LEVEL OF INTERLEUKIN-6 IN OBESE PEOPLE WITH AND WITHOUT INSULIN RESISTANCE

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ABSTRACT

Background. *Obesity is one of the risk factor for type 2 diabetes mellitus characterized by insulin resistance, decrease insulin secretion and hyperglycemia. Chronic inflammation has been proposed to have an important role in the pathogenesis of obesity related insulin resistance. A number of studies have indicated that several humoral markers of inflammation are elevated in people with obesity and type 2 diabetes mellitus, because adipose tissue secretes a number of proinflammatory cytokines, including interleukin-6. The level of plasma IL-6 is increase in obese people.*

Objective. *To investigate the mean of difference in the level of IL-6 in obese people with and without insulin resistance.*

Subjects and Method. *The study design was cross-sectional. It was conducted in obese people with BMI ≥ 25 kg/m2. Insulin resistance were measured with HOMA-IR methode, calculated using the following formula: fasting serum glucose X fasting plasma insulin/22,5. Insulin resistance was defined when HOMA-IR > 2,77. Interlukin-6 was measured with Quantikine High Sensitivity human IL-6 ELISA. Difference of mean of IL-6 level was analyzed by student's t-test for normal distribution and Mann- Whitney U-test if distribution was not normal.*

Results. *There were 56 subjects, 24 (42,9%) subjects with insulin resistance and 32 (57,1%) subjects without insulin resistance. Obese people with insulin resistance had higher mean level of IL-6 than obese people without insulin resistance, although the difference was not significant (20,05±8,59 vs 18,98±8,15 pg/ml; p=0,639; 95% CI -5,58-3,46).*

Conclusion. *There was no difference in the mean of IL-6 level in obese people with and without insulin resistance.*

Keywords. *IL-6, obese, insulin resistance*

INTRODUCTION

Obesity is well known as one of risk factor for type 2 Diabetes Mellitus, with sign such as insulin resistance, decrease insulin secretion and hyperglycemia. It is estimated that about 80% type 2 Diabetes mellitus is obese[1].

In obese subjects, resistance to insulin cellular action could develop, with insulin failure to inhibit glucose production from liver and increase glucose uptake in adipose tissue and muscle. Insulin resistance is main etiologic factor for type 2 Diabetes Mellitus. In United States, insulin resistance prevalence in obese subjects is 59,6%. It needs understanding about cellular mechanism which is underlying insulin resistance so we could overcome epidemic of Diabetes mellitus and cardiovascular disorder due to obesity[2,3,4].

Chronic inflammation has key role on pathogenesis of insulin resistance in obese subjects. Several studies show that inflammatory marker increase in obese subjects and type 2 diabetes mellitus. This is because adipose tissue produce some pro-inflammatory cytokine including tumor necrosis factor-α (TNF-α) and interleukin-6 (IL-6)[5,6].

Interleukin-6 is mainly produced by macrophage and adipose, Plasma IL-6 level would increase in obese. About 30% plasma IL-6 level derived from adipose tissue and its release is influenced by sympathetic nervous system. Interleukin-6 is a major circulated cytokine which has effect on development and change of cells, acute phase response protein, lipid and carbohydrate metabolism[7]. Fasting plasma IL-6 level has positive relationship with body fat presentation and negative relationship with insulin action. Relationship between IL-6 and insulin action is mediated by adiposity[6].

The aim of this study is to investigate the IL-6 levels difference in obese subjects with and without insulin resistance.

METHOD

This study is a cross sectional study. The study was conducted at RSUP Dr Sardjito, Yogyakarta on October until November 2006. Study population was RSUP Dr Sardjito obese employee who presented in RSUP Dr Sardjito general check up department. During this study, there were 56 obese subjects which fulfill the criteria. Obese criteria for Asian are BMI > 25 kg/m2 [8].

Inclusion criteria were obese with BMI > 25 kg/m2, age 22 until 60 years old, agreed to participate and signed informed consent. Exclusion criteria were having hypertension, acute infection, smoking, cancer, coronary heart disease, congestive heart failure, renal failure, thyroid disease, and liver disease.

Statistical analysis was done with student's t test to see the difference in mean IL-6 level between obese with and without insulin resistance. If data distribution was not normal, Mann Whitney U test was done. Categorical data was analyzed with Chisquare test. Significance limit is p < 0,05.

