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Studying the genotype of Aryl Hydrocarbon Receptor-Interacting Protein (*AIP*) Gene (rs641081C>A) in Iraqi Samples with Acromegaly Pituitary Adenoma

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Abstract:

Pituitary adenomas are the anterior pituitary tumors. Patients with an *Aryl Hydrocarbon Receptor-Interacting Protein (AIP)* mutation (*AIP*- mut) tend to have more aggressive tumors occurring at a younger age. Single nucleotide polymorphisms (SNPs) in many studies have been related to metabolic comorbidities in the general population. Study aims investigated the role of AIP gene SNPs with susceptibility to acromegaly pituitary- adenoma, with levels of LH, FSH, TSH, Testosterone, IGF1,GH, FT4 , Prolactin hormones and blood sugar levels. The study was conducted on a group of acromegaly patients, including 50 patients) both Genders) with hyperplasia of the ends, and apparently healthy control group. Genotyping of *AIP* gene SNP (rs641081C >A) was indicated significant differences in frequency percentage between the study groups. The frequency of heterozygous CA genotype was significantly ($p<0.01$) higher in the patients' group when compared with control. The Means of IGF1, GH, prolactin, testosterone, and FBS were significantly higher in patients at first treatment than that in control. While, the means of IGF1, GH, prolactin, and testosterone increased significantly in patients at last treatment than control. Patients mean level of TSH decreased significantly at last treatment. No significant differences ($P \geq 0.05$) were detected between study groups in all other values. The Means of IGF1, GH, and prolactin at first and last treatment were significantly decreased, while LH was significantly increased at last treatment. In patients with heterozygous mutant and wild genotype, means of IGF1, GH, and prolactin decreased significantly at last treatment compared to first treatment. In this study heterozygous rs641081C>A showed a risk factor for susceptibility of acromegaly. Also, serum IGF1, GH, prolactin, were affected by the SNP of *AIP* gene within carriers of genotypes of rs641081C>A. IGF1, prolactin and GH decreased significantly at last treatment compared to first treatment.

Keywords: Acromegaly, Aryl hydrocarbon receptor-interacting protein, Gene polymorphism, Pituitary adenoma.

Introduction:

Pituitary tumors are anterior pituitary tumors. Most of these tumors are benign, which can be classified according to the size or cell of origin. They can be classified according to size to micro_ adenoma, macro_ adenoma, and giant tumor where micro_ adenoma is less than 10 mm while macro_ adenoma is greater than 10mm and giant tumor more than 40mm. There are tumors in which the pituitary gland leads to the hormonal secretion of one or more hormones, while the pituitary gland tumors do not secrete hormones, but they put pressure on the anterior pituitary gland peripheral areas, which leads to a lack of hormone[1].The term acromegaly is derived from the Greek words akron, meaning extremity, and megas meaning great. Acromegaly is a chronic endocrine disease [2]. It was first described by the French neurologist Pierre Marie in 1886in adults. It is an infrequent chronic disease resulting from increase in releasing of GH and hence of IGF-1. When hypersecretion of GH happens in childhood, the resulting disease is referred to as gigantism rather than acromegaly. In

the vast majority of cases, the underlying cause of acromegaly has been detected to be micro- or macro-adenoma of pituitary gland[3]. Benign tumor in the pituitary gland causing Acromegaly, manifests many symptoms, signs and complexes which tumor cause such as headaches and relating to vision or sight due to high level HGH / IGF-1 effecting on several tissues and organs, and causing morphological changes in hands, enlargement in feet and features of face, anterior orientation, rib cage deformation with back kyphosis, cardiovascular disorders, bone joints and metabolism, sleep apnea, respiratory diseases, nerve diseases,sexualdisorders
and intestinal manifestations). The severity of clinical manifestations depends on the levels of IGF-1 and GH, diagnostic time and tumor size [4].

