Total Antioxidant Activity and Insulin Resistance in Iraqi Male Patients with Active Acromegaly: A Case Control Study

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Received 12 August 2016; Accepted 17 December 2016; Available online 22 December 2016

ABSTRACT
Acromegaly is an endocrine hormonal disorder, characterized by progressive somatic deformation, lead to excessive secretion of the growth hormone (GH) and consequent increase of IGF-1 (insulin-like growth factor 1), most commonly produced by benign pituitary adenomas. Active acromegaly is characterized by increased levels of free radical and insulin resistance (IR). The aim of the study is to compare the correlate TAA/MDA with (IR) and the correlate of GH/IGF-1 with (IR). This study includes 30 male patients with active acromegaly, their ages ranged (30-40) years, and 30 normal healthy males, their ages ranged (30-40) years as a control group. Patients and volunteers with osteoporosis, osteomalesia, cardiovascular disease, renal failure, hypertension alcoholics, DM2 and smokers were excluded from the study. Blood was collected and the sera were separated from attended the National Diabetes Centre /Baghdad during the period from December 2015 to June 2016. Serum of GH, IGF-1, insulin were determined by (ELISA) kits, glucose, MDA TAA catalase enzyme were estimated colorimetric methods, (IR) = glucose × insulin /405 = IR. The results show that there were increase significant difference of GH, IGF-1, GH/IGF-1, insulin, glucose, (IR), MDA, between acromegaly patients and control group (p<0.001), and decrease significant difference of TAA, TAA/MDA, catalase, between acromegaly patients and control group (p<0.001) We conclude that, increase levels of GH, IGF-1, GH/IGF-1 leads to increase lipid peroxidation, through increase MDA levels, and increase oxidative stress, through decrease total antioxidant and catalase enzyme activities in patients with active acromegaly, in a result of elevated ROS production. GH has negative effect on the level of TAA; reduce level of TAA leads to elevated ROS. GH and IGF-1 have more effect on (IR) than TAA/MDA. Our recommendation is giving patients with acromegaly antioxidants as a complementary therapy to reduce the incidents of IR.

KEYWORDS: acromegaly, TAA, MDA, GH, insulin resistance

INTRODUCTION

Acromegaly is a chronic disease, caused by a pituitary adenoma; lead to excessive secretion of the growth hormone (GH). High level of GH causes hepatic secretion of insulin-like growth factor-1 (IGF-1), which in turn causes changes in the appearance of patient, metabolic disorders and many skeletal deformities [1].

Growth hormone acts at several levels to block actions of insulin, including inhibition of the insulin receptor phosphorylation and one of its principal signaling molecules in response to insulin administration. This result in decrease sensitivity of cells to insulin in the periphery in stimulating peripheral glucose uptake and to increased resistance to insulin's ability to suppress gluconeogenesis. So excess of (GH) leads to mobilization of free fatty acids and inhibit insulin stimulated glucose oxidation by acting as a competitive energy source thus leading to further worsening of insulin resistance[2,3].
Patients with active acromegaly are characterized by increased levels of free fatty acids and other lipid intermediates together with markedly increased lipid oxidation rates \[4\]When lipid oxidized with oxygen, the oxygen molecule itself becomes reduced and forms intermediates, and reactive oxygen species (ROS) are always produced \[5\].  

Reactive oxygen species (ROS) plays a major role in the pathogenic of many disorders including cancer, diabetes and brain edema \[6\].  

The blood antioxidant system is impaired in patients with active acromegaly, what indicates the development of oxidative stress \[7\].  

The aim of the current paper was to correlate study of TAA/MDA with insulin resistance and compare to the correlate of GH/IGF-1 with insulin resistance in patients with active acromegaly.

**MATERIALS AND METHODS**

1. **Subjects:**  
The patients were selected from from the National Diabetes Centre /Baghdad during the period from December 2015 to June 2016. This study includes 30 male patients with active acromegaly, their ages ranged (30-40) years, and 30 normal healthy males, their ages ranged (30-40) years as a control group. Patients and volunteers with osteoporosis, osteomalesia, cardiovascular disease, renal failure, diabetic mellitus, hypertension alcoholics and smokers were excluded from the study. All patients were diagnosed by physicians.