RESULT

During study, there were 56 obese subjects which meet the criteria, 24 (42,9%) men and 32 (57,1%) women. Based on insulin resistance status which was determined according to HOMA-IR value, there were 24 subjects (42,9%) with insulin resistance (HOMA-IR ≥ 2,77) and 32 subjects (57,1%) without insulin resistance (HOMA-IR < 2,77). Insulin resistance also influenced by age and obesity. Mean age in this study was 46,48 ± 6,32 years old and mean BMI was 29,6 ± 3,54 kg/m2.

Table 1. Shows Study Subject Characteristics

Variable	Mean=SD	Frequency (%)
Gender (%)		
Man (n=24)		42.9
Woman (n=32)		57.1
Age (year old)*	46.48±6.32	
BMI (Kg/m^2)	29.63±3.54	
Fasting Blood Glucose (mg/dL)	125.95±47.18	
Blood glucose 2hours *post prandial* (mg/dL)	172.43±86.70	
HOMA IR	2.83±2.01	
Insulin (µU/ml)	9.04±6.92	
IL-6 (pg/ml)	19.44±8.28	
Total Cholesterol (mg/dL)	220.69±39.45	
LDL (mg/dL)	138.05±35.31	
Triglyceride (mg/dL)	52.75±97.19	
Insulin resistance status (%)		
With Insulin Resistance (n=24)		42.9
Without Insulin Resistance (n=32)		57.1

Note:
BMI = body mass index;
SD =standard deviation;
HOMA-IR = *homeostasis model assessment insulin resistance*;
IL-6 = interleukin-6;
LDL = *low density lipoprotein*;
HDL = *high density lipoprotein*,
* = abnormal distribution.

In this study, clinical and laboratory variable analysis based on insulin resistance status (Table 2), though not statistically significant, showed that group with insulin resistance have older age than those without insulin resistance (48,00±5,56 years old and 45,34±6,70 years old; p=0,129). For BMI, the mean was higher in group with insulin resistance in compare with group without insulin resistance, but not statistically significant (30,55±4,21 kg/m2 and 28,95±2,92 kg/m2; p=0,940; 95% CI -3,49-0,28). Mean IL-6 level in obese subjects with insulin resistance though not statistically significant was higher than those without insulin resistance (20,05±8,59 pg/ml and 18,98±8,15 pg/ml; p=0,639; 95% CI -5,58-3,46).

Table 2. Clinical Laboratory Variable Differences Based on Insulin Resistance Status

Variable	Without Insulin Resistance (n=32)	With Insulin Resistance (n=24)	P	95%CI
Age (year old)*	45.34±6.70	48.00±5.56	0.129*	
BMI (Kg/m^2)	28.95±2.92	30.55±4.12	0.094	-3.49-0.28
IL-6 (pg/ml)	18.98±8.15	20.05±8.59	0.639	-5.58-3.46
FBG (mg/dL)	101.88±27.43	158.04±49.26	<0.001	-76.89- -35.44
BG2HPP(mg/dL)	124.81±51.39	235.92±83.97	<0.001	-147.5- -74.70
Insulin (µU/ml)	6.06±2.44	13.01±8.81	<0.001	-10.21- -3.67
Total Cholesterol (mg/dL)	218.81±41.39	223.21±37.43	0.722*	
LDL (mg/dL)	139.72±34.69	135.83±36.73	0.817*	
HDL (mg/dL)	52.72±9.06	52.79±9.29	0.980*	
Triglyceride (mg/dL)	153.88±60.49	209.5±125.73	0.032	-106.6- -4.82

Note:
BMI = Body mass index;
CI = confidence interval;
FBG = fasting blood glucose;
BG2HPP = blood glucose 2 hours post prandial;
IL-6 = interleukin-6;
LDL = *low density lipoprotein*;
HDL = *high density lipoprotein.*
* *Mann Whitney U Test.*

From table 2 we could see that mean fasting blood glucose, 2 hours post prandial, insulin and triglyceride levels in group with insulin resistance in compare with those without insulin resistance (158,04±49,26 mg/dL and 101,88±27,43 mg/dL; 235,92± mg/dL and 124,81±51,43mg/dL; 13,01±8,81 µU/ml and 6,06±2,44 µU/ml; 209,58±125,73 mg/dL and 153,88±60,49 mg/dL), haves statistically significant differences (p<0,001; p<0,001 p<0,01 and those p=0,032, respectively). Mean cholesterol and HDL level in group with insulin resistance are higher than group without insulin resistance (223,21±37,43mg/dL and 218,81±41,39 mg/dL; 52,79±9,29 mg/dL and 52,72±9,06 mg/dL, respectively) but the differences were not statistically significant (p=0,722 and p=0,980, respectively).