Some studies have been done in a group of patients with acromegaly who are reviewing National Diabetes Center (NDC), one of the largest specialized centers in endocrinology in Iraq, including acromegaly .There are several common mutated genes associated with acromegaly such as the hydrocarbon receptor- Albright syndrome reactive peptide receptor (AIP), , McCune- (MAS) and multiple endocrine neoplasia type 1 (MEN1) . Mutations in *AIP* gene give important severe characteristics in acromegaly. As compared with patients have non-mutated acromegaly, those with mutations in *AIP* have younger age, have larger tumor size, and high levels of GH., so treatment of acromegaly patients *AIP*-mutation is more difficult because the severity of the profile disease [5]. Aims of the study are to: evaluate SNP polymorphisms frequency in AIP gene of Iraqi samples with acromegaly in comparison with controls, study the association between the polymorphisms of this SNPs and the incidence of acromegaly in Iraqi patients and study the effect of AIP gene polymorphisms on the level of some hormones and parameters.

Materials and Methods:

Subjects and blood specimens' collection:

This study was carried out at the National Diabetes Center (NDC)/Al-Mustansiriya University during the period from 1/6/2020 to 1/9/2020. The study was conducted on a group of acromegaly, including fifteen patients with hyperplasia of the ends of both sexes (male and female). Apparently the health control group consists of fifteen healthy people. They were chosen on the basis of the absence of a tumor in the pituitary gland that causes acromegaly and facial features and hand size are free from enlargement due to pituitary tumor that causes acromegaly. Each patient on acromegaly has a record of his disease and the analyses he conducted at the National Diabetes Center. These records were used to obtain the results of their first visit to the center before receiving treatment comparing them with the analyses that we conducted for patients for the last treatment dose they were treated with. The samples collected from the patients of acromegaly was in a fasting state, as were the samples from healthy people. 10 ml of venous blood samples were collected. It was divided into 2 parts: the first part was an EDTA tube to isolate the DNA (molecular genetics study) , the second part included the serum obtained by placing the blood in a sterile tube gel and allowing it to clot at 37 ° C for 30 minutes before centrifugation . The tubes were centrifuged at 6000 revolutions per minute for a period of 5 minutes, then we collected the serum and distributed to five parts to make hormonal tests (GH analysis, IGF1 analysis, fertility hormones which contain Prolactin, LH, FSH, Tetestron and Thyroid hormones which contain TSH, FT4) and fasting blood sugar test.

Gene polymorphism of *AIP* gene SNPs (rs641081)

Total DNA was isolated from fresh whole blood collected in tube containing EDTA for molecular studies and applied with DNA purification kits (The WizPrep DNA Extraction kit)(W71050-100) . The primers in this study were used to amplify specific regions of *AIP* gene that contains rs641081 SNP. These primers were designed by (Deacon Designer). Primers were supplied by Alpha DNA Company as a lyophilized product of different picomoles concentrations, the sequences of primer (F: GCAGTAGTTGAGCAGCAG; R CCCATACTCCCAGGAACA). According to the instructions of the manufacturing company.Polymerase –Chain Reaction Components and Programs Polymerase Chain reaction were carried out after several attempts of optimization to detect the best temperature for annealing with a total volume of 20 . DNA samples from acromegaly patients (n=50) and apparently healthy subjects (n=50) were genotyped for the *AIP* gene SNPs (rs641081) with a EVA Green SNP genotyping assay using real time thermo cycler according to the protocol **was**recommended by the manufacturer (Table 1). The hormonal analysis for LH, FSH,PROLACTIN, TESTERON, TSH and F T4 was performed by using Automated Immune Assay (AIA) by the VIDAS auto analyzer, (bioMérieux Company) France, GLUCOSE GOD-PAP Reagent for quantitative determination of glucose in human serum and plasma. In vitro assay for the quantitative determination of IGF-I (Insulin-like Growth Factor I) and GH in human serum was done. The tests have to be performed on the Analyzer

family (LIAISON). The data were analyzed by using Statistical Package for Social Sciences (SPSS) version 25, as mean, standard deviation and ranges. Categorical data were presented by frequencies and percentages. Independent t-test (two tailed) was used to compare the continuous variables accordingly. To compare the hormonal traits levels between last and first treatment, Paired t-test was used. To assess the association between study groups and certain information, Chi square test was used. A level of P – value less than 0.05 was considered significant. Expected frequency was less than 5.

