Research Article

Evaluation of Some Pro-inflammatory and Anti-inflammatory Factors in Patients with Acromegaly

Refif Sabih Al-Shawk, *PhD*
Department of Microbiology; College of Medicine; Mustansiriyah University, Iraq/Baghdad

Date Submitted: 13/4/2017
Date Accepted:15/10/2017

Address for Correspondence:
Dr Refif Sabih Al-Shawk, Department of Microbiology; College of Medicine; Mustansiriyah University, Baghdad, Iraq.
e-mail: refifalshawk@uomustansiriyah.edu.iq

Abstract

Background: Inflammation is an important risk factor in disorders such as cardiovascular, metabolic syndrome, diabetes mellitus and insulin resistance and there is a controversy about the role of pro and anti-inflammatory factors in acromegaly and their relationships with each other. Evidence showed that IL-10 level is associated with insulin resistance.
Objectives: To investigate the association of GH with IL-10, TNF-α and hs-CRP in patients with active acromegaly and the association of IR with IL-10, TNF-α and hs-CRP.
Methods: The study was conducted on thirty Iraqi acromegalic patients (age 35-57 years) attending the endocrine clinic of National Diabetes Center of Mustansiriyah University and were diagnosed with active acromegaly. For the purpose of comparison, 28 apparently healthy control subjects age matched to the patients were included in this study. Serum was obtained from all subjects for the determination of the GH , hs-CRP,IL-10 and TNF-α levels which was measured by Enzyme Linked Immunosorbent Assay (ELISA) in both study groups.
Results: The results in this study revealed that mean levels of serum TNF-α, hs-CRP levels were significantly decreased in active acromegaly as compared with control group and this significantly correlated with GH, while IL-10 and IR were significantly increased in active acromegalic patients when compared with control group and IL-10 showed no significant correlation with GH, but significantly correlated with IR in patients group.
Conclusion: There were significant negative correlations between TNF-α and hs-CRP levels with GH in patients with active acromegaly, while IL-10 is non-significantly correlated with GH. A positive correlation of both TNF-α and IL-10 was observed with IR in patients with acromegaly. TNF-α and IL-10 can play a role in IR in patients with acromegaly and might be involved in the inflammatory network of acromegalic patients.

Key words: *GH , hs-CRP,IR,TNF-α, IL-10, active acromegaly*

INTRODUCTION

Acromegaly is a rare disease of chronic endocrinopathies which can be caused by a benign tumor of pituitary gland, hypothalamic tumors, nonendocrine tumors, and manifested by hypersecretion of growth hormone (GH) and results in growth and swelling of some organs.[1]

The highr level of GH affect the metabolism by stimulating the breaking-down of triglycerides leading to inhibition of adipocytes from accumulating and uptaking triglyceriedes and causing a decrease in fat mass. The GH as a result induce reduction in adipocyte mass and this reduction affect inflammatory cytokines, as adipose tissue is a source of interlukin-6 (IL-6) synthesis.[2,3] As known, the hepatocytes produce their acute phase reactants in to the blood after an induction by IL-6, such as C-reactive protein and fibrinogen, the elevated level of these reactants used clinically as signs of infection or some inflammatory processes. As a

result, IL-6 is known as major regulator of this acute phase proteins production. Increased level of GH consequently suppresses C-reactive protein (CRP) production and this suppression in active acromegaly may reflect the lower proportion of fat.[4-6]

Indirectly, GH affect bone resorption by stimulating release of mediators that regulate osteoclastic resorption which are specialized cells derived from haematopoietic lineage such as monocytes/macrophage and the elevation in number and/or activity of bone-resorbing osteoclasts could reflect the excessive bone resorption.[7,8] Several mediators like insulin like growth hormone, growth factors, IL-10, IL-6, IL-1, tumor necrosis factor (TNF-α) and prostaglandins play a role on bone cells.[9] Increased levels of these mediators are associated with enhanced osteoclastogenesis and a negative remodeling balance [10]. GH increase due to osteoblastic production of IL-6 [11], TNF-α produced by activated T- cell as a result of increased number of T-cell in the bone marrow which is necessary for the increase in osteoclast.[12]

