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DETERMINE THE GPS MUTATIONS IN ACROMEGALIC SAMPLE OF IRAQI PATIENTS

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INTRODUCTION

Acromegaly is a syndrome that results when the anterior pituitary gland produces excess growth hormone (GH) after epiphyseal plate closure at puberty (Hodish and Barkan, 2008).

Acromegaly most commonly affects adults in middle age, and can result in severe disfigurement, complicating conditions, and premature death if unchecked. Because of its pathogenesis and slow progression**,** the disease is hard to diagnose in the early stages and is frequently missed for years until changes in external features, especially of the face, become noticeable (Miller et al., 2011).

In over 90 percent of acromegaly patients, the overproduction of growth hormones is caused by a benign tumor of the pituitary gland, called an adenoma (Fieffe et al., 2011). Most pituitary tumors arise spontaneously and are not genetically inherited. Many pituitary tumors arise from a genetic alteration in a single pituitary cell which leads to increased cell division and tumor formation. This genetic change, or mutation, is not present at birth, but is acquired during life. The mutation occurs in a gene that regulates the transmission of chemical signals within pituitary cells; it permanently switches on the signal that tells the cell to divide and secrete growth hormones. The events within the cell that cause disordered pituitary cell growth and growth hormone over secretion (Tamburrano *et al.*, 2002). The mutations of the gene for the α-subunit of G protein, (Gsα) have been identified in human growth hormone (GH)-secreting pituitary adenomas (Vallar *et al.* 1987, Landis *et al.* 1989, Clementi *et al.* 1990, Lyons *et al.* 1990) and clinically non-functioning pituitary adenomas (Williamson *et al.* 1994).

The Gsα mutations, that have been reported, (*gsp* mutations) inhibit the guanine triphosphatase activity of Gsα. Inhibition of guanine triphosphatase leads to persistent activation of adenylyl cyclase and continually elevated intracellular cAMP levels in pituitary tissue, leading to cellular proliferation, differentiation and hypersecretion. Thus, *gsp* mutations in pituitary tissues result in hyper functioning and non-functioning gland adenomas (Landis *et al.* 1989, Dhanasekaran *et al.* 1995). These mutations are detected in either codon 201 in exon 8 (arginine replaced by cysteine, serine or histidine) or codon 227 in exon 9 (glutamine replaced by arginine or Lucien) of the *Gsp_* gene.

The aim of this study was to find the rate of reported mutation and search for if there is another mutation are related with our cases.

MATERIAL AND METHOD

Subject

Sixty patients with active acromegaly were examined in the national diabetic centre in Baghdad from September 2013 to December 2014. Patients had diagnosis of active acromegaly that comprised clinical examination and an increase in the serum levels of GH and or IGF-1 (Trinder, 1969). Each patient was asked to fill a special prepared formula of inquiry which included a meticulous history. All of them have history of pituitary adenoma at least more than one year. The enrolled patients are taking monthly long acting octreotide injections. The control group was twenty apparently healthy individuals were randomly selected as non acromegalic counter parts.

Methodology

Genomic DNA was extracted from EDTA blood or clotted blood samples using the Relia Prep Blood gDNA Miniprep kit (Promega, Madison, USA).

PCR was used to amplify DNA fragments including Exon 8 and Exon 9 of the Gas gene were amplified using a pair of oligonucleotides: forward primer (Ex8-F), 5’-CAGAAACCATGATCTCTGTTA -3’; and reverse primer (Ex8-R), 5’-TCGGTTGGCTTTGGTGAGATCCAT -3’. Primers for exon 9 of the Ga s gene were: forward (Ex9-F), 5’- CCAGTCCCTCTGGAATAACCAG -3’; and reverse (Ex9-R), 5’-CAGCGACCCTGATCCCTAACA -3’. Genomic DNA was amplified in a 50µl PCR mixture containing 2X Go Taq Green Master Mix (Promega), 0.4µM of each primer, 50ng of target DNA. Thermal cycling was performed in a MyCycler (BioRa). The reaction included an

initial 95°C denaturation step for 5 min, followed by 35 cycles of denaturation at 94°C (30 s), annealing at 60°C (30 s), and extension at 72°C (30 s), with a final extension step of 10 min at 72°C. All PCR products were analyzed on 1% agarose gel, stained by ethidium bromide.

PCR products had prepared and sending, for sequencing, to National Instrumentation Center for Environmental Management (NICEM), Korea. 25µl of PCR product has prepared for each sample, 2 directions are analyses (forward and reverse) for significant analyses.

RESULTS AND DISCUSSION

For the 60 acromegalic patient and the 20 non acromegalic counterparts DNA was extracted according the manufacturing protocols. PCR amplification of exon 8 and 9 of Gsp gene were performed for the genomic DNA of the patients and control subjects using specific primers. All fragments showed the expected size on a 1% agarose gel (Fig 1 and Fig2). The purified PCR products were analyzed to determine the sequence of genomic DNA.

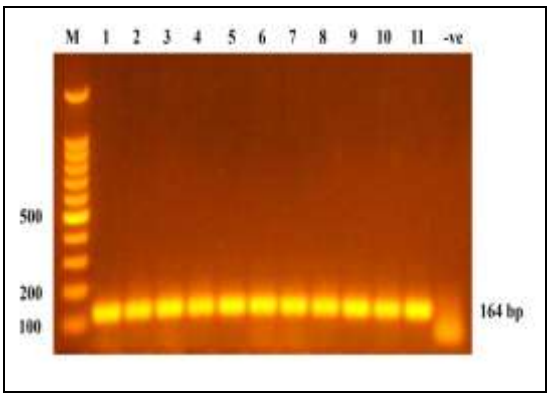

Fig.1: PCR product for amplification of exon 8 of Gsα gene.