Table 3. Interleukin-6 Level in Obese With and Without Insulin Resistance with Limit ≤9,96 pg/ml and >9,96 pg/ml

IL Level (pg/ml)	Without Insulin Resistance	With Insulin Resistance	p
≤9,96 pg/ml	7 (12,5%)	5 (8,9%)	0,925
>9,96 pg/ml	25 (44,6%)	19 (33,9%)	

From above table it was shown that IL-6 levels ≤ 9,96 pg/ml obtained in 7 (12,5%) obese without insulin resistance and 5 (8,9%) in obese with insulin resistance.

Meanwhile, IL-6 levels > 9,96 pg/ml obtained in 25 (44,6%) obese without insulin resistance and 19 (33,9%) obese with insulin resistance and this differences were not statistically significant with p=0,925.

Table.4 Variable Influencing Insulin Resistance

Parameter	B	SE	P	Exp.B
FBG	0,004	0,022	0,852	1,004
BG2HPP	0,038	0,020	0,059	1,039
Insulin	0,839	0,367	0,022	2,314

Note: B= beta estimation, SE= *standard error*, Exp= exponential, FBG = fasting blood glucose, BG2HPP= blood glucose 2 hours *post prandial*.

From table 4 it is shown that insulin resistance was influenced by insulin level, fasting blood glucose and 2 hours post prandial.

DISCUSSION

In this study, 42,9% obese have insulin resistance, which is lower than 59,6% that was obtained by Renolds and He(14). Mean BMI is 29,6±3,54 kg/m2, according to WHO criteria for Asian, belong to obese I (BMI 25-29,9 kg/m2) with moderate morbidity risk. Amount of women (57,1%) is larger than men (42,9%). This is in accordance to Waspadji *et al*.[9] study which included women more than men.

Interleukin-6 is cytokine which has close relationship with obesity and insulin resistance. Interleukin-6 could inhibit insulin signal so insulin resistance could develop. About 30% circulated IL-6 is produced by adipose tissue and it has positive correlation with obesity, glucose intolerance and insulin resistance[10]. Plasma IL-6 levels could predict type-2 diabetes mellitus development[6]. In this study there is no differences of mean IL-6 in obese subjects with and without insulin resistance (20,05±8,59 pg/ml and 18,98±8,15 pg/ml; p=0,639). Kern *et al.*(10) reported their study which obtain 19 IL-6 levels increase in obese subjects with insulin resistance in compare with obese without insulin resistance (3,21±0,79 pg/ml and 1,43±0,30 pg/ml; p< 0,05).

In Kern *et al.* [6] study, the subject had been matched in age, gender, or BMI between those with and without insulin resistance, while in this study the subjects had not been matched so the result is different. In Kern *et al.* [10] study, subject with diabetes mellitus was excluded while in this study not due to limited sample size.

Insulin resistance measurement technique in Kern *et al.* [10] was FSIVGTT method with result S1 4,6±0,34 for subject without insulin resistance and S1 1,5±0,12 for subject with insulin resistance. This measurement showed good correlation with hyperinsulinemia euglycemia clamp (r= 0,84) in non diabetic subject but its accuracy was getting worse in diabetic patient. Plasma insulin response which appears soon after glucose supply disappears. This was overcome with exogenous insulin or secretagogue (ex., tolbutamide) in the beginning of measurement[11]. In this study, insulin resistance measurement with HOMA method is a simpler method. This HOMA method is based on fasting glucose and insulin level, has strong correlation with glucose clamp in type 2 diabetes mellitus (r = 0,83) or non type 2 diabetes mellitus (r= 0,92)[12].

In this study, with upper limit IL-6 in normal subjects which was IL-6 levels ≤ 9,96 pg/ml and > 9,96 pg/ml, there was no significant difference

between obese with and without insulin resistance (p=0,925).

Some factor influencing IL-6 level in this study was controlled by including it in exclusion criteria. Interleukin-6 also is produced locally from contracting skeletal muscle and could increase plasma IL-6 level during exercise. Interleukin-6 production produced in exercise has relationship with training intensity and duration, and could be stimulated by the low muscle glycogen level[13]. In healthy young person, extension training in inferior extremity for 3 hours would increase plasma Il-6 level 20 fold[14]. In this study we did not measure physical activity, the subject had previously been informed for not doing extreme exercise one day prior sampling so its influence on IL-6 could not be measured.

Obese could have multiple lung disorder, including decreased residual capacity, inadequate ventilation, perfusion, and hypoxemia. Hypoxemia itself, without blood lost or damage causes IL-6 release by 313%. Roytblat *et al*. [15] IL-6 level increase in obese with obstructive sleep apnea syndrome (OSAS) (5,58±0,37 pg/ml; p<0,0005) and obesity hypoventilation syndrome (OHS) (43,13±24,27; p=<0,005) in compare with normal people (1,28±0,85 pg/ml). In this study, there was no measurement for sleep apnea so that its influence on IL-6 could not be measured.