IL-10 plays a central role in regulating immune response and it suppresses inflammation. IL-10 also down regulates the release of reactive oxygen species (ROS) and nitrogen intermediates antigen presentation capacity and suppression of proliferative and cytotoxic T-cell responses.[28] Bone homeostasis, in homeostatic and inflammatory conditions are regulated by IL-10.[13] There is growing evidence linking IL-10 to obesity, metabolic syndrome cardiovascular disease, type 2 diabetes mellitus (T2DM) and insulin resistance (IR).[14]

The aim of this study is to determine the level of IL-10, TNF-α, IR, high sensitive-CRP (hs-CRP) and lipid profile in Iraqi acromegalic patients and their associations with GH and IR.

PATIENTS AND METHODS

The study was conducted on thirty Iraqi acromegalic patients (age 35-57 years) attending the endocrine clinic of National Diabetes Center of Mustansiriyah University/Baghdad, during the period of December 2013 to June 2014 and were diagnosed by physicians as active acromegaly. The exclusion criteria were presence of complication such as renal failure, hypertension, or osteomalesia, osteoporosis, and cardiovascular disease. Alcoholics and smokers were also excluded. For the purpose of comparison, 28 control subjects matching to patients in age and gender were selected from subjects who were healthy subjects free of acute illnesses at time of sampling.

Blood samples were taken from all subjects and serum was obtained for the determination of the GH, hs-CRP, IL-10, TNF-α and insuline levels which are measured by enzyme-linked immunosorbent assay (ELISA) test sandwich method ELISA using commercially available kits (abcam Company, UK) and the other biochemical tests "Total cholesterol (TC), triglyceride (TG) and glucose" were analyzed by colorimetric method of enzyme-catalyzed. While serum HDL was assayed using HDL-C kit. Calculation of the low density lipoprotein (LDL) was done by the Friedwald equation "(LDL) = TC- [TG/5 + HDL]", very low density lipoprotein "(VLDL)=TG/5". Body mass index was calculated:

"BMI = Mass (kg)/Height^2 (m^2)"[15]

Insulin resistance was measured by a mathematical formula that was used to measure IR by an updated HOMA model of (HOMA2 model):

"Fasting glucose (mg/dL) × fasting Insulin (µU/mL)/405 = IR"[16]

Statistical Analysis: The SPSS program version 20 was used to analyze data into mean ±SD. "T- test used to assess the differences between two groups and P-value =< 0.05 was considered significant. To examine correlations between (GH),(IR) and other parameters in acromegaly and control groups Pearson's correlation coefficient was used".

RESULTS

The means of BMI and TG showed highly significant differences between patients with active acromegaly compared to control subjects (P<0.01, P< 0.008 respectively), and both TC and LDL couldn't reach a significant difference of comparison of acromegalic patients with controls (P=0.14, P=0.8 respectively). The HDL level in acromegaly group was lower than the controls but this difference wasn't significant. (Table 1)

The serum level of both GH and IL-10 were significantly higher in active acromegalic patients as matched up against control subjects (P<0.0001, P< 0.01 respectively), while TNF-α and hs-CRP were significantly lower in patients as compared to controls (P<0.01, P< 0.001 respectively). (Table 2)

Table 1: Comparison of BMI and lipid profile in patients and control subjects

groups / parameters	Mean ± SD		P-value
	C(n=28)	Acr(30)	C-Acr
BMI (Kg/m2)	29.6±6.2	32.3±5.1	0.01*
chol(mg/dL)	181.8±46.3	215.5±72.01	0.14
TG(mg/dL)	98.7±27.3	186.9±142.8	0.008*
HDL(mg/dL)	56.9±48.9	39.5±13.3	0.18
LDL(mg/dL)	112.1±59.0	118.6±65.9	0.8
VLDL(mg/dL)	19.9±4.4	28.7±20.3	0.95

