# The Insulin Like Growth Factor-1(IGF-1) and Insulin Binding Protein-3 (IGFBP-3) Level As Predictor Biomarker of Chronic Kidney Disease (CKD) Patients in Indonesia

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***Abstract*—This research aimed to identify (i). the IGF-1 and IGFBP-3 level of ESRD and non ESRD populations in Indonesia and (ii). The correlation between IGF-1 and IGFBP-3 level and ESRD incidences.This case control study was carried out in Yogyakarta among 159 volunteers. The result showed that among respondents whose data were successfully and completely collected involved 159 volunteers. The result of bivariate analysis showed level, IGF-1 and IGFBP-3 plasma level, DM history and hypertensive illness history had correlation with ESRD incidences. Lower IGF-1 level and higher IGFBP-3 level have highest OR value and can be used as predictor biomar ESRD incidence. Based on the research result, it is concluded that there difference in IGF-1 and IGFBP-3 plasma level between ESRD and non- ESRD patients in Indonesia. The IGF-1 and IGFBP-3 plasma level have correlation with ESRD incidence in Indonesia.**

***Index Terms*—IGF-1 level; IGFBP-3 level; ESRD; biomarker predictorl; odds ratio**

## I. Introduction

End stage renal disease (ESRD) has been a global health problem. Mortality rate becaused of ESRD has increased in many countries, including some developing countries such as Indonesia [1]. In 2025, the number of United states's CKD patients is estimated 2 times number of patients with CKD in 2000[2]. The CKD incidence in Indonesia is estimated at 100-150 per 1 million population annualy [3]. In line with the decline in the quality of life of ESRD and CKD patients, the number of patients show increasing risk of mortality (ROM). The ROM among ESRD patients in the first year reached 20 % and increased to 60 % in the fifth year of illness [4]. Patients diagnosed with CKD with or without diabetes have the possibility of experiencing the death of a 5 to 10 times greater compared to patients with cardiovascular disease [2], [5].

The treatment of patients with ESRD conduct on three measures, i.e. hemodialysis (HD), peritoneal dialysis (PD) and kidney transplantation (KT). In Indonesia the treatment of ESRD patients relies on costly hemodialysis. ESRD requires high cost treatment with less satisfactory[2], therefore, handling ESRD problem in Indonesia needs to be reviewed, from the curative to promotive and preventive oriented. The results of epidemiological studies have shown that the levels of IGF-1 has associated with the incidences of cardiovascular disease, cancer, depression, impaired growth and the levels of fitness and health and hospital stay. The bioactivity of IGF-1 in the body is controlled by various factors, both genetic and non-genetic, such as growth hormones, nutrition, environment and lifestyle including smoking. The biological effects of IGF-1 is iniated from the signaling pathway activation through its receptor called as IGF-1R. In laboratory, IGF-1 may reduce the progression of atherosclerosis, prevent LDL oxidation, inhibit the infiltration of macrophages into lesion area, reduce the expression of IL-6 proinflammatory cytokines and TNF alpha and decrease superoxide formation in aorta. On the other hand, IGF-1 may increase the expressions of eNOS and pAkt as well as the development of aortic endothelial progenitor cells [6]. IGF-1 activity is affected by 6 types of IGF -binding protein (IGFBP). Among the six IGFBPs, the IGFBP-3 mostly influences on the activity, bioavaibility and degradation of IGF-1. More than 90 % of IGF-1 in plasma is bound by IGFBP-3 and only about 1% of free IGF-1 [7]. Decreased levels of IGF-1 affected by increasing IGFBP-3 levels is suggested associated with glomerulosclerosis, which is in line with clinical evidence that changes in the levels of IGF-1 associated

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with an increasing incidences of microalbuminuria and cardiovascular disease risk in CRF or ESRD patients with hemodialysis.

Preventive and promotive efforts are necessary to be developed in handling ESRD problems in Indonesia. Uncomplaint patient in controlling blood sugar level has been proven to reduce levels of IGF-1 and increase IGFBP-3 and escalate the risks of retinopathy and IHD among patients with DM. It is strongly suspected that the decrease of plasma IGF-1 level or the rise of IGFBP-3 may serve as initial marker of the damage process of blood vessels in the glomerulus that will culminate in ESRD [7]-[9]. Study to identify the relationship of plasma IGF-1 and IGFBP-3 levels with ESRD incidences in Indonesia has not been carried yet. The present study aimed to identify the potentials of IGF-1 and IGFBP-3 level as a biomarker estimating the CKD incidence in Indonesia.

