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The response of acromegalic patients (Diabetic versus non-diabetic) to Long - acting - repeatable Octreotide (LAR) in the presence or absence of Glutathion S transferase (GSTM1,GSTT1) Genes

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Abstract

Across sectional study was conducted in the National Diabetes Center -Mustansyriah University during the period from May to December 2017 The study included 70 acromegalic patients who were diagnosed and treated with transphenoidal and hypophysectomy or radiotherapy or both ,however all of them are receving monthly LAR injections.

The response to treatment was given symbols :R1, R2, and R3 for the sake of clarification where R1 points to decrement in the size of the pituitary adenoma by ≥ 20% and R2 points to reduction of IGF-1 to a normal level while R3 represents decrement of (growth hormone GH) to less than 2.5 ng /ml which reflects disease control

Keywords:GSTM1,GSTT1 genes, Acromegaly**,** IGF-1**,** GH.

INTRODUCTION

Acromegaly is a rare metabolic disease resulting from hypersecretion of growth hormone (GH) from pituitary somatotroph cells . This high GH stimulates the secetion of Insulin-like factor-1 (IGF-1) mainly from the liver which induces the net results of the fully blown acromegalic signs and symptoms mainly enlargement of the acral parts (hands , feet , and head)(1). It is prevalence approoches 130 per million while it is annual incidence is 4 - 6 per million per year (2,3) Its symptoms are very slow therefore its diagnosis delayed for years , the age of diagnosis is usually after 40 years (2,3,4).Diagnosis based on : the clinical picture , high IGF-1, plus high nonsuppressible GH during oral glucose tolerance test (5,6,7). Comorbidities as hypertension and diabetes are common among acromegalic subjects(8,9)Glutathione S-transferases (GSTs) constitute multifunctional enzymesthat are coded by at least eight distinct loci: α (GSTA), μ (GSTM),θ (GSTT), π (GSTP), σ (GSTS), κ (GSTK),ω(GSTO), and ζ (GSTZ), each onecomposed by one or more homodimeric or heterodimeric isoforms(8,9).These enzymes are involved in the conjugation reactions duringphase II of the xenobiotic metabolism, catalyzing reactions betweenglutathione (GSH) and a variety of potentially toxic and carcinogenicelectrophilic compounds (10,11), besides, GSTs also display peroxidaseactivity and can thus protect from oxidative damage (12) The deficiencyin the activity of this enzyme can be derived from the inheritedGSTs polymorphisms, e.g., GSTT1 (22q11.23), GSTM1 (1q13.3) (13).These genes were studies in all enrolled patients to find out the impact of presence of such gene on the response of our enrolled patients to octreotide LAR ,injections .The enrolled subjects are subdivided to diabetic and nondiabetic. The study was designed in a way to detect any relation between those harboring GSTM1 or GSTT1 or both to control of their disease in the presence or absence of diabetes.The gold standard therapy is trans - sphenoidal hypophysectomy ,however it is not the only modality as patient have to be treated individually keeping in mind the role of medical treatment with somatostatin analogues as octreotide LAR or Lanreotide and there is a role for gamma-knife radio surgery in some patients.The main problem which is faced by the physician is selction of the ideal modality of treatment in order to achieve the three goals which are :reduction of the adenoma size if its removal is not fcosible ,reduction of IGF-1 to the age and sex matched control and reduction of GH to ≤1 ng/ml that marks cure or ≤ 2.5 ng/ml that marks disease control