2. **Samples Collection:**  
Blood samples were collected and the sera were separated. GH, IGF-1 and insulin levels were determined by (ELISA) kits, FSG was determined using enzymatic colorimetric method (Glucose oxidase-peroxidase). Serum MDA was estimated by methods of \[8\], TAA was estimated by methods of (Koracevic D. 2001) and catalase enzyme was estimated by methods of \[9\]. \( \text{IR} = \text{glucose} \times \text{insulin} / 405 = \text{IR} \)

3. **Ethics:**  
Informed parental consent was obtained to be eligible to enrollment for the study, which was done according to the rules of the Local Ethics Committee of Chemistry Department, College of Science, Al-Mustansiriyah University, Iraq.

4. **Statistical Analysis:**  
Data were presented as mean ±SD using SPSS program version 20. The differences between two groups were analyzed by independent t-test, \( P \)-value ≤0.05 considered significant. Correlation coefficient was calculated between (IR) and other parameters.

**RESULTS AND DISCUSSION**

Table (1) shows the (Mean ±SD) of GH, IGF-1, GH/IGF-1 in serum of patients with acromegaly and control group. The results show that there were significant difference of GH, IGF-1, GH/IGF-1 between acromegaly patients and control group (\( p<0.001 \)). We suggest that the result of excessive secretion of the (GH) in patients with active acromegaly due to hormonally active adenoma of the pituitary gland and the excessive production of GH from interior loop of pituitary gland and IGF-1 increase parallel to GH, since IGF-1 production and release under GH stimulation effect. So, significant GH levels in present study are associated by significant IGF-1 levels.

[10] Showed reduction in mean GH levels after 3 months octreotide therapy, however, GH level remain higher than control group. [11] found that (GH) levels were markedly raised. [12] and other workers found that (GH) was higher than the normal value.[14] showed that significant increase in GH level in acromegalic patients group compared to control group.

[15], showed significant high levels in GH among active acromegalic group with respect to control acromegalic group are accompanied by significant elevation in IGF1 level in active acromegalic group with respect to control acromegalic. Many studies showed elevation in IGF1 levels which is reflect of GH elevation is also associated with several biochemical changes, as illustrated by laure E. Boreo and her co-worker [16].

Although there was study showed increased IGF1 level in acromegaly, disease can also occur in normal IGF1 level as showed in study by Lee HM. [17] that found acromegaly with normal IGF-1 levels this difference because systemic illness, may lower the IGF-1 level and result in false-negative values and its might be rare cases and difficult to diagnose.

Mumby C. and others and Pedro W. and other co-workers,(2014) (18,19) showed elevated IGF1 level in acromegalic patients than normal value before treatment, and after treatment found within the normal range.
CerdàGabaroi D. and other co-workers [20] found that IGF-1 level was high value than normal value in acromegalic patients. S. Tabur and others [14] showed that significant increase in IGF-1 concentration in acromegalic patients group compared to control group.

Pure somatotroph adenomas patients were significantly likely to present with abnormal glucose metabolism, independent of GH/IGF-1 levels or tumor invasiveness [21].

Table (2) show that serum glucose, insulin and IR were significantly increase in patients group in comparison to healthy subject, p<0.01. We suggest that prolonged GH hypersecretion results in pancreatic β-cell dysfunction which predisposes acromegalic subjects to elevation in insulin hormone in body.

The effect of GH on glucose and insulin sensitivity include initially that GH enhances insulin sensitivity and this assume thought IGF-1 pathway that act on insulin receptors and increase the receptor sensitivity. In addition when the levels of GH increase cause enhance the synthesis and so elevation of IGF-1 levels [22], which in turn inhibit GH release via negative feedback mechanism, and so control the GH level and therapy of glucose and insulin levels. But in high GH level as in adenoma this mechanism are overcome by continuous release of GH that increase glucose level. In response there is elevation in glucose level and so hyperinsulinemia with insulin resistance are develop.

We suggest that due to excess of GH secretion by a somatotroph adenoma, which can cause insulin resistance through the failure of a compensatory increase in β-cell function, the decrease in the insulin binding to the receptor due to a reduction of the receptor concentration (reduction in the receptor number per cell) without alteration on receptor affinity state. Endocrine disorder with excess growth hormone (GH) production results in important metabolic effects in insulin resistance. Insulin resistance is an important component of the pathogenesis of type 2 diabetes mellitus.

The results of the study are in agreement with what found by others, Rodrigues et al. [23] showed the prevalence of diabetes among acromegalic patient was 38%. However other study gave different results like the study by Fieffe S. et al. [24] showed the prevalence of diabetes among acromegalic patient was 22.3%.

In another study in acromegaly patients, the prevalence of diabetes was not different, these results confirm exists of diabetes is the most implication of GH excess [24].

Previous studied conclude that the risk factors for progression to diabetes in patients with acromegaly include higher GH level and a family history of diabetes [25].