## II. Material and Method

The case-control study design research was conducted at the PKU Muhammadiyah Yogyakarta Hospital, PKU Gamping Sleman Hospital and RSUD (District Public Hospital) of Sleman, RSUD of Bantul - Yogyakarta as the hemodialysis center in Yogyakarta Special Province and its surrounding areas. The research was carried out from March 2012-May 2013. The stage 1 in the first year of this research aimed to collect demographic, geographic, economic-social data, and the IGF-1 and IGFBP-3 level among 53 research subjects of case group and 106 research subject of control group.

Population involved community members of Yogyakarta aged 15-75 years. The criteria of CKD diagnosis involved creatinine clearance <5ml/minute or the creatinine level of blood serum higher than or equal to 10 mg /dl, which was identified from their medical records. Inclusion criteria: Indonesian (Javanese, Sundanese, Malay, aged between 15-75 years old, willing to participate in this research by completing and signing informed consent sheet, cooperative. The case group consisted of patients diagnosed as having CKD that underwent hemodialysis at the PKU Muhammadiyah Yogyakarta Hospital, PKU Gamping Sleman Hospital, RSUD Sleman, RSUD Bantul Yogyakarta, Indonesian, living in Yogyakarta and willing to be research subjects in this research as evidenced by his willingness to complete the informed consents. The control group comprised those with no ESRD diagnosis based on the characteristics of age, gender and hospital of origin in each case, and willing to be research subjects, not still under hospitalization due to DM, heart and lung diseases, that were proved from their medical records. The selected subjects based on the inclusion criteria were excluded when they (a). suffered from congenital kidney disease, (b). underwent kidney transplants, (c). suffered from mental illness. Based on the calculation, the minimum total sample was 40. The estimated OR was = 2.5 proportion of the group $\alpha$ of 0.2 with the significance $\beta$ of 0.05 and a power of 80%.

The primary data were collected through interviews using a questionnaires stating the respondent identity, socio-economic status, history of hypertension, history of diabetes mellitus, smoking activity, treatment history of anti DM, treatment history of anti-hypertensives and other data related to the variables of this research. The primary data collection was carried out through laboratory examination of plasma IGF-1 and IGFBP-3 level, hemoglobin level and sugar level, and serum creatinine level. The laboratory procedures were conducted as the following: Blood sampling, the 5 ml heparinized blood was taken by hospital personnel from the respondents. Blood sampling was carried out at the cubital fossa using 5 ml injection syringe and by maintaining safety and sterility principle. Blood samples were introduced in tubes labeled with code number of the tube, the name, sex and age of the respondent; then, they were kept in a flask containing cold pack, to be transported to the laboratory for IGF-1 and IGFBP-3. Elisa-based test was carried out on IGF-1 and IGFBP-3. The bloods in vacutainer tubes that contained anticoagulant, were ready for plasma IGF-1 and IGFBP-3 level test by using Elisa kit. The procedures for IGF-1 and IGFBP-3 level test was performed as those conducted by the previous researchers. Routine blood and chemical blood tests were carried out by using hematocitoanalyzer.

ANOVA testing (mean) was conducted to identify the mean differences of IGF-1 and IGFBP-3 level, serum creatinine level, serum blood sugar level and Hb level between CRF/ ESRD patients and the control group. The relationship between IGF-1 and IGFBP-3 level, hypertensive illness history, DM illness history, education status and other risk factors with the GGK/ ESRD incidences was identified through the use of bivariate tests (chi-square) by 2x2 table; therefore, odds ratio (OR) of individual factor risk was identified.

## III. Results

The general description of the case and control respondents is showed in Table I. Of 159 volunteers, 105 and 54 were male and female respondent, respectively. Of the 105 male respondents, 35 and 70 were ESRD and non ESRD patients as cases and controls, respectively.