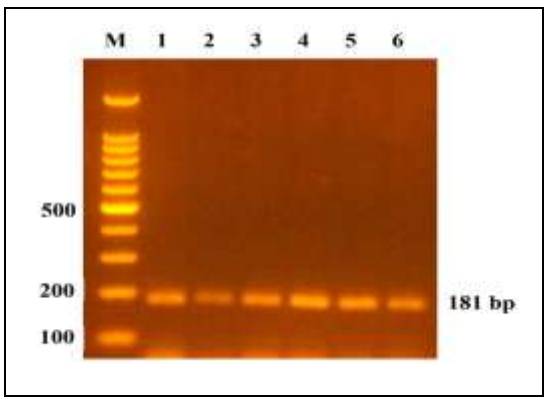

Fig.2: PCR product for amplification of exon 9 of Gsα gene.

Nine mutations were identified in 28 patients' samples from 60 which are A, B, C, D, E, F, G, H and I.

The C and I mutations has been reported in several acromegalic patients having pituitary adenoma from different populations. While A, B, D, E, F, G and H are novel. The remaining 32 acromegalic patients have no such mutations, so 28 out of 60 patients have the above mentioned mutants and the remaining were found to have normal work up on exons 8 and 9 of chromosome 20.

In figure 4-3 and table 4-1 have represent the mutations with its abbreviation and positions.

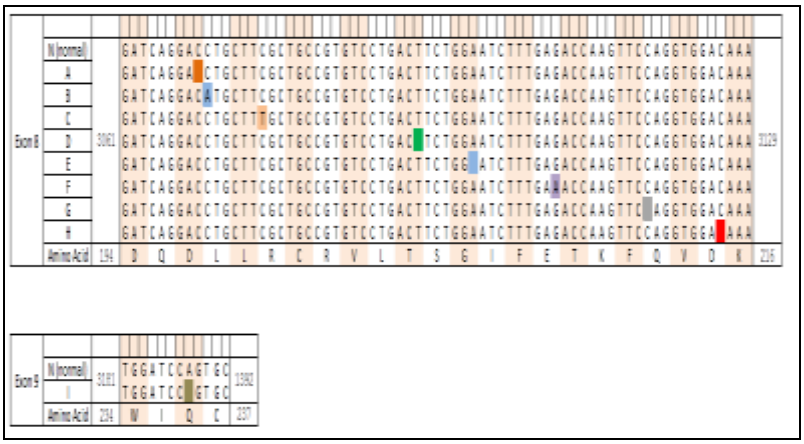

Fig 3: the comparison between normal sequence of exon 8 and 9 from side and the patients sequence from the other side. The result show that we have several variation in exon 8 take the symptoms (A, B, C, D, E, F, G and H) while just one.

Table (1): Positions of Mutations.

Exon	Codon	Wild Type	Variation	Name of variation	Variation Form
8	196	GAC	GA-	A	c196 del C
	197	CTG	ATG	B	c197 C>A
	201	CGC	TGC	C	C201 C>T
	204	ACT	AC-	D	c204 del T
	206	GGA	GG-	E	c206 del A
	209	GAG	GAA	F	c209 G>A
	213	CAG	-GA	G	c213 del C
	215	GAC	GA-	H	c215 delC
9	227	CAG	C-G	I	c277 delA

From table (4-1) and fig. (4-3) which represented the variations in nitrogenous bases were found in different codons of exons 8 and 9 of Gsp gene on chromosome no. 20 of human.

Table (2): Distribution sample study according to sequencing.

Sequencing		Number.	Percentage (%)
No Mutation		32	53.33
Mutations	A	20	33.33
	B	1	1.67
	AD	1	1.67
	AE	1	1.67
	AF	2	3.33
	ACE	1	1.67
	F	1	1.67
	GHI	1	1.67
	Total	60	100%
	Chi-square –χ2	---	12.439 **
	** (P<0.01).		

As shown in table 4-2 C and I mutations were detected in 2 of 60-GH secreting pituitary adenomas (3.34%) of Iraqi acromegalic patients' samples. Both C and I mutations are well known in acromegalic patients (recorded previously) and those C and I mutations are found in combination with A and E mutation in a single female patient and found as well in combination with G and H mutation in another male patient. The reported frequencies of GSP mutations in patients with GH-secreting pituitary adenomas ranged from 4.3 to 4.4% of examined cases (Lyon, et al. 1990, Hosoi, et al. 1993) in Caucasians, The prevalence of GSP mutations was 27-43% of GH-secreting pituitary adenomas (Lyon et al1990, Barlier et al 1998), but in Japanese, the prevalence of GSP mutations was considerably lower (4.4- 9.3%) (Hosoi et al 1993, Yoshimoto et al. 1993). By contrast, Yang et al. (1996) reported that mutation of the GSP gene of GH-secreting adenomas in Korean acromegalic patients is as common as that found in Caucasian patients. But the results also revealed that the most common A mutation which was detected in 20 acromegalic patient (33.33%) followed by AF mutation as 2 (33.3%) so the prevalence of these types of mutations in Iraqis patients' samples are higher than C and I mutations.

We also found 6 samples show more than one mutations as AD, AE, AF, ACE and GHI and also we notice A mutation occurnase with other mutation in patients samples.

A number of acromegaly samples showed none of the previos mutations and sequence was normal that could be due to existence of mutation in other region rather than exon 8 and 9.

So 32 (53%) acromegalic patients samples shows normal sequence without mutations in exons 8 and 9 while 28 (47%) acromegalic patients in exons 8 and 9 of Gsp gene of GNAS chromosome.

We conclude the prevalence of Gsp mutations in GH- secreting pituitary adenomas was thought to differ geographically or racially.

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