*Significant difference

Table 2: Comparison of GH, hs-CRP, IR,TNF-α and IL-10 in patients and control subjects.

groups / parameters	Mean ± SD		P-value
	C(n=28)	Acr(30)	C-Acr
GH (mU/L)	1.6±1.2	10.4±10.3	0.000*
IR	1.95±0.7	5.8±2.7	0.01*
hs-CRP(g/L)	4.3±4.4	0.08±0.001	0.001*
TNF-α	3.57±1.6	0.882±0.28	0.01*
IL10	4.408±2.3	7.33±2.13	0.01*

*Significant difference

Table (3) showed that, there were negative correlations between GH and both hs-CRP and TNF-α in acromegalic patients and the correlations were significant, while the positive correlations were observed between GH and IL-10 in both patients and controls but with no significance.

Another non-significant negative correlation was observed between GH and hs-CRP and TNF-α in control subjects. (Table 3)

Table 3: Correlation between GH and hs-CRP, TNF and IL-10 in controls and acromegalic patients

GH	(Acr)r	(Acr) P-value	(C) r	(C) P-value
hs-CRP	-0.67	0.008*	-0.21	0.058
TNF-α	-0.41	0.05*	-0.15	0.062
IL10	0.35	0.052	0.11	0.077

*Significant difference

Insulin resistance (IR) showed significant positive correlations with TNF-α and IL-10 in both patients and controls. (Table 4)

Table 4: Correlation between IR and hs-CRP, TNF and IL-10 in controls and acromegalic patients

IR	(Acr)r	(Acr) P-value	(C) r	(C) P-value
hs-CRP	-0.25	0.2	-0.11	0.8
TNF-α	0.33	0.047*	-0.27	0.34
IL10	0.35	0.03*	-0.11	0.09

*Significant difference

The IR showed a non-significant negative correlation with hs-CRP in acromegalic patients. The IR in control subjects also gave a non-significant negative correlation with hs-CRP, TNF-α and IL-10. (Table 4)

DISCUSSION

In this study we determined some pro-inflammatory proteins such as hs-CRP, TNF-α and anti-inflammatory one (IL-10) levels in Iraqi patients with active acromegaly compared with control group. Those patients were acromegalic with a high level of GH as compared with control groups. Growth hormone has anabolic action on various body organ systems and this is well observed by stimulating longitudinal bone growth.[23] The GH effect on BMI (increase) in patients with acromegaly in this study, is unclear if this is related to persistent soft tissue hypertrophy as a result of acromegaly or the effect of bone mineral density (BMD) and body weight which was studied by Giuseppina *et al.*, who found body weight is positively correlated with bone mineral density (BMD) and observed significantly reduced BMD in 42% of treated patients suggesting

obesity is not the reason,[1] while Reid. had observed that the effect of body weight is maybe affected by both fat mass and lean mass "a number of mechanisms for the fat-bone relationship appear to be included" suggesting that the adipose tissue and the bones are responsible for the secretion of their mediators,[17] and affecting the body as obesity is associated with moderate but chronic increase of many inflammatory markers including IL-1ß, IL-6, IL12, IL-18, TNF-α produced by infiltrating macrophages.[25] In acromegaly it's important to distinguish between the fat mass and the body mass index to understand the cytokine network.

This study found a significantly higher means of TC, TG, LDL and VLDL in active acromegaly patients as compared with control groups and low level of HDL, this could be explained that growth hormone activates the adipocytes degradation of TG and inhibit their absorption leading to accumulate lipid in circulation [26]. While Berg *et al.*, have showed that the acromegaly patients had lower LDL and HDL levels, whereas TG levels were not affected as compared to the normal population.[18]

The decreased level of CRP in patients was observed in this study as compared with controls with significant negative correlation between GH and hs-CRP which could be explained by the high level of growth hormone lead to the regression in the mass of adipocyte as GH promotes lipolysis and reduction in the fat mass and accretion of lean tissue,[26] consequently leading to the reduction in the production of inflammatory cytokines produced by infiltrating macrophages, such as TNF- α and IL-6 which is an important regulator of CRP production. Low hs-CRP levels in active acromegaly may be a reflection of a lower proportion of fat. A study conducted by Johan *et al.*, have observed a significant lower level of hs-CRP shown in patients with active acromegaly compared to patients with controlled disease and even lower level of hs-CRP compared with controls.[4]