Most respondents belonged to productive age; 16 respondents aged less than 40 years old; 74 respondents aged between 41-60 years, and 24 respondents aged over 60 years. Based on marital status, most respondents were married, i.e. 66 of 72 respondents. 42 respondents graduated from junior high school; only 30 respondents graduated from senior high school and college. 96 respondents were from Bantul; 35 were from Sleman; 13 were from Yogyakarta Municipality, 9 were from Kulon Progo and 6 were from Wonosari.

### *A. The Description of Clinical Status, BMI, IGFBP-3 and IGF-1 Level*

The average ages of the respondents in the case and the control groups were relatively similar or statistically were not significantly different ($p > 0.05$). The average

body heights (BH) of the respondents in case and control groups were significantly different (p <0.05); however, the medians of body weight (BW) of the respondents in case and control groups were not different (P>0.05); this also true with the average size of body mass index (BMI) of the respondents in case and control groups that were not different (P>0.05). The results of measurements on the serum IGF-1 and IGFBP-3 level in ESRD patients as the case group were different with those of non ESRD patients as a control group. The test results on BMI and serum IGF-1 level are presented in Table II.

TABLE I. DESCRIPTION OF DEMOGRAPHY AND BIOLOGICAL RESPONDEN CHARACTERISTIC

| No | Responden characteristic | | Responden status | | P |
|---|---|---|---|---|---|
| | | | Cases | Control | |
| 1 | Hospital | RS PKU Yogyakarta | 19(35.85%) | 38(35.85%) | 1 |
| | | RS PKU Gamping | 6(11.32%) | 12(11.32%) | |
| | | RSUD Bantul | 25(47.17%) | 50(47.17%) | |
| | | RSUD Sleman | 3(5.66%) | 6(5.66%) | |
| 2 | Sex | Male | 35(66,30% ) | 70(66.3%) | 1 |
| | | Female | 18(33.70% ) | 36(33.70% ) | |
| 3 | Age group | 16-30 yr | 5 (9,43%) | 11(10,38%) | 1 |
| | | 31-45 yr | 15(28,30%) | 29(27,36%) | |
| | | 46-60 yr | 25(47,17%) | 50(47,17%) | |
| | | > 60 yr | 8(15,10%) | 16(15,09%) | |
| 4 | Recidens | Yogyakarta | 4(7,55%) | 9(8,49%) | 0.2 |
| | | Sleman | 11(20,75%) | 24(22,64%) | |
| | | Bantul | 29(54,72%) | 67(63,21%) | |
| | | Kulonprogo | 5(9,43%) | 4(3,77%) | |
| | | Gunung Kidul | 4(7,55%) | 2(1,89%) | |
| 5 | Educatio n | No | 3(5,66%) | 11(10,38%) | 0.3 |
| | | Elementary school | 14(26,42%) | 36(33,96%) | |
| | | Yunior high school | 5(9,43%) | 18(16,98%) | |
| | | Senior high school | 21(39,62%) | 26(24,58%) | |
| | | Diploma | 3(5,66%) | 6(5,66%) | |
| | | S1-S3 | 7(13,20%) | 9(8,49%) | |
| 6 | Occupati on | Public sector | 13(24,53%) | 12(11,32%) | 0.1 |
| | | Privat sector | 9(16,98%) | 16(15,10%) | |
| | | Labor/carpenter/ farmer | 15(28,30%) | 48(45,28%) | |
| | | No | 16(30,19%) | 30(28,30%) | |
| 7 | Marital status | Yes | 52(98,11%) | 103(97,17%) | 0,6 |
| | | No | 1(1,89%) | 3(2,83%) | |

The average serum IGF-1 level of ESRD patients was lower than those of non ESRD patients (20.83 ±10.56 vs. 73.97 ± 23.57) (p <0.05). The average serum IGFBP-3 level of ESRD patients was heigher than those of non ESRD patients (3381 ±1140.00 vs. 1501 ± 577.41) (p <0.05).