Table 1. PCR amplification program

Steps	Temperature	Time	Cycles
Hold	**95**	**15 min.**	**1 cycle**
Denaturation	**95**	**5 sec.**	**5 cycles**
Annealing	**60**	**20 sec.**	
Extension	**72**	**10 sec.**	
Denaturation	**95**	**5 sec.**	**40 cycles**
Annealing	**60 ***	**20 sec.**	
Hold	**55**	**60 sec.**	**1 cycle**
Melting temperature	**60-90 ***	**0.5 degrees each step Hold 2 sec. each step**	

*Dye detection (Filter HRM) for EVA green) Tm: 60

Result and Discussion:

Age and gender

The study group's distribution according to age and gender is shown in Figs 1, 2. Studying patients' age ranged from 23 - 66 years, and the mean is 42-37 years with standard deviation (SD) ± 45 years. The proportion of study patients is the highest in patient and control groups aged ≤ 45 years (66% and 62% respectively). That means the pituitary adenomas is anaggressive disease in young patients, and this result agrees with another study by Daly et.al [6], while it disagrees with the study of Rostomyan et.al [5] who found that the median age at the onset of rapid growth was younger in females than in males (11 (3; 14) *versus* 13 (10; 15) years, respectively, significantly; P=0.003).Regarding gender, 50% of acromegaly patients were males and 50% were females; while 60% of control group were female(6).. These results disagree with the same study of Daly et.al [6], which showed that the pituitary gigantism consisted of 208 patients, the majority were male (78%; n=163), and 22% female[5]. It also disagreed with Lenders et.al [7], who found that Acromegaly is more common in female and present in older ages [7]. It agreed with the study Colao et.al [8], who found no interaction between age and gender on biochemical and morphological parameters and explained that the treatment was different between two sexes, asserting that women require treatment with a higher dose for amounting to response [8]. Based on the considerations mentioned previously, this research will examine whether there is a gender effect on acromegaly by critically examining, biochemistry, demography, symptomatology, natural history, management, morbidities, mortality of this disease and treatment outcomes. Search was indexed in Pubmed and Medline using the terms, acromegaly, and, gender. Journal articles were individually reviewed for, quality, relevance, exclusion of duplicate data and size.

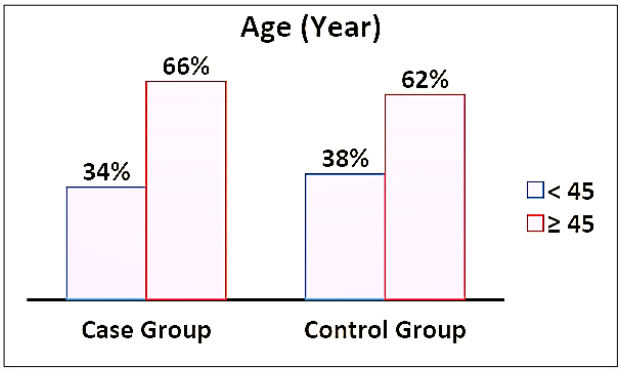

Figure 1. Distribution of study groups by age

Study groups comparison according to age and gender, no significant differences in gender and age (P ≥ 0.05) between study groups as shown in Tables 2, 3.

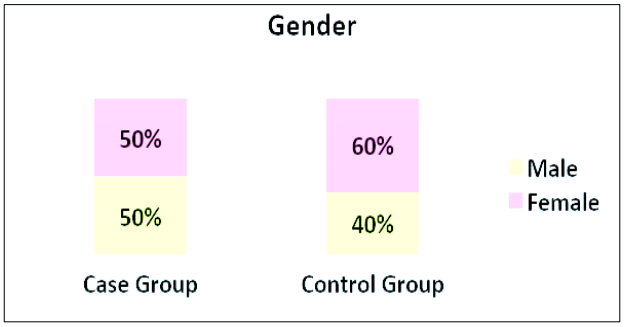

Figure 2. Distribution of study groups by gender

Table 2. Comparison between study groups by age.

Age (Years)	Study Group		P – Value
	Patients Mean ± SD	Control Mean ± SD	
	43.62 ± 9.5	7.2 ± 41.12	**0.917**

Table 3. Comparison between study groups by gender.

Gender	Study Group		Total (%) n= 100	P – Value
	Patients n= 50	Control n= 50		

Male	25 (50.0)	20 (40.0)	45 (45.0)	**0.314**
Female	25 (50.0)	30 (60.0)	55 (55.0)	

Fig 3 shows the type of adenoma in patient group. In this study half of acromegaly patients were diagnosed with macroadenoma and the other half were diagnosed with micro-adenoma.

