.900 and 0.053 respectively when compare normal allele (G) with mutant allele (A) among patients these finding disagree with Michaela Granfors *et al* whom found the association between the polymorphism and high levels of TSH and low free T4 levels, indicating relative hypothyroidism, is found in homozygous carriers of A/A⁽¹³⁾ also Anna Grandone *et al* and Arnaud *et al*. found significant increase in TSH level^(7, 14) while TSH level in this study was unaffected *p*. value 0.070 which may due to different population.

Conclusion:-

The study concluded that PDE8B A allele is associated with euthyroid goiter where as no association observed with FT4, FT3 and TSH levels.

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