Study by Rizza R. A. and co-workers [26] tried to be determine the mechanism of insulin resistance in acromegaly. They concluded that the increase in plasma GH can cause insulin resistance, which is due to decrease in both hepatic and extra hepatic effects of insulin and this decrease in insulin action can be explained on the basis of post receptor defect. The effect of free fatty acid (FFA) on partitioning of intra-cellular glucose fluxes was originally describe by Randle et al [27] according to this hypothesis (the glucose/fatty acids cycle), oxidation of FFA initiate an up-stream, chain-reaction-like inhibition of glycolytic enzymes, which ultimately inhibits glucose uptake(GH enhance mobilization and catabolism of FFA, increase catabolism of FFA cause increase production of acetyl-CoA that in turn inhibit pyruvate dehydrogenase and cause increase pyruvate level which in turn inhibit phosphofructokinase and cause increase in glucose-6-phosphate level that in turn inhibit hexokinase and glucose uptake).

Soo Kyoung Kim. and others [28] found of the 184 acromegalic patients the prevalence of diabetes was 37.5%, including those who were previously known diabetic patients. S. Tabur and others [14] showed that significant increase in fasting plasma glucose (FSG) concentration in acromegalic patients group compared to control group. Hochberg et al. [27] showed significant difference in FSG between acromegalic and patient group (p<0.013). Hochberg et al. [29] show a significant difference in fasting insulin level between acromegalic and patient group (p<0.012), all previous results are agreement with presenting study.

Hochberg et al. [29] showed higher significant difference in fasting insulin resistance between acromegalic and patient group (p<0.001).

Also table (3) showed that MDA level showed significant increase in patients group when compared to control group , TAA and TAA/MDA showed a significant decrease in patients group when compared to control group P<0.05.

We suggest that the elevated levels of MDA in acromegalic patients is a marker of increased oxidative stress or LPO due to the excessive production of GH, which inhibits glucose uptake and oxidation of FFA initiate an up-stream and then increase level of free radical.

Malondialdehyde (MDA), one of the most popular markers, consider a biomarker for oxidative stress and was designed to indicate lipid peroxidation [30]. The decrease in TAA indicates a lack of balance between elevated ROS production and antioxidant activity, which is inefficient. This situation can lead to deterioration function of different organ. We suggest that impair antioxidant system in acromegalic patients so leading to reduce TAA/MDA comparing to control group.

Faassen M.V. and others [31] Baizhumanov A.A., showed that plasma TAA in patients was on the average 20% lower than in the control subjects. These result confirm the imbalance in the antioxidant system of blood at
acromegaly patients, which leads to the development of oxidative stress in these patients, these result are agreement with our present study.

LhanYaylim. And other co-workers [32] show TAA levels are significantly decreased in patient groups (controlled and uncontrolled) compared to healthy subjects (p<0.001). These results are agreement with our present study.

Table (3) showed that catalase activity showed a significant decrease in patients group when compared to control group p<0.05. We suggest reduced levels of catalase, confirm an increased susceptibility to oxidative damage and this is an agreement with the study that found relation between LPO and catalase status. Catalase depletion can impair the cell defense against the toxic action of catalase and may lead to cell injury/death, this lead to increased lipid peroxidation and reduced antioxidant status in acromegaly patients. The decrease in the activity of catalase observed in the present work could be due to less availability of NADH and hence, no beneficial effect on catalase activity to reduce lipogenesis/oxidative stress [33].

Anagnostis P and others [12], also study by Faassen M.V. and others [31] found that acromegalic as compared with controls had significantly lower levels of catalase activity.

Table (4) showed the correlation between IR and TAA/MDA, GH, IGF-1 for acromegaly patients and which were revealed that in patients with acromegaly correlation between GH and TAA, IGF-1. Significant positive correlation of GH, IGF-1 with (IR) and significant negative correlation of TAA/MDA with (IR). In the result from this study we suggest that GH, IGF-1 have more effect on (IR) than TAA/MDA and GH has negative effect on the level of TAA, reduce level of TAA leads to elevated ROS.

Although IR is common in acromegaly, few studies report the effect of GH and IGF-1 on IR, and their results were not consistent Kasayama et al. and Fukuoka et al. reported no correlation between IR and serum GH or IGF1 levels. In contrast Stelmachowska-Banas et al. and Puder et al. reported their associations. In the previous study, one indicator of IR, HOMA-IR, was not correlated with GH or IGF-1 in a linear regression analysis, and the other indicator. Together with previous studies, other study by Kinoshita Y. et al. [3] results suggested that the GH/IGF1 axis and IR are not closely correlated in Japanese patients with acromegaly, even if acromegaly is involved in the increase in clinical parameters of IR.