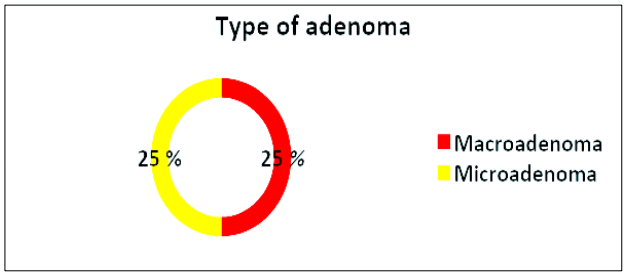

Figure 3. Type of adenoma in patient group

This classification may provide an accurate tool for selection criteria in clinical studies and important indications to identify patients have acromegaly with disease aggressiveness and outcome [9].

Genotype

The comparison between both groups by genotype is explained in Table 4. The results found that 84% of acromegaly patients had Heterozygous mutant genotype compared to 18% of control group and this difference was statistically significant (P= 0.001).

Table 4. Comparison between study groups by genotype -

Genotype	Study Group		Total (%) n= 100	P – Value
	Patient n= 50	Control n= 50		
Heterozygous Mutant (CA)	42 (84.0)	9 (18.0)	51 (51.0)	**0.001**
Wild (CC)	8 (16.0)	41 (82.0)	49 (49.0)	
	Allele Frequency			
C	0.58	0.91	-	-
A	0.42	0.09	-	-

These results agreed with the study of Yaman et.al [10], who suggested that the *AIP* gene variants at rs641081 and rs4930195 might be caused pituitary adenomas in the Turkish population (sporadic and familial), (and somatotrophinoma corticotrophinoma) [10]. The *AIP* encoding gene the chromosomal location (11q13.3) and many mutations (inactive) have been founded, which cause the development of sporadic and familial pituitary - adenomas (FIPA) [11] The first *AIP* germline mutations were identified in Finnish samples with FIPA. The types of mutation are (nonsense mutation splice site mutation Q14X and IVS3-1G>A, respectively) and Italian samples of patients have nonsense mutation (R304X) pituitary adenoma[12]. When using sequencing of AIP and assays of Multiplex Ligation dependent Probe Amplification (MLPA), No *AIP* mutation was found in a Turkish samples as a family[13,14]. That 50% of patients with FIPA had no mutation in *AIP* gene.

These polymorphisms are found in the database of dbSNP within the NCBI. Within the *AIP* locus in six ethnic populations samples of DNA there was 14 exonic SNPs which have been identified [15.16].The function of *AIP* gene as a tumor suppressor and composed of 6 exons, encode a 330 amino acid. The C-terminal region of the molecule composed of 3 tetratricopeptide repeat (TPR) domains that are 34 amino acid long conserved structures consisting of an α-7 helix that is important for protein–protein interactions [17]. The *AIP* gene mutations majority was altered structure of C-terminal, and they change the sequence of amino acid due to different mutations such as insertion, nonsense, or deletion mutations, which results in the protein truncation of and the C-terminus loss [18]. *AIP* polymorphisms cause AIP-phosphodiesterase-4A5 disruption of binding important for the structure of TPR, but it is play akey role in pituitary tumorigenesis [19].

Hormonal traits, blood sugar levels

At first treatment

Table 5 shows the comparison in hormonal traits and blood sugar levels between patient group at first treatment and control group. Means of IGF1, GH, prolactin, testosterone, and FBS were significantly higher in acromegaly patients at first treatment than that in control healthy people (945.04 *versus* 306.44 ng/ml, P= 0.001; 12.58 *versus* 1.5 ng/ml, P= 0.001; 16.66 *versus* 9.2 ng/ml, P= 0.001; 1.75 *versus* 0.89 ng/ml, P= 0.005; and 225.9 *versus* 96.5 mg/dl, P= 0.001, respectively).

No significant differences detected between both groups in all other values, statistically (P ≥ 0.05).