Patients with active acromegaly have significantly lower values of TNF-α when compared with control group, and negative correlation with GH, so this study suggest that, GH excess can affect TNF-α secretion by suppressing its production , the same effect of GH on hs-CRP by promoting the lypolysis and affecting the infiltrated macrophages. A study conducted by Lu *et al.*, reported a novel action of GH on adipocyte macrophage, demonstrate that GH action on macrophage increases adipocyte differentiation which was mediated via decreased secretion of IL-1β by the macrophage and indicate that GH-dependent inhibition of NF-κB could play a role in the decreased secretion of pro-inflammatory cytokines after GH stimulation of macrophage.[27]

Other worker (Longobardi *et al.*,) showed that the level of circulating cytokines such as IL-1 and TNF-α are not affected and stay in the normal range suggest that cytokines are not involved in the pathogenesis of GH-excess induced osteoporosis.[19] In contrast Bolanowski *et al.*, observed that the TNF-α and IL-6 were elevated in patients with acromegaly, and correlated with BMD concluding that levels of all studied cytokines (TGF-beta ,IL-6 and TNF-α) correlated with the BMD differences in different bone sites.[20] Others also found that serum TNF-α and IL-8 levels were increased in acromegalic pateints than in controls and these cases were newly diagnosed. Acromegaly is correlated with inflammatory conditions independent of body mass index and fat mass of the body.[22]

IL-10 is immuneregulator cytokine with multiple, pleiotropic effects. It downregulates the expression of major histocompatibility antigens complex II, cytokines of Th1 , and co-stimulatory molecules on macrophages and can block NF-κB activity, and is involved in the regulation of the JAK-STAT signaling pathway.[28] GH affects a broad spectrum of physiological functions, ranging from carbohydrate and lipid metabolism to the immune response.[29] The interesting observation in this study was the higher significant difference levels of IL-10 in patients with active acromegaly when compared with control group and as far as our knowledge this is the first study to document in Iraq. A non-significant positive correlation between GH and IL-10 was observed and supporting the existence of abnormalities of the immune system in patients with chronic GH/IGF-1 excess.[29] Moreover Interleukin-10 reduced monocyte chemoatractant protein -1(MCP-1) secretion from adipocytes,[24] the effect that could explain the decreased level of TNF- α in acromegalic pateints.

A significant positive correlation between IR and both cytokines IL-10 and TNF-α was observed in this study. Cytokines produced by skeletal muscle fibers termed myokines and IL-10 is one of them,[31] was shown to be a classical anti-inflammatory cytokine. But studies have shown that IL-10 plays a role in modulating glucose metabolism and is expressed in skeletal muscle,[32] independent of obesity. Nishida *et al.*, Showed that cytokine (IL-10) positively associated with the adipokines that are predominant in subjects with metabolic syndrome and there is an association linking this cytokine (IL-10) to diabetes mellitus, obesity and insulin resistance.[21] TNF-α impairs insulin signaling, by increase serine phosphorylation of insulin receptor

substrate-1 and -2, making them poor substrates for insulin receptor and increasing their degradation.[30]

Conclusion:- this study concludes that there are significant negative association between pro-inflammatory factors (TNF-α, hs-CRP) levels with GH in patients with active acromegaly. While anti-inflammatory factor (IL-10) is non-significantly associated with GH. But there is a significant positive correlation between IR and with both cytokines (TNF-α and IL-10). Thus TNF-α and IL-10 can play a role in acromegalic patients with insulin resistance. So TNF-α might be involved in the inflammatory network of acromegaly patients and IR.