The increasing production of free radicals and changes in activity levels of antioxidant enzymes in order to scavenge free radicals has an important effect in insulin resistance. [12] The GH/IGF-1 axis was surely involved in the exacerbation of IR.

**Conclusion:**

From this study, we conclude that, increase levels of GH, IGF-1, GH/IGF-1 leads to increase lipid peroxidation, through increase MDA levels, and increase oxidative stress, through decrease total antioxidant and catalase enzyme activities in patients with active acromegaly, in a result of elevated ROS production. GH has negative effect on the level of TAA; reduce level of TAA leads to elevated ROS. GH, IGF-1 have more effect on (IR) than TAA/MDA. Our recommendation is giving patients with acromegaly antioxidants as a complementary therapy to reduce the incidents of IR.

**Table 1:** Mean±SD, P-value of GH, IGF-1, GH/IGF-1 in control and patients with acromegaly

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Control Mean±SD</th>
<th>Patients Mean±SD</th>
<th>t-test</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>GH (ng/mL)</td>
<td>1.065±0.258</td>
<td>11.173±4.601</td>
<td>9.776</td>
<td>0.000*</td>
</tr>
<tr>
<td>IGF-1 (ng/mL)</td>
<td>274.47±73.153</td>
<td>493.41±83.506</td>
<td>5.429</td>
<td>0.000*</td>
</tr>
<tr>
<td>GH/IGF-1</td>
<td>0.027±0.012</td>
<td>0.05±0.003</td>
<td>8.058</td>
<td>0.000*</td>
</tr>
</tbody>
</table>

**Table 2:** Mean±SD, P-value of F.S,G insulin, IR in control and patients with acromegaly

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Control Mean±SD</th>
<th>Patients Mean±SD</th>
<th>t-test</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>F.S,G (mg/dL)</td>
<td>97.22±9.117</td>
<td>137.25±21.439</td>
<td>7.289</td>
<td>0.000*</td>
</tr>
<tr>
<td>Insulin (µU/mL)</td>
<td>6.37±2.182</td>
<td>15.43±3.999</td>
<td>3.086</td>
<td>0.005*</td>
</tr>
<tr>
<td>IR</td>
<td>1.95±0.579</td>
<td>7.98±3.074</td>
<td>6.960</td>
<td>0.000*</td>
</tr>
</tbody>
</table>

**Table 3:** Mean±SD, P-value of MDA, TAA, TAA/MDA, catalase enzyme in control and patients with acromegaly

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Control Mean±SD</th>
<th>Patients Mean±SD</th>
<th>t-test</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>MDA (μmol/L)</td>
<td>3.6±0.82</td>
<td>4.42±1.29</td>
<td>-0.91</td>
<td>0.026*</td>
</tr>
<tr>
<td>TAA (μmol/L)</td>
<td>0.79±0.27</td>
<td>0.30±0.14</td>
<td>-0.15</td>
<td>0.000*</td>
</tr>
<tr>
<td>TAA/MDA</td>
<td>0.91±0.21</td>
<td>0.39±0.11</td>
<td>1.06</td>
<td>0.01*</td>
</tr>
<tr>
<td>Catalase kU/L</td>
<td>36.82±9.9</td>
<td>13.10±1.3</td>
<td>1.09</td>
<td>0.007*</td>
</tr>
</tbody>
</table>
Table 4: Correlation coefficient between some parameters

<table>
<thead>
<tr>
<th>Correlations</th>
<th>R</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>IR - TAA/MDA</td>
<td>-0.38</td>
<td>0.046*</td>
</tr>
<tr>
<td>IR – GH</td>
<td>0.530</td>
<td>0.008*</td>
</tr>
<tr>
<td>IR – IGF-1</td>
<td>0.498</td>
<td>0.008*</td>
</tr>
<tr>
<td>TAA – GH</td>
<td>-0.449</td>
<td>0.028*</td>
</tr>
<tr>
<td>GH - IGF-1</td>
<td>0.37</td>
<td>0.048*</td>
</tr>
</tbody>
</table>

**ACKNOWLEDGEMENTS**

The authors would like to thank the staff of National Diabetes Centre for his assistance in collecting the blood samples and helpful in measurements and the staff of Department of Chemistry, College of Science, Al-Mustansiriyah University for helpful in measurements.

**REFERENCES**