MATERIAL AND METHODS

Subjects and Sample Collection

In the National Diabetic Center (NDC) ,more than 250 acromegalic patients are registered and followed up for variable time but the registry starts in 2004. These patients attend The NDC regulary monthly to be followed up and given their injection of LAR. Seventy patient are selected randomly during their regular visits, their mean age (40.1±12) years, 35 are diabetics representing group A while the remaining 35 acromegalic patients are non diabetic group B at the time of enrollement .The duration of acromegaly in groub A is (1-34) with a mean duration of (10.43±8.81) years while the a duration of acromegaly in the group B counterparts ranges from 2 -28 years with a mean of (7.09 ± 6.22) ,these subjects are nondiabetic at the time of enrollement .The aim is to find out their response to octreotide LAR and its relation to being diabetics or not plus the reflection of Glutathion S transferase gene on their response to octreotide LAR,radiologically,by IGF-1 decrement and by GH reduction. Serum GH ,and IGF-1 levels were measured using Enzyme Linked immunostbent assay (ELISA).The gentic studies were conducted by collecting 5 ml of venous blood to be divided into 2 parts , 2 ml are kept in a test tube and EDTA was added, this sample is utilized for DNA extraction after being stored at -20˚C till the time of the test while the remaining 3ml were collected in another tube waiting for the clotting followed by centerifugation at at 3000 rpm for 10 minutesthe serum is utilized for biochemical workup. Going back the collected blood sample for DNA extraction ,genomic extraction is conducted by using DNA mini kit which was supplied by Relia Preptm ™ Blood g DNA miniprep kit, USA .primer used in this study were obtained from Promega company The GSTM1 gene Forward strand primers 5'-GTTGGGCTCAAATATACGGTGG -3' and Reverse strand primers 5'- GAACTCCCTGAAAAGCTAAAGC -3' while The GSTT1 geneForward strand primers 5'-TCACCGGATCATGGCCAGCA -3' and Reverse strand primers 5'- TTCCTTACTGGTCCTCACATCTC -3',.[14] the next step is Polymerase Chain Reaction(PCR) for the sake of amplification which is conducted followed by gel electophoresis utilizing 1%agrose gel then UV transilluminator is utilized to detect the expected bands which are reflective of the presence of the gene (GSTM1,GSTT1).

Stathistical method

Chi-square was used to compare percentage and student -T test was used to compare means P value of <0.05 is regarded to be significant.

RESULTS:

Figure 1 showed positive results for using GSTM1 and CYP1A1genes. Figure 2 showed positive results for using GSTT1 and CYP1A1genes.

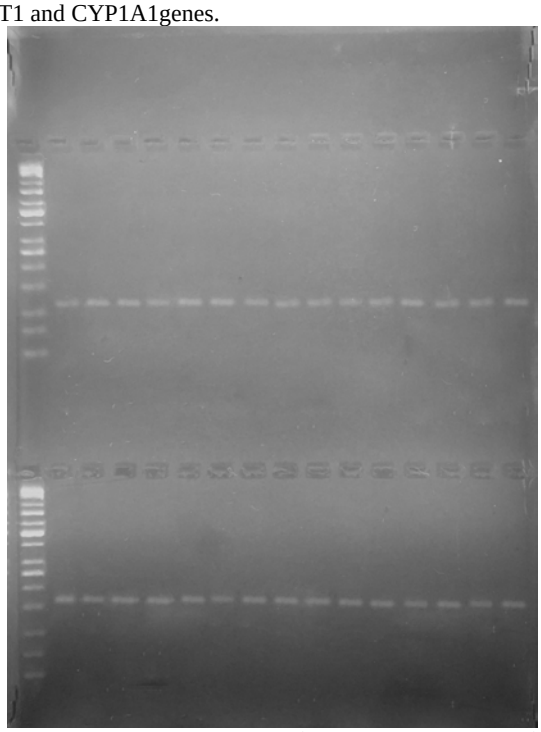

Figure 1:- Agarose gel electrophoresis for *GSTM1* gene (215bp) of first lane and*CYP1A1*gene(312 bp) in second lane . Bands were fractionated by electrophoresis on a 2% agarose gel (2 h., 5V/cm, 1X TBE) and visualized under U.V. light after staining with Ethidium bromide. Lane: 1 (M: 100bp ladder), Lane: 1to 30 product PCR positive.