REFERENCES

1. P. Giuseppina, B. Graziella, I. Laura et al., "Prevalence of osteoporosis and vertebral fractures in acromegalic patients,"*Clinical Cases in Mineral and Bone Metabolism* 2011,8 (3) :37–43.
2. Mohamed V, Goodrick S, Rawesh A, Katz D, Miles J,Yudkin J, Klein S & Coppack S. Subcutaneous adipose tissue releases interleukin-6, but not tumor necrosis factor-a,in vivo. *Journal of Clinical Endocrinology and Metabolism* 1997, 82: 4196–4200.
3. Jakab L & Kalabay L. The acute phase reaction syndrome: the acute phase reactants (a review). *Acta Microbiologica et Immunologica Hungarica* 1998, 45: 409–418.
4. Johan V, Brigitte V, Dominique M, Patrick H, Guy T, Ernst R, Bernard C, Pascale A, Frank N, Roger A and Marie B. Active acromegaly is associated with decreased hs-CRP and NT-proBNP serum levels: insights from the Belgian registry of acromegaly .*European Journal of Endocrinology* 2013,168:177-184.
5. Andreassen M, Vestergaard H, Kristensen LØ. Concentrations of the acute phase reactants high-sensitive C-reactive protein and YKL-40 and of interleukin-6 before and after treatment in patients with acromegaly and growth hormone deficiency.2007 . *clinical endocrinology* vol67 iss 7p;
registry of acromegaly .*European Journal of Endocrinology* 2013,168:177-184.
6. Daniela I. Koleva1, Maria M. Orbetzova1, Pepa K. Adipose tissue hormones and appetite and body weight regulators in insulin resistance.*Folia Medica*, 2013,55(1):25-32.
7. D. L. Cochran, "Inflammation and bone loss in periodontal disease," Journal of Periodontology, vol. 79, no. 8, pp. 1569–1576, 2008.
8. T. M. Post, S. C. L. M. Cremers, T. Kerbusch, and M. Danhof, "Bone physiology, disease and treatment: towards disease system analysis in osteoporosis," Clinical Pharmacokinetics, vol. 49, no. 2, pp. 89–118, 2010.
9. Cheung, J., Mak, YT., Papaioannou, S., Evans, BAJ., Fogelman, I. and Hampson, G.Interleukin-6 (IL-6), IL-1, receptor activator of nuclear factor-kB ligand(RANKL) and osteoprotegerin production by human osteoblastic cells: comparison of the effects of 17-b oestradiol and raloxifene. *Journal of Endocrinology*. 2003,177:423-433.
10. Bernard-Poenaru O, Roux C, Blanque R, Gardner C, de Vemejoul MC & Cohen-Solal ME. Bone-resorbing cytokines from peripheral blood mononuclear cells after hormone replacement therapy: a longitudinal study. *Osteoporosis International* 2001 12, 769–776.
11. Swolin D & Ohlsson C. Growth hormone increases interleukin-6 produced by human osteoblast-like cells. *Journal of Clinical Endocrinology and Metabolism* 1996 81, 4329–4333.
12. Cenci S, Weitzmann MN, Roggia C, Namba N, Navack D, Woodring J & Pacifici R. Estrogen deficiency induces bone loss by enhancing T-cell production of TNF-alpha. *Journal of Clinical Investigation* 2000 ,106 1229–1237.
13. S. F. Lee, E. Andrian, E. Rowland, and I. C. Marquez, "Immune response and alveolar bone resorption in a mouse model of Treponema denticola infection," Infection and Immunity, vol. 77, no. 2, pp. 694–698, 2009.
14. Nasser M Al-Daghri,1,2 Abdulrahman SM Al-Ajlan,3 Hanan Alfawaz,1,4 Sobhy M Yakout,1,2 Naji Aljohani,1,5 Sudhesh Kumar,6 Majed S Alokail1,2. Serum cytokine, chemokine and hormone levels in Saudi adults with pre-diabetes: a one-year prospective study .*Int J Clin Exp Pathol*. 2015; 8(9): 11587–11593.
15. Freedman D.S., Horlick M. & Berenson G.S., (2013): A comparison of the Slaughter skinfold-thickness equations and BMI in predicting body fatness and cardiovascular disease risk factor levels in children. *Am. J. Clin. Nutr.*, 98.(6).1417–24.
16. Wallace, T.M.; Levy, J.C. & Matthews, D.R. (2004). Use and abuse of HOMA modeling. Diabetes Care, 27 (6): 1487-95.
17. Reid IR. Relationships between fat and bone. *Osteoporos Int* 2008, 19(5):595–606
18. Berg C, Petersenn S, Lahner H, Herrmann BL, Buchfelder M, Droste M, Stalla GK, Strasburger CJ, Roggenbuck U, Lehmann N et al. Cardiovascular risk factors in patients with uncontrolled and long-term acromegaly: comparison with matched data from the general population and the effect of disease control. *Journal of Clinical Endocrinology and Metabolism* 2010 ,95 :3648–3656.
19. Longobardi S1, Di Somma C, Di Rella F, Angelillo N, Ferone D, Colao A, Merola B, Lombardi G. Bone mineral density and circulating cytokines in patients with acromegaly. *J Endocrinol Invest*. 1998;21(10):688-93.
20. Bolanowski M1, Daroszewski J, Zatonska K, Arkowska A. Circulating cytokines in relation to bone mineral density changes in patients with acromegaly. *Neuro Endocrinol Lett*. 2006;27(1-2):183-8.
21. Nishida M, MD; Toshiki Moriyama, MD; Yoshiro Sugita, MD; Keiko Yamauchi-Takihara, MD. Interleukin-10 Associates With Adiponectin Predominantly in Subjects With Metabolic Syndrome. *Circ J* 2007; 71: 1234 – 1238
22. Arikan S, Bahceci M, Tuzcu A and Gokalp D. Serum tumour necrosis factor-alpha and interleukin-8 levels in acromegalic patients: acromegaly may be associated with moderate inflammation. *Clinical Endocrinology* (2009) **70** , 498–501
23. Devesa J, Almengló C and Devesa P. Multiple Effects of Growth Hormone in the Body: Is it Really the Hormone for Growth? Clinical Medicine Insights: Endocrinology and Diabetes 2016:9 47–71.