### *B. The Results of the Bivariate Analysis of IGF-1 and IGFBP-3 Level, Smoking Status, and History of Illness with the ESRD Incidence*

TABLE II. DESCRIPTION OF THE MEASURED PARAMETERS OF AGE,BODY WEIGHT (BW) (KG), BMI (KG/M2), UREUM, CREATININ, GFR, LIPID LEVEL AND SERUM IGFBP-3 & IGF-1 LEVEL

| No | Responden characteristic | Cases | controll | p |
|---|---|---|---|---|
| 1 | Age | 50.26 ± 10.70 | 50.93 ± 10.13 | 0.962 |
| 2 | Body weight | 57.23 ± 8.06 | 57.62 ± 9.01 | 0.787 |
| 3 | Body mass index (BMI) | 22.21 ± 2.79 | 22.33 ± 3.27 | 0.809 |
| 5 | Hb level | 8.24 ±1.46 | 12.93 ± 1.81 | 0.000 |
| 6 | Erytrocyte count | 2.89 ±0.56 | 4.58 ±0.58 | 0.000 |
| 7 | Leucocytes count | 7.08±2.06 | 9.24 ± 3.21 | 0.000 |
| 8 | Thrombocytes count | 242.26 ± 65.23 | 249.33 ± 68.17 | 0.533 |
| 10 | Serum glucose level | 120.87 ± 23.22 | 107.72 ± 13.87 | 0.000 |
| 11 | Lipid level | 118.46 ± 56.18 | 115.63 ± 38.18 | 0.742 |
| 12 | Ureum | 155.76 ± 42.81 | 21.65 ± 7.82 | 0.000 |
| 13 | Creatinin | 8.83 ±2.97 | 0.75 ±0.24 | 0.000 |
| 14 | Glomerular filtration rate | 5.81 ±2.97 | 104.89 ± 20.05 | 0.000 |
| 15 | Nicotine level (pg/ml) | 69.19 ± 32.09 | 10.08 ± 2.85 | 0.000 |
| 16 | IGF-1 level (ng/ml) | 20.83 ±10.56 | 73.97 ± 23.57 | 0.000 |
| 17 | IGFBP3 level (pg/ml) | 3381 ±1140.00 | 1501 ± 577.41 | 0.000 |

TABLE III. THE RESULTS OF THE BIVARIATE ANALYSIS OF IGF-1 LEVEL, PASSIVE SMOKING STATUS AND DM HISTORY AND HYPERTENSIVE ILLNESS AS THE RISK FACTORS OF ESRD INCIDENCE

| Karakteristik | OR (95% CI; p) | |
|---|---|---|
| | *Un match analysis* (UMCC) | *Match analysis* (MCC) |
| DM History | 34 (7,58 – 153; p<000) | 41 (6,95 – 156; p<000) |
| Hipertension hystory | 8 (4,2 – 18,7; p<000) | 11 (4 – 35; p<000) |
| Hipercholesterolemia history | 3 (1,6 – 8,3; p<0,001) | 5 (1,07 – 47; p<0,001) |
| ESRD phamily history | 8 (0,93 – 78,7; p>0,05) | 8 (1,07 – 355; p<0,05) |
| hyperglicemia | 2,79 (1,34 – 8,92; p<0,05) | 2,69 (1,39 – 5,54; p<0,001) |
| Hipertension status | 7,64 (3,65 – 16;p<0,001) | 12,5 (6,1 – 29,75; p<0,000) |
| Dislipidemia | 5,42 (1,01 – 8,92; p<0,05) | 5 (1,07 – 46,9;p<0,05) |
| Obesity | 0,8 (0,4-1,8; p>0,05) | 0,9 (0,45 – 1,7; p>0,05) |
| Low IGF-1 level (<53 ng/ml) | 46 (16 -137; p<0,000) | 36 (10 – 299; p<0,000) |
| hight IGFBP-3 level (pg/ml) | 38,5 (14 – 106;p<0,000) | 16 (6 – 61; p<0,000) |

DM history, hypertension, hypertensive illness history, obesity, ESRD phamily history, dislipidemia, lower levels of IGF-1, higher IGFBP-3 level associated with the ESRD incidences. DM history, hypertensive disease history, lower IGF-1 level and higher IGFBP-3 level

turned out to be the four factors having strongest relationship with the ESRD incidences, with odds ratio (OR) of 24 (CI: 4.2 to 110.5, 95%),8 (CI: 3.7-41, 95%), 46 (CI:16-137) and 38,5 (CI:14 -106, 95%). Obesity, dislipidemia, hypertension status and DM history and ESRD history also indicated a strong relationship with the ESRD incidences.The results of the bivariate analysis between IGF-1 level, smoking status and history of illness are showed in Table III.