Table 5. Comparison in hormonal traits and blood sugar levels between Patient group at first treatment and control group

Variable	Study Group		P-Value
	Patient at 1st Rx Mean ± SD	Control Mean ± SD	

IGF1 (ng/ml)	945.04 ± 845.6	± 306.44 158.5	**0.001**
GH (ng/ml)	12.58 ± 14.7	1.2 ± 1.5	**0.001**
LH (mIU/ml)	3.6 ± 2.1	1.8 ± 3.91	**0.42**
Prolactin (ng/ml)	16.66 ± 12.3	9.2 ± 5.8	**0.001**
FSH (mIU/ml)	6.96 ± 4.9	6.54 ± 2.7	**0.589**
Testosterone (ng/ml)	1.75 ± 1.6	0.89 ± 1.3	**0.005**
TSH (umol/L)	1.96 ± 8.4	1.37 ± 0.89	**0.621**
FT4 (Pmol/L)	15.88 ± 4.2	15.99 ± 2.0	**0.872**
FBS (mg/dl)	225.9 ± 94.8	96.5 ± 7.2	**0.001**

Hormonal studies focus on prolactin, GH, FSH, LH, TSH, adrenocorticotropic hormone (ACTH), and IGF-1, cortisol, T3/T4 and sex hormones. These results were reported by [20] agreed with this study. Several studies showed the relationship between acromegaly hormonal traits such as the study is done by [21], which reported the examination of Hormonal assessed severe (LH 0.5 mIU/mL, FSH < 1.0 mIU/mL and estradiol 13 pg/mL), along with an increase in the levels of serum prolactin 43.2 ng/mL. About %18 of the patients were at age of 30 years or younger [21]. Among the young patients, there was a shift in the balance of gender, with more male than female patients. They were taller, in some young patients, GH have started early during puberty before growth plate closure. Levels of IGF-1 were high, the fact that IGF-1 peak during puberty and adolescence. More young patients had a macro adenoma and extrasellar extension, were treated with radiotherapy, or treated by medical combination therapy, which proved that the clinical course may be more severe in young patients [22], and the study was done by [23], which reported that the increase in GH, IGF-1, BLAP, LOX, and LPO can be associated with active acromegaly [23].

Table 6: shows the comparison in hormonal traits levels between patient group at last treatment and control group. Means of IGF1, GH, prolactin, and testosterone were significantly higher in acromegaly patients at last treatment than that in control healthy people (448.26 *versus* 306.44 ng/ml, P= 0.003; 4.69 *versus* 1.5 ng/ml, P= 0.007; 12.9 versus 9.2 ng/ml, P= 0.025; 1.44 versus 0.89 ng/ml, P= 0.04 respectively).

Mean of TSH was significantly lower in patients at last treatment than in control healthy people (0.92 versus 1.37 µmol/L, P= 0.012). No significant differences between both groups in all other values, (P ≥ 0.05).

Table 6. hormonal traits levels Comparison between patient group at last treatment and control group

Variable	Study Group		P - Value
	Patient at last Rx. Mean ± SD	Control Mean ± SD	
IGF1 (ng/ml)	448.26 ± 283.2	± 306.44 158.5	**0.003**
GH (ng/ml)	4.69 ± 8.2	1.2 ± 1.5	**0.007**
LH (mIU/ml)	4.44 ± 2.9	1.8 ± 3.91	**0.277**
Prolactin (ng/ml)	12.9 ± 9.9	9.2 ± 5.8	**0.025**
FSH (mIU/ml)	8.63 ± 8.5	6.54 ± 2.7	**0.102**
Testosterone (ng/ml)	1.44 ± 1.3	0.89 ± 1.3	**0.04**
TSH (µmol/L)	0.92 ± 0.8	1.37 ± 0.89	**0.012**
FT4 (Pmol/L)	17.01 ± 3.33	15.99 ± 2.0	**0.066**

The comparison in hormonal traits levels between last and first treatment among acromegaly patients is shown in Table 7. Means of IGF1, GH, and prolactin were significantly decreased while LH was significantly increased (P < 0.05) at last treatment compared to that at first treatment. No significant changes (P ≥ 0.05) detected in all other traits at last treatment compared to that at first treatment.

Acromegaly is a disease which causes systematic abnormalities such as cardiovascular, metabolic, endocrine, osteoarticular, respiratory and neoplastic morbidities and increasing mortality (1.26 to 3 times the mortality of general population) [24], Beside, the diagnosis is made(7–10) years when the symptoms were begin. So, the diagnosis is fixed and acromegaly treatment is explained, and the function of anterior pituitary and acromegaly is evaluated, the complications related are key role in this disease management.