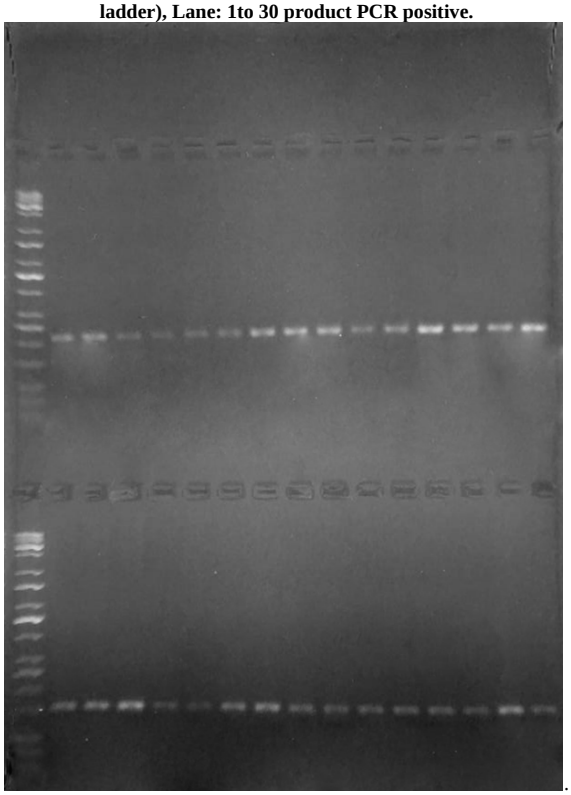

Figure 2:- Agarose gel electrophoresis for *GSTT1* gene (480bp) of first lane and*CYP1A1*gene(312 bp) in second lane . Bands were fractionated by electrophoresis on a 2% agarose gel (2 h., 5V/cm, 1X TBE) and visualized under U.V. light after staining with Ethidium bromide. Lane: 1 (M: 100bp ladder), Lane: 1to 30 product PCR positive.

As shown in table 1, the mean age of diabetes is (51.37±11.66) years while it is 40 ± 10.25) years in nondiabetic counterparts .The dose of octreotide LAR is higher in diabetics versus nondiabetic acromegalic subjects (701.14 ± 373.44) mg and 518 ± 330.79) mg respectively.

Growth hormone GH and IGF-1 are found to be lower among diabetics versus nondiabetics as shown in table 2. Table 3 show non stathistically significant difference in diabetic versus non-diabetic acromegalic subjects in the presence of GSTM1 or GSTT1 gene.

Table 4 shows the response by radiology (adenoma regression) tooctrotide LAR thus the response was found to be in diabetic: 63.3% if both GSTM1 and GSTT1 are presence, 50% if GSTM1 only presence,66.7% if GSTT1 only presence,20% if both GSTM1 and GSTT1 are null . so the best response by radiology is found to be the best among the GSTT1 presence in diabetics.In nondiabetics acromegalic patients the radiological response was found to be 36.4% if both GSTM1 and GSTT1 are presence,50 % if GSTM1 presence,33.3% if GSTT1 presence and 80% when both of them are null So the best response to octreotide LAR in nondiabetics was found to be in those GSTM1 and GSTT1 null, however the difference is responsiveness in diabetics versus nondiabetics is found to be non significant stahistically but numerically evident P= 0.371. Table 5 shows that in diabetics IGF-1 drops to normal in 57.1% if GSTM1 and GSTT1 presence , 50% if GSTM1 presence , 28.6% if GSTT1 presence, 40% if GSTM1 and GSTT1 null, while in nondiabetics 21.4 % are responsive by IGF-1 decrement if GSTM1 and GSTT1 presence, 33.3% if GSTM1 presence, 23.1% when GSTT1 presence however the response jumps to 60% when GSTM1 and GSTT1 are negativity thus the negativity of GSTM1 and GSTT1 makes 40% of diabetics diabetics, and 60% of non diabetics responsive to octreotide LAR by adenoma regression when they are GSTM1 and GSTT1 negative but the difference is not stathistically significant P=0.392. Table 6 shows that 72.7 % of diabetics are responsive when GSTM1 and GSTT1 presence but only 27% are responsive if they are non diabetic .