## IV. Discussion

In general, the results of the present research indicated that the ESRD incidence in Bantul was higher than any other cities in Yogyakarta Special Province. These results are consistent with the results of previous researches that investigator reported[10]. Many factors may affect CRF incidence [11], [12]. In general, the IGF-1 level measured in this research was lower than those of found in other researches. The reported results of geriatric IGF-1 level in Brazil that was 80.6 ± 28 ng / ml [13]. The results of IGF-1 level amoing DM patients in Malaysia also showed higher IGF-1 level compared to the results of this research [14]. One of the results in IGF-1 level which was quite similar with the results of the this research, was reported by Zuppi *et al.* [15] who conducted a research on healthy populations in Africa. Some results of this research and previous research have suggested there are several factors affecting IGF-1 level; among others; they are health condition, age, activity and body mass index.

Insulin-like growth factor-1 (IGF-1) is a growth factor or endocrine and paracrine activity serving as the main mediator of growth hormone (growth hormone/GH) on the development and growth process. IGF-1 is expressed by vascular cells and monocytes or macrophages. The conditions of oxidative stress that may result in cell damage, may cause the accelerated aging process, and also may lead to coronary heart disease (CHD), diabetes mellitus (DM), kidney demage and cancer. The endogenous antioxidant activity of ESRD patients has decreased, leading to the susceptibility against the oxidative stress. It is assumed that chronic oxidative stress and decreasing IGF-1 level that will provide an impact on the various growth and development mechanisms, reparation and degenerative processes. IGF-1 is a mitogen for endothelial cells and vascular smooth muscle cells. IGF-1 may increase the activity of cJun and NFkB-induced endothelial cells. In addition, IGF-1 has the effect of maintaining the life of blood vessels and prevents the oxidation of LDL-induced apoptosis in vascular smooth muscle cells. The oxidized LDL may reduce IGF-1 and IGF-1 receptor expression in vascular smooth muscle cells. The expression of IGF-1 is reduced in plague areas with positive staining for oxidized LDL. These condition indicates that the decreasing IGF-1 activity may contribute to the process atherosclerosis[6]. IGF-1 may lessen the progression of atherosclerotic plaque formation, inhibit macrophage infiltration into the lesion, decrease the expression of proinflammatory cytokines IL-6 and TNF alpha and reduce superoxide formation in the aorta. On the contrary, IGF-1 may increase the expression of eNOS and pAkt aorta and endothelial progenitor cells [6].

Signaling pathway activation may induce different biological effects of IGF-1, including cell growth, differentiation, migration and survival [16]. Maturation and maintenance of T lymphocytes survival in the body are also affected by IGF-1. IGF-1 may reduce the progression of atherosclerotic plaque formation, inhibiting the infiltration of macrophages into the lesion, decrease the expression of proinflammatory cytokines IL-6 and TNF alpha and lessen superoxide formation in the aorta. On the other hand, IGF-1 may increase the expression of eNOS and pAkt aorta and endothelial progenitor cells [6], [8]. Different from the previous research results, the most recent research in animal models demonstrate that the decreasing level of IGF-1 has been associated with glomerulosclerosis.

Free IGF-1 level in plasma is associated with the incidence and morbidity of ESRD patients. Lower levels of IGF-1 and higher IGFBP-3 level associated with the ESRD incidences, having strongest relationship with the ESRD incidences, with odds ratio (OR) of 46 (CI:16-137) and 38,5 (CI:14 -106, 95%). This is consistent with the clinical evidences that change in IGF-1 level is associated with microalbuminuria incidences and increasing cardiovascular disease risk among CRF or ESRD patients with hemodialysis [8]. In conclusion, lower IGF-1 level and higher IGFBP-3 level can be used as predictor biomarker of ESRD.

## V. Conclusion and Recommendations

Needs to make modeling biomarker terminal chronic renal failure (GGKT) by increasing the amount of sample and analysis of IGF-1 polymorphism.