Table 7. Comparison in hormonal traits levels between last and first treatment among acromegaly patients

Variable	Time		P – Value
	At First Treatment Mean ± SD	At Last Treatment Mean ± SD	
IGF1 (ng/ml)	945.04 ± 845.6	448.26 ± 283.2	**0.001**

GH (ng/ml)	12.58 ± 14.7	4.69 ± 8.2	**0.001**
LH (mIU/ml)	3.6 ± 2.1	4.44 ± 2.9	**0.019**
Prolactin (ng/ml)	16.66 ± 12.3	12.9 ± 9.9	**0.036**
FSH (mIU/ml)	6.96 ± 4.9	8.63 ± 8.5	**0.127**
Testosterone (ng/ml)	1.75 ± 1.6	1.44 ± 1.3	**0.098**
TSH (µmol/L)	1.96 ± 8.4	0.92 ± 0.8	**0.389**
FT4 (Pmol/L)	15.88 ± 4.2	17.01 ± 3.33	**0.134**

Acromegaly treatment should be done by a professional team; this team contains a neurosurgeon, endocrinologist, and a radiotherapist. The Treatment composed of: adenoma surgical resection by transsphenoidal approach, medical and radiotherapy[25]. [26] showed other details on these treatment modalities. The treatment role is to normalize the secretion and action of GH, decrease the levels of IGF-I to gender and age matched controls, the signs and disease symptoms relieve, complications reducing, the tumor growth control, the function of anterior pituitary is preserved and the recurrence of tumor is prevented. The goal is to reduce mortality by

Obtaining GH levels less than 2.5ng/mL and normalization of IGF-I for sex and age [27]. Many studies have founded that lower GH levels should be followed (1ng/mL) [28].

Comparison in hormonal traits levels between first and last treatment among acromegaly patients according to genotype is shown in Tables 8, 9 respectively. In patients with heterozygous mutant genotype, means of IGF1, GH, and prolactin were significantly decreased ($P < 0.05$) at last treatment compared to that at first treatment. In patients with wild genotype, means of IGF1 and GH were significantly decreased ($P < 0.05$) at last treatment compared to that at first treatment. No significant differences ($P \geq 0.05$) detected in all other traits at last treatment compared to that at first treatment in wild and heterozygous mutant genotypes. Mutations of *AIP* involved at younger age pituitary adenomas with larger tumors than in adenomas with non-AIP-associated [29.] They usually are somatotropinomas or mixed GH and prolactin secreting adenomas and this secretory profile, combined with the young age at onset childhood and adolescence means that the *AIP* gene mutations of the most recurring known cause of pituitary gigantism [6].These characterizations of typical AIP related-pathology and FIPA come from large international series of hundreds of acromegaly patients (29.18). Clinicians need less typical pituitary presentations, like potential cases of pituitary apoplexy or prolactinoma. Moreover, these conditions can develop at an early age but may not be linked to the genetic condition until decades later. The penetrance of pituitary disease in *AIP* mutations patients has been assessed in a few families[30,31].

Table 8. Comparison in hormonal traits level among acromegaly Patients according to genotype at first treatment

Variable	In Heterozygote Mutant genotype	In Wild genotype	P-value
IGF1 (ng/ml)	996.23 ± 909.4	676.25 ± 251.5	0.263 NS
GH (ng/ml)	12.24 ± 15.2	14.37 ± 12.4	0.502 NS
LH (mIU/ml)	3.51 ± 2.1	4.06 ± 2.3	0.571 NS
Prolactin (ng/ml)	15.61 ± 10.5	22.2 ± 19.3	0.636 NS
FSH (mIU/ml)	6.93 ± 4.5	7.17 ± 7.2	0.593 NS
Testosterone (ng/ml)	1.67 ± 1.6	2.13 ± 1.9	0.379 NS
TSH (µm/L)	2.22 ± 9.2	0.61 ± 0.2	0.421 NS
FT4 (Pmol/L)	15.34 ± 4.4	18.62 ± 1.4	0.107 NS

NS: Non-Significant.