One factor that influences IL-6 in insulin resistance is genetic factor. Genetic study in different population reveal that promoter polymorphism (G/C) from IL-6 gene on -174 position is a predispose factor for type 2 diabetes mellitus and related symptoms. On several studies, expression and Il-6 level which is high in plasma has relationship with G/C (-174) genotype which is often found in Indian Pima and Caucasian type 2 diabetes mellitus patient[16,17]. Study by Real *et al*., [18] in healthy Caucasian women obtained IL-6 level which is higher in G/C and C/C genotype in compare with G/G (9,9±6,9 pg/ml and 6,8±1,7 pg/ml; p=0,09). In this study genetic influence could not be controlled.

Interleukin-6 production could directly stimulated by depression, negative emotion and stress condition. Depression could activate response to inflammation. From several study, IL-6 increase is obtained in acute phase of depression. Interleukin-6 could influence HPA (*hypothalamic-pituitary-adrenal*) by increase *hypothalamic corticotrophin-releasing hormone* (CRH) stimulation which is released in stress condition[19,20]. Study by Kahl *et al*. [21] in depressed young woman in major depressive disorder obtain higher IL-6 level than normal woman (1,72±1,5 pg/ml and 0,76±0,33 pg/ml; p<0,05). Based on *Beck Depression Inventory Score* ≥ 10 show major depression. Depression influence on this study could not be controlled.

Interleukin-6 activates inflammatory pathway through JNK and IKKβ. Inhibition JNK and IKKβ/NF-κ B could repair insulin resistance. Some drugs could inhibit IKKβ/NF-κB such as salicylic, thiazolidinedione and statin[22,23]. In this study, influence of drugs could not be controlled.

In obese group with insulin resistance, there are increased insulin levels, fasting blood glucose or post prandial, total cholesterol and triglyceride, this is due to the role of obesity in glucose intolerance, hyperinsulinemia, type 2 diabetes mellitus and dyslipidemia[24]. Periphery interleukin-6 supply induces hyperlipidemia, hyperglycemia, and insulin resistance in mice and human[25].

There are some limitations in this study. First, the application of cross sectional study give limitation in driving cause and effect conclusion due to the single expose and outcome measurement. This could make bias because the existing data only represent condition when study is held. Second, sample size is not meet defined calculation because of time and cost limitation. Third, subject in both group did not matched. Fourth, there are some factor influencing IL-6 levels that could not be controlled such as physical activities, possibility of sleep apnea, genetic, depression and drug use such as salicylic acid, thiazoldinedione, and statin.

CONCLUSION

There is no difference in IL-6 level on obese with and without insulin resistance in this study.

REFERENCES

1. Suastika, K. 2003. Mekanisme Molekuler Diabesitas Fokus pada Disfungsi sel β. *Dalam:* S. Adi, S. Murtiwi, A. Tjokroprawiro, Hendromartono, A. Sutjahjo, A. Pranoto (eds.). *Naskah Lengkap National Obesity Symphosium II 2003*. Surabaya. Perkeni. 9-16.
2. Saltiel, A.R., & Kahn, C.R. 2001. Insulin Signaling and The Regulation of Glucose and Lipid Metabolism. *Nature*, 414: 799-809.
3. Zimmet, B., Alberti, K.G., Shaw, J. 2001. Global and Societal Implications of The Diabetes Epidemic. *Nature*, 414: 782-7.