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## References

[1] V. Kher, "End stage renal disease in developing countries," *J. Kidney International*, vol. 62, pp. 350-362, 2002
[2] A. S. Go, G. M. Chertow, D. Fan, C. Y. Hsu, "Chronic kidney disease and the risk of death, cardiovascular events and hospitalization," *NEJM*, vol. 351, pp. 1296-305, 2004.
[3] S. Bakri, "Deteksi dini dan upaya-upaya pencegahan progresifitas penyaki gagal ginjal kronik," *Jurnal Medika Nusantara*, vol. 26, no. 3, pp. 36-39, 2005.
[4] L. S. Dalrymple and A. S. Go, "Epidemiology of acute infections among patients with chronic kidney disease," *CJASN*, vol. 3 no. 5, pp. 1487-1493, 2008.
[5] W. M. Mcclellan and W. D. Flanders, "Risk Factor for progressive chronic kidney disease," *J Ant Soc Nephrol*, vol. 14, pp. s65-s70, 2003.
[6] S. Sukhanov, Y. Higashi, S. Y. Shai, C. Vaughn, *et al.*, "IGF-1 reduces inflammatory responses, suppresses oxidative stress, and decreases atherosclerosis progression in ApoE-deficient mice," *Arterioscler Thromb Vasc Biol*., vol. 27, no. 12, pp. 2684-90, 2007.
[7] S. Kawachi, N. Takeda, A. Sasaki, and Y. Kakubo, "Circulating IGF-1 and IGFBP-3 are associated with early carotid

 

atheroschlerosis," *Arterioscler Tromb Vasc Biol*, vol. 25, pp. 617-621, 2005.
[8] A. Juul, T. Scheike, and M. Davidsen, "Low serum IGF-1 is associated with increased risk of ischemic hearth disease," *Circulation*, vol. 106, pp. 939 – 944, 2002
[9] I. Rietveld, A. Hofman, J. A. M. Janssen, and H. A. P. Pols, "A Polymorphisme in the IGF-1 gene influences the age –related decline in circulating total IGF-1 levels," *European Journal of Endocrinology*, vol. 148, pp. 171–175, 2003.
[10] T. Hidayati, H. Purnomo, and Suhardi, "Hubungan hipertensi, merokok dan minuman suplemen dengan kejadian GGKT di RSU PKU muhamadiyah Yogyakarta," Thesis, Pasca Sarjana UGM, Yogyakarta, 2007.
[11] R. Agarwal and M. J. Andersens, "Correlates of systolic hypertension in patients with chronic kidney disease, hypertension," *J. Am Soc Nephrol*, vol. 46, pp. 514-520, 2005.
[12] S. J. P. Siestma, J. Mulder, W. M. T. Janssen, and H. L. Hillege, "Smoking is related to abnormal renal function in nondiabetic persons," *Ann Intern Med*, vol. 133, pp. 585-91, 2000.
[13] R. G. De Souza Vale, R. D. De Oliveira, C. S. Pernambuco, Y. P. Da Silveira, F. De Meneses, and J. da Silva Novaes, "Correlation between basal serum IGF-1 levels and functional autonomy in elderly women," *International Journal of Sport Science*, pp. 11-18, 2009
[14] S. Adzura, M. Muhaya, M. Normalina, A. M. Zaleha, W. P. S. Ezat, and I. Tajunisah, "Correlation of serum insulin like growth factor-I with retinopathy in Malaysian pregnant diabetics," *Int J Ophthalmol*, vol. 4, no. 1, 2011.
[15] C. Zuppi, J. Simpore, B. Zappacosta, C. Carrozza, M. Malaguarnera, S. Musumeci, Somatomedin C (IGF-1), Dehydroepiandrosterone Sulphate (DHEA-S) and Hcy metabolism in postmenopausal African women, *J. Med. Sci*., vol. 6, no. 5, pp. 734-742, 2006.
[16] P. Delafontaine, Y. H. Song, and Y. Li, "Expression, regulation and function of IGF-1, IGF-1R and IGFBP-1 in blood vessel," *Arterioschler Thromb Vasc Biol*, vol. 24, pp. 435-444, 2004.

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