Table 9. Comparison in hormonal traits level among acromegaly patients according to genotype at last treatment

Variable	In Heterozygote Mutant genotype	In Wild genotype	P-value
IGF1 (ng/ml)	461.14 ± 289.5	380.62 ± 253.4	0.492 NS
GH (ng/ml)	5.0 ± 8.8	3.02 ± 3.11	0.677 NS

LH (mIU/ml)	4.01 ± 2.3	6.66 ± 4.6	0.302 NS
Prolactin (ng/ml)	11.83 ± 8.8	18.5 ± 13.8	0.584 NS
FSH (mIU/ml)	7.04 ± 4.6	17.01 ± 17.1	0.219 NS
Testosterone (ng/ml)	1.49 ± 1.4	1.18 ± 1.3	0.755 NS
TSH (µmol/L)	0.96 ± 0.9	0.71 ± 0.5	0.698 NS
FT4 (Pmol/L)	16.71 ± 3.4	18.37 ± 2.7	0.327 NS

NS: Non-Significant.

In two families, 30 to 50% of asymptomatic mutation carriers have been diagnosed with high prolactin or IGF1levels with normal pituitary magnetic resonance imaging (MRI) findings. [30, 31]These percentages are based on very preliminary investigations. Extra pituitary disorders related to mutation of *AIP* could be suspected because of the *AIP* expression of non-endocrine specificity. It has been found that an AIP mutation child aged 2-year gives a premature the larches [31]. Levels of Glucose in acromegaly patients increased with increasing the levels of IGF-1, and showed no GH correlation. Although GH recruit insulin resistance and increase the glucose levels, IGF-1 may be considered a good marker of the metabolic burden of acromegaly; this is fit in other national samples analyses [32]. The IGF-I receptors presence was assessed in normal and neoplastic thyroid tissue in humans. There is scientific evidence that IGF-I reveals an essential, TSH–independent effect in the processing of growth in humans thyroid [33,34]. There are several studies which describe the goiter increasing prevalence diffuse and nodular in acromegaly patients, and many researchers have assessed a positive correlation between the concentration of serum IGF-I volume of thyroid. The study published by [33] has proved that thyroid carcinoma and thyroid nodular disease are more frequent in patients with acromegaly than in general population, significantly [35]. Results of the retrospective study by Turkish authors [36], included 64 patients with acromegaly who were subjected to thyroid US examination and thyroid function tests (distribution of thyroid diagnoses). [36] found out an increased prevalence of thyroid cancer, significantly high when compared to control group [37]. Thus, it is suggested that acromegaly patients should be routinely submitted to thyroid ultrasound evaluation, followed by Fine Needle Aspiration Biopsy (FNAB) of nodules when indicated. Results of relationship of *AIP* mutation and Testosterone levels showed no significant correlation between study groups, but this does not mean there is no association between acromegaly and high levels of testosterone, there are several studies which reported the relation between them. [37] explained that the testosterone and estradiol mean as well as the ratio of testosterone to estradiol showed a higher significant difference in acromegaly patients when compared with the control. [37] assessed aromatase activity and hormones such as estradiol, testosterone and another parameters such as, GH, IGF-1and BMI in Iraqi patients with active acromegaly and contrasted with control Serum GH level very highly significant increase ($P \geq 0.001$) was remarked in this study in active acromegaly patients when contrasted with control groups. Acromegaly were disorders created by increased secretion of growth hormone on account of tumor in the pituitary [38]. GH activities anabolic on numerous organs systems were very much recorded. GH invigorates bone growth during the childhood, and it induces skeletal maturation [39]. [40] found the GH stimulates longitudinal bone growth and BMI and expanded in acromegaly patients, because of positive correlation with the weight of body with bone mineral density, the reason is not obesity [40]. The results in our study are in conformity with the results explained by [40], who assessed that difference noted inter alia both in healthy control and acromegaly group and no variance particularly active acromegaly and control [40] . But the result disagreed with the results acquired by [41], who found that no significant differences were shown when compared with physiological levels [41]. The testosterone concentrations were significantly lower in patients with other types of tumor such as benign prostatic hyperplasia (BPH) [42, 43].

Conclusion:

According to the findings, this study concluded: *AIP* gene at (rs641081C<A) SNP has a risk role in the acromegaly pituitary adenoma incidence in Iraqi samples. Iraqi samples with CA genotype are more susceptible to acromegaly pituitary adenoma. Serums of IGF1, GH, prolactin, are affected by the studied SNP of AIP gene within carriers of genotypes of rs641081C<A (IGF1, prolactin and GH are decreased at last treatment compared to that at first treatment. and this study a proofed that the heterozygous mutants at rs641081C>A of AIP *gene* that showed a risk factor for susceptibility of acromegaly. Also, it indicated that serum IGF1, GH, prolactin, were affected by the studied SNP of AIP *gene*.