Being GSTM1 presence makes 50% of diabetics and non diabetics are responsive to octreotide LAR , While GSTT1 positivity makes 57.1% of diabetics and 42.9% of non diabetics responsive to LAR but if both GSTM1 and GSTT1 are null 40% of diabetics and 60 % of non diabetics are responsive to LAR by IGF-1 reduction down to normal age and sex - matched control but the difference is found to be stathistically nonsignificant P= 0.641.

Table 7 shows that being GSTM1 and GSTT1 presence makes 64.3 % of diabetics responsive to LAR by GH regression while only 21,4% of non diabetic are found to be responsive. 50% of diabetics who are GSTM1 presence are responsive while 66.7% of non diabetics are found to be responsive . Being GSTT1 presence makes 35% of diabetics and 30.8% of non diabetics responsive by GH regression.

Negativity of GSTM1 and GSTT1 makes 40% of diabetics and 100% of non diabetics responsive to LAR by GH regression which is found to be stathistically significant P= 0.012. Aa shown in table 8, 75% of diabetics are responsive when GSTM1 and GSTT1 are presence but it drops to 33.3% when GSTM1 is presence and 55.6% when GSTT1 is presence while when both genes are null the response drops to 28.6% in diabetics.

The scenario in non diabetics is different thus GSTM1 and GSTT1 presencesmakes only 25% responsive by GH decrement while when GSTM1 presence 66.7% become responsive but GSTT1 presence makes 44.4% of non diabetics responsive. If both genes are null 71.4 of non diabetics become responsive to octreotide LAR by GH reduction . The difference in response although numerically evident but stathistically nonsignificant P = 0.214.

Table 1: Demographic data ,duration of acromegaly and total dose of octreotide in diabetic and nondiabetic acromegalic subjects

Groups / Parameters		Diabetic Acromegaly		Non-Diabetic Acromegaly		
		No	%	No	%	
Age (years)	<30y	1	29	5	14.3	
	30---39	5	14.3	13	37.1	
	40---49	11	31.4	7	20.0	
	50---59	8	22.9	8	22.9	
	=>60y	10	28.6	2	5.7	
	Mean±SD (Range)	51.37±11.66 (29-72)		40.89±10.25 (24-63)		
Gender	Male	16	45.7	23	65.7	
	Female	19	54.3	12	34.3	
Governorate	Baghdad	23	65.7	23	65.7	
	Other Gov	12	34.3	12	34.3	
						P value
Duration of disease (years)	<5y	10	28.6	17	48.6	0.226
	5---9	12	34.3	9	25.7	
	=>10y	13	37.1	9	25.7	
	Mean±SD (Range)	10.43±8.81 (1-34)		7.09±6.22 (2-28)		
Drugs of Sandostatin 20 mg	<500	11	31.4	21	60.0	0.056
	500---	15	42.9	9	25.7	
	=>1000	9	25.7	5	14.3	
	Mean±SD (Range)	701.14±373.44 (160-1380)		518.57±330.79 (100-1300)		

Table 2 :Growth hormone and IGF-1 in diabetic and nondiabetic acromegalic patients.

Groups / Parameters	Diabetic Acromegaly N=35	Non-Diabetic Acromegaly N=35	P value
Growth hormone	4.61±4.85 (0.20-14.9)	5.85±6.55 (0.14-20.0)	0.370
IGF-1	651.42±347.76 (171.0-1683.0)	800.03±371.51 (232.0-1481.0)	0.089

Table 3: Radiological response by adenoma regression (R1) in diabetics versus nondiabetics in the presence of GSTM1 or GSTT1 gene.