4. Reynolds, K., & He, J. 2005. Epidemiology of Metabolic Syndrome. *Am J Med Sci*, 330: 273 9.
5. Hotamisligil, G.S., Arner, P., Caro, J.F., Atkinson, R.L., Spiegelman, B.M. 1995. Increased Adipose Tissue Expression of Tumor Necrosis Factor-α in Human Obesity and Insulin Resistance. *J Clin Invest*, 95: 2409-15.
6. Vozarova, B., Weyer, C., Hanson, K., Tataranni, P.A., Bogardus, C., Pratley, R.E. 2001. Circulating Interleukin-6 in Relation to Adiposity, Insulin Action, and Insulin Secretion. *Obesity Res*, 9 (7): 414-7.
7. Mohamed-Ali, V., Flower, L., Sethi, J., Hotamisligil, G., Gray, R., Humphries, S.E., York, D.A., Pinkney, J. 2001. β-Adrenergic Regulation of IL-6 Release from Adipose Tissue: In Vivo and in Vitro Studies. *J Clin Endocrinol Metab*, 86: 5864- 9.
8. Adam, J.M.F. 2006. Obesitas, Pengertian dan Kriteria Diagnosis. *Dalam*: J.M.F. Adam (ed.). *Obesitas dan Sindroma Metabolik*, Bandung. 1-8.
9. Waspadji, S. 2006. Sindrom Resistensi Insulin dan Prediabetes: Apa yang Harus Dikerjakan. *Dalam*: Sumaryono, S. Setiati, R. Gustaviani, L. Sukrisman, K.N. Sari, A. Lydia, I.K. Wijaya, P.W. Laksmi (eds.). *Pertemuan Ilmiah Tahunan Ilmu Penyakit Dalam* 2006. Jakarta. FK-UI. 156-62.
10. Kern, P.A., Ranganathan, S., Li, C., Ranganathan, G. 2001. Adipose Tissue Tumor Necrosis Factor and Interleukin-6 Expression in Human Obesity and Insulin Resistance. *Am J Physiol Endocrinol Metab*, 280: E745-E51.
11. Cefalu, W.T. 2001. Insulin Resistance: Cellular and Clinical Concepts. *EBM,* 266: 13-26.
12. Bonora, E., Targher, G., Alberiche, M., Bonadonna, R.C. 2000. Homeostasis Model Assessment Closely Mirror the Glucosa Clamp Technique in the Assessment of Insulin Sensitivity. *Diabetes Care*, 23 (1): 57-63.
13. Pedersen, B.K., Steensberg, A., Schjerling, P. 2001. Muscle-derived Interleukin-6: Possible Biological Effects. *J Physio,* 536 (2): 329-37.
14. Petersen, A.W., & Pedersen, B.K. 2005. The Anti-Inflammatory Effect of Exercise. *J Appl Physio*, 98: 1154-64. 23
15. Royblat, L., Rachinsky, M., Fisher, A., Greemberg, L., Shapira, Y., Douvdevani, A., Gelman, S. 2000. Raised Interleukin-6 Level in Obese Patients. *Obesity Res,* 8: 673-5.
16. Real, J.M.F., & Ricart, W. 2003. Insulin Resistance and Chronic Cardiovascular Inflammatory Syndrome. *Endocrine Review*, 24: 278-301.
17. Yang, X. Jansson, P., Pelme, F., Laakso, M., Smith, U. 2005. Effect of the Interleukin -6 (-174) G/C Promoter polymorphism on Adiponectin and Insulin Sensitivity. *Obesity Res*, 13: 813-7.
18. Real, J.M.F., Broch, M., Vendrell, J., Richart, C., Ricart, W. 2000. Interleukin-6 Gene polymorphism and Lipid Abnormalities in Healthy Subjects. *J Clin Endocrinol Metab*, 85: 1334-9.
19. Kiecolt-Glaser, J.K., Preacher, K.J., MacCallum, R.C., Atkinson, C., Malarkey, W.B., Glaser, R. 2003. Chronic Stress and Age-related Increases in the Proinflammatory Cytokine IL-6. *PNAS*, 15: 9090-5.
20. Pasic, J., Levy, W.C., Sullivan, M.D. 2003. Cytokines in Depression and Heart Failure. *Psychosom Med*, 65: 181-93.
21. Kahl, K.G., Bester, M., Greggersen, W., Rudolf, S., Dibbelt, L., Stockelhuber, K.M., Gehl, H., Sipos, V., Hohagen, F., Scheiger, U. 2005. Viseral Fat Deposition and Insulin Sensitivity in Depressed Women With and Without Comorbid Borderline Personality Disorder. *Psychosom Med*, 64: 407-12.
22. Hirosumi, J., Tuncman, G., Chang, L., Gorgun, C.Z., Uysal, K.T., Maeda, K., Karin, M., Hotamisligil, G.S. 2002. A Central Role for JNK in Obesity and Insulin Resistance. *Nature*, 420: 333-6.
23. Shoelson, S.E., Lee, J., Goldfine, A.B. 2006. Inflammation and Insulin resistance. *J Clin Invest.*, 116: 1793-801.
24. McFarlane, S.I., Banerji, M., Sowers, J.R. 2001. Insulin Resistance and Cardiovascular Disease. *J Clin Endocrinol Metab*, 86 (2): 713-8.
25. Kershaw, E.E., & Flier, J.S. 2004. Adipose Tissue as an Endocrine Organ. *J Clin Endocrinol Metab*, 89 (6): 2548-56.