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Authors' declaration:

- Conflicts of Interest: None.
- We hereby confirm that all the Figures and Tables in the manuscript are mine ours. Besides, the Figures and images, which are not mine ours, have been given the permission for re-publication attached with the manuscript.
- Authors sign on ethical consideration's approval
- Ethical Clearance: The project was approved by the local ethical committee in Mustansiriyah University.
- The author has signed on animal welfare statement. N:725 Date 2020\9\22

Authors' contributions statement:

Y. S. A. A. This is a first author, she did all the methods in this study beginning in collect samples and writing it.S. J. K. she is the supervisor, made the proposal, help in review the search (writing and explanation the results and discus them. A. Y. A. S. help in diagnosis acromegaly patients and collection the samples from them.

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دراسة الطرز الوراثية لجين (*AIP*) Aryl Hydrocarbon Receptor-Interacting Protein في عينات عراقية ضخمة الاطراف مصابة بورم الغدة النخامية (A<rs641081 C)

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الخلاصة:

اورام الغدة النخامية هي اورام في الغدة النخامية الامامية. يميل المرضى الذين يعانون من طفرة AIP- mut إلى الإصابة بأورام أكثر عدوانية تحدث في سن أصغر. هدفت هذه الدراسة لمعرفة دور تعدد الطرز الوراثية الـ SNPs لجين الـ AIP بالإصابة بالورم الحميد النخاعي عند ضخام الاطراف وتأثيرها في مستويات الهرمون المنبه للجريب FSH الهرمون المحفز للجسم الاصفر، الهرمون المنبه للدرقية، التستوستيرون، IGF1 -1، GH، FT4 فضلا عن البرولاكتين ومستوى السكر في الدم. أجريت الدراسة على مجموعة المرضى ضخام الأطراف ، من بينهم 50 مريضاً (ذكورا واناثا) ومجموعة السيطرة 50 من الاصحاء ظاهريا. تم تحديد التنميط الجيني لجين *AIP* (SNP>A rs641081C). بينت النتائج وجود اختلافات معنوية بين مجموعات الدراسة لهذه التراكيب الجينية والترددات. اما فيما يخص النمط الوراثي CA المتغاير الاليل كان تردده عالي (P <0.01) في عينات المرضى. اما النمط الوراثي AA عند rs641081C> كان تردده اقل (p <0.01) في عينات المرضى مقارنة بالأصحاء. عوامل IGF1 و GH والبرولاكتين والتستوستيرون و FBS كانت أعلى بشكل ملحوظ في مرضى ضخام الاطراف في العلاج الأول مقارنة بالأشخاص الأصحاء و لم يتم الكشف عن فروقات معنوية احصائياً بين مجموعتي الدراسة في جميع القيم الأخرى. بينما كانت عوامل IGF-1 و GH والبرولاكتين والتستوستيرون أعلى بشكل ملحوظ في مرضى ضخام الاطراف في العلاج الأخير مقارنة بالاصحاء . متوسط TSH أقل بشكل ملحوظ في المرضى في العلاج الأخير منه في الأصحاء وتم الكشف عن فروق بدلالة إحصائية بين مجموعات الدراسة في جميع القيم الأخرى. ووسائط IGF-1 و GH والبرولاكتين في العلاج الأول والأخير، انخفضت النتائج بشكل ملحوظ بينما زاد LH بشكل كبير عند آخر علاج مقارنة بالعلاج الأول. اظهرت الدراسة أن طفرات متغايرة الاليل لـجين AIP عند A<rs641081C تزيد خطر الاصابة بضخامة الاطراف، وتاثر مستوى IGF – 1 و GH والبرولاكتين بـالطرز الوراثية المدروسة لـجين AIP عند A<rs641081C. ولوحظ ايضا انخفاض IGF- 1البرولاكتين و GH في العلاج الأخير مقارنةً بالعلاج الاولي.

الكلمات المفتاحية: تضخم الأطراف، بروتين اريل الهيدروكاربوني المتفاعل مع مستقبلات الهيدروكربون، تعدد الاشكال الجيني، ورم الغدة النخامية.