GST	Diabetic Acromegaly				Non-Diabetic Acromegaly			
	R1				R1			
	Positive		Negative		Positive		Negative	
	No	%	No	%	No	%	No	%
M&T	7	50.0%	7	50.0%	4	28.6%	10	71.4%
M	1	50.0%	1	50.0%	1	33.3%	2	66.7%
T	4	28.6%	10	71.4%	2	15.4%	11	84.6%
Negative	1	20.0%	4	80.0%	4	80.0%	1	20.0%
P value	0.531				0.069			

Table 4: Resposiveness by adenoma regression(R1) in diabetic and non-diabetic acromegalic patient in relation with GSTM1 , GSTT1 positivity.

GST	Positive				Negative			
	R1				R1			
	Diabetic Acromegaly		Non-Diabetic Acromegaly		Diabetic Acromegaly		Non-Diabetic Acromegaly	
	No	%	No	%	No	%	No	%
M&T	7	63.6%	4	36.4%	7	41.2%	10	58.8%
M	1	50.0%	1	50.0%	1	33.3%	2	66.7%
T	4	66.7%	2	33.3%	10	47.6%	11	52.4%
Negative	1	20.0%	4	80.0%	4	80.0%	1	20.0%
P value	0.371				0.543			

Table 5: The response to octreotide LAR by regression of IGF-1 to the age and sex - matched control (R2)in diabetics and non diabetics acromegalic patients in relation with GSTM1 and GSTT1 positivity and negativity.

GST	Diabetic Acromegaly				Non-Diabetic Acromegaly			
	R2				R2			
	Positive		Negative		Positive		Negative	
	No	%	No	%	No	%	No	%
M&T	8	57.1%	6	42.9%	3	21.4%	11	78.6%
M	1	50.0%	1	50.0%	1	33.3%	2	66.7%
T	4	28.6%	10	71.4%	3	23.1%	10	76.9%
Negative	2	40.0%	3	60.0%	3	60.0%	2	40.0%
P value	0.495				0.392			

Table 6:Comparison of responsiveness of diabetic and non diabetics to octreotide LAR by IGF-1 regression in relation with GSTM1 and GSTT1 positivity or negativity .

GST		Positive				Negative			
		R2				R2			
		Diabetic Acromegaly		Non-Diabetic Acromegaly		Diabetic Acromegaly		Non-Diabetic Acromegaly	
		No	%	No	%	No	%	No	%
	M&T	8	72.7%	3	27.3%	6	35.3%	11	64.7%
	M	1	50.0%	1	50.0%	1	33.3%	2	66.7%
	T	4	57.1%	3	42.9%	10	50.0%	10	50.0%
	Negative	2	40.0%	3	60.0%	3	60.0%	2	40.0%
P value		0.641				0.690			

Table7: The response to octreotide LAR by GH reduction < 2.5 ng/ml in diabetics and non diabetics acromegalic patients in relation with being GSTM1 and GSTT1 positive and negative.

GST		Diabetic Acromegaly				Non-Diabetic Acromegaly			
		R3				R3			
		Positive		Negative		Positive		Negative	
		No	%	No	%	No	%	No	%
	M&T	9	64.3%	5	35.7%	3	21.4%	11	78.6%
	M	1	50.0%	1	50.0%	2	66.7%	1	33.3%
	T	5	35.7%	9	64.3%	4	30.8%	9	69.2%
	Negative	2	40.0%	3	60.0%	5	100.0%	-	-
P value		0.483				0.012*			

Table 8: Response of diabetic and non diabetic acromegalic patients by GH rduction in relation to GSTM1 and GSTT1 gene.

GST		Positive				Negative			
		R3				R3			
		Diabetic Acromegaly		Non-Diabetic Acromegaly		Diabetic Acromegaly		Non-Diabetic Acromegaly	
		No	%	No	%	No	%	No	%
	M&T	9	75.0%	3	25.0%	5	31.3%	11	68.8%
	M	1	33.3%	2	66.7%	1	50.0%	1	50.0%
	T	5	55.6%	4	44.4%	9	50.0%	9	50.0%
	Negative	2	28.6%	5	71.4%	3	100%	-	-
P value		0.214				0.168			

DISCUSSION

This study was conducted to find out the impact of Glutathion S transferase (GSTM1and GSTT1) polymorphism on the response of acromegalic patients to octreotide LAR when they are diabetic or non diabetic.