24. Lumeng C. N., Bodzin J. L., Saltiel A. R. Obesity induces a phenotypic switch in adipose tissue macrophage polarization. J. Clin. Invest. (2007) 117, 175–184
25. Silswal, N.; Singh, A.K.;Aruna, B.;Mukhopadhyay, S.;Ghosh, S.; Ehtisham, N.Z.. human resistin stimulate the pro-inflammatory cytokines TNF-alpha and IL-12 in macrophage by NF-kB pathway.Biochem Biophys Res Commun 2005;344:1092-101
26. Møller N, Jørgensen JO . Effects of growth hormone on glucose, lipid, and protein metabolism in human subjects. Endocr Rev.2009; 30:152–177.
27. Lu C, Kumar P, Fan Y, Sperling M A and Menon R K. A Novel Effect of Growth Hormone on Macrophage Modulates Macrophage-Dependent Adipocyte Differentiation. Endocrinology. 2010 May; 151(5): 2189–2199
28. Saraiva M, O'Garra A. The regulation of IL-10 production by immune cells. Nature Reviews Immunology. 2010; 10 (3): 170–81.
29. colao, A. Ferone, D., Marzullo, P., et al. Lymphocyte subset pattern in acromegaly.J Endocrinnol Invest. 2002; 25(2) : 125-128.
30. Hotamisligil, G.S. Inflammatory pathways and insulin action. Int J Obes Relat Metab Disord. 2003; 27 (suppl 3): S53-55
31. Pedersen BK, Akerstrom TCA, Nielsen AR, Fischer CP. Role of myokines in exercise and metabolism. J Appl Physiol 2007; 35: 1295–1297.
32. Hacham M, White RM, Argov S, Segal S, Apte RN. Interleukin-6 and interleukin-10 are expressed in organs of normal young and old mice. Eur Cytokine Netw. 2004; 15: 37– 46