Non diabetic subjects were found to be highly responsive to LAR as defined by GH decrement to the target when they are absent for both GSTM1and GSTT1.GSTM1 and GSTT1 presence makes diabetics acromegalic patients more responsive by GH reduction GSTM1presence and GSTT1 null were found to make non diabetic subjects more responsive to LAR by GH reduction in non diabetics(15).The Duration of acromegaly is longer among those with diabetes, this is realy logical because of the fact that diabetes is expected to increase with the advance of age as well as the known effect of GH being diabetogenic(16).The regression of pituitary adenoma size is the best when both GSTM1 and GSTT1 genes are absent among non diabetic subjects so absent for these genes is expected to be predictor of regression of adenoma in non diabetic acromegalic patients(15-). Regression of IGF-1 down to normal is found to be better in non diabetic subjects when they are null for GSTM1 and GSTT1 gene but the difference in response by IGF-1 reduction in this scenario between non diabetic and diabetic acromegalic subjects is marginal.The impact of GSTM1 and GSTT1 geneon the response of acromegalic patients is variable in the presence of diabetes(17) .The impact is found to be affected being GSTM1or GSTT1 presence or absent and the affect vary among various parameters of response to octreotide LAR. To compare the current study with others a comparable study is not available after on extensive search in the literature however the impact of presence of GSTM1 or GSTT1 have been studied to find out its association with diabetes in North India(18).They observed presence association of GSTM1 genotype with type 2DM so GSTM1 gene polymorphism may be predictive marker of type 2 DM(18).The relation of these genes have been studied to fixed out any sussceptibility to astrocytoma and meningioma ,polymorphism in these genes encodes for carcinogen metabolizing enzymes thus having an unfavorable effect by increasing the risk to develop astrocytoma and meningloma thus there are on going studies to find out any detoxifying agent that can reduse the chance of development of thus tumors.(19)Lee et al. has found an association between GSTM1,GSTT1 and GSTP1 polymorphism and the risk of advanced colorectal cancer in smokers and non smokers, as smoking is known risk factor for colorectal adenoma and Glutathion S transferase can detoxify such carcinogens in tobacco smoke.GSTM1 inactive allels reduce the chance of Colorectal Cancer while having inactive GSTT1 allel was found to increase the risk of Colorectal Cancer among smokers only(19).The relation of these genes with breast cancer have been studied in Iraq to find out the impact of GSTP1 gene polymorphism on cancer of the breast. The workers try to find out the effect of Glutathion S transferase P1 being known detoxifying agent that protect DNA from exogenous and endogenous DNA destructive compounds.The frequency of GSTP1 polymorphisim in breast cancer group is significantly higher than the control group and the odds ratio is found to be 16.3 indicating that GSTP1 (Val /Val) genotype was associated with increased risk of breast cancer (20).

CONCLUSION:

The duration of acromegaly is found to be longer among diabetics versus non diabetics as well as the dose of octreotide LAR Growth hormone and IGF-1were found to be lower among diabetics.Absence of both GSTM1 and GSTT1 makes the response by adenoma regression very high among non diabetics however presence of these genes makes the response more favorable by adenoma regression in diabetics.Regression of IGF-1is better among non diabetics if GSTM1and GSTT1 are null but the difference from diabetics is marginal.Presence of GSTM1 and

GSTT1makes reduction of IGF-1 in diabetics very clear and favorablehowever if one of them is presnce the difference is marginal however it was found to be better in diabetics versus nondiabetics.Growth hormone decrement is very significant among non diabetics 100% when they are GSTM1 and GSTT1 null but presence GSTM1 makes diabetics better responsive than non diabetics.GSTM1 presence alone surprizingly better in non diabetics which was realy not in line with other parameters.

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