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Resistin Protein Analysis Using Receiver Operating Characteristics Curve in Predicting the Occurrence of Type 2 Diabetes Mellitus in Respondents of Cohort Study of Risk Factors for Noncommunicable Diseases in Bogor

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ABSTRACT

Resistin is a hormone that involved in obesity and type 2 diabetes mellitus. High resistin level in pre-DM respondents with obesity will cause insulin resistance. ROC analysis is used as an initial screening to predict chronic diseases precisely and accurately to look for high specificity values. The method was carried out in cross sectional method by examining protein in 744 respondents in the cohort study. Blood plasma was examined with an ELISA kit and analyzed with a Receiver Operating Characteristic curve (ROC Curve). The cutoff point results of pre-DM Resistin levels of respondents compared to normal respondents were 5.9 ng / ml with AUC (Area Under Curve) 0.68 (0.63-0.73), (P <0.001), sensitivity 74.8% and specificity 62.4%. While the Area Under Curve in the combined protein (resistin, insulin, adiponectin and c-peptide) increased to 0.86 (0.82-0.9) (P <0.001), sensitivity 77.7% and specificity 78.8%. Conclusion = resistin can be used as a biomarker in Prediabetes respondents to predict becoming diabetes.

Keywords*: diabetes mellitus, ROC, resistin*

1. INTRODUCTION

Pre diabetes is a disease state which requires recognition because of its high prevalence particularly among middle-aged individuals with sedentary life. It is associated with increased mortality and morbidity. The available data recommends frequent screening for its diagnosis and possibly early intervention, including changes in lifestyle as well as possible pharmacological treatment if necessary. If ignored, subjects will develop the more sinister disease state of diabetes (type 2 diabetes) [1]. Impaired fasting glucose (IFG) and impaired glucose tolerance (IGT) are mild metabolism glucose disorders that are thought to be intermediate stages in the progression towards type 2 diabetes. IFG is characterized by an elevated fasting glucose concentration which is consistently above the normal range but it is also not high enough to be defined as diabetes [2].

Prediabetes condition is more likely develop in individual who are insufficiently active. Exercise training often in combination with other lifestyle strategies, has beneficial effects on preventing the onset of prediabetes and type 2 diabetes [3].

Prediabetes criteria are those who are classified as impaired fasting glucose (IFG) or impaired glucose tolerance (IGT) or Interrupted Glucose Tolerance (TGT). Some of them have also found abnormalities such as those found in diabetes mellitus, namely micro vascular disorders. Globally worldwide there are an estimated 314 million people with prediabetes at present, and will increase to 418 million by 2025 [4].

Resistin (or 'resistance to insulin') is a hormone that involved in obesity and type 2 diabetes mellitus. High resistin level in pre-DM respondents with obesity will cause insulin resistance [5]. The great potential to be a marker of diabetes makes resistin worth being a predictor of diabetes.

ROC curve analysis is used as an initial screening to predict chronic diseases precisely and accurately to look for high specificity values [6]. Receiver Operating Characteristic curve (ROC Curve) analysis is being used as a method for evaluation and comparison of classifier. The ROC gives complete description of classification accuracy as given by the area under the ROC curve. The curve shows how the receiver operates the existence of signal in the presence of noise. The ROC curve plots the probability of detecting true signal (sensitivity) and false signal (1-specificity) for an entire range of possible cut points. The sensitivity and specificity of a classifier depend on the definition of the cut-off point for the probability of predicted classes [7]. The objective of the paper is finding good diabetes biomarker that can be easier to administrate and have reliable results in predicting diabetes by using ROC curve analysis.

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2. METHOD

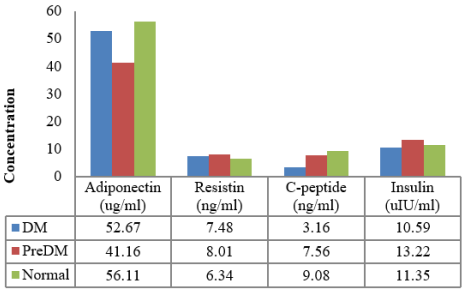
The research design is cross sectional study from the respondent of Cohort study of risk factor for non-communicable diseases, Bogor, West Java in 2016. The study was approved by the ethics committee of the Indonesia Agency of Health Research and Development Ministry of Health of Republic Indonesia, number: LB.02.01/2/KE.235/2017.

Samples are stored biological material (BBT/Bahan Biologi Tersimpan) from respondents of the Non-communicable Disease Risk Factor Cohort Study Bogor in the form of blood plasma from blood taken in 2016. The blood taken is processed and stored in the form of PBMC (Peripheral Blood Mononuclear Cells) and blood plasma. BBT samples were stored in the biorepository Laboratory of Infectious Disease Research Prof. dr. Sri Oemiati, NIHRD. PBMC is genetically examined (in other research examinations) and blood plasma is checked for protein levels in 2017. The number of samples examined came from 744 respondents (adult people, 25-65 years old) who were grouped into three groups, diabetes 88 respondents, prediabetes 280 respondents and normal 376 respondents. The group classification is based on fasting blood glucose test.

Protein tests were carried out on respondents' plasma with normal prediabetes and DM as control. Protein tests were carried out by the Elisa method using a commercial kit from Sigma Aldrich based on the manufacturer's instructions and optimization results. Storage instructions, contents of kit components and buffer dilution are the same for all proteins. Human protein of Resistin, Adiponectin, Insulin and C-Peptide ELISA kits for serum, plasma and cell culture supernatant according to the examination instructions provided in the kit. Protein examination results will be processed using logistic regression and ROC (receiver operating characteristic) curve analysis of the four proteins in diabetes, prediabetes and normal respondents to test their closeness in predicting the respondent's incidence of diabetes.

3. RESULTS AND DISCUSSION

The results on the examination of proteins related to diabetes for Resistin, Insulin, Adiponectin and C-Peptide shows in figure 1.



	Adiponectin (ug/ml)	Resistin (ng/ml)	C-peptide (ng/ml)	Insulin (uIU/ml)
DM	52.67	7.48	3.16	10.59
PreDM	41.16	8.01	7.56	13.22
Normal	56.11	6.34	9.08	11.35

Figure 1. Protein Level in Diabetic and Prediabetic Respondents

The ROC curve analysis was performed on all four proteins. The analysis was carried out to predict the possible sensitivity and specificity of each protein. The ROC curve could predict, if the protein can be used as a marker then the results show an AUC value of more than 0.5 ($p<0.05$) and an estimated cut off point will appear (figure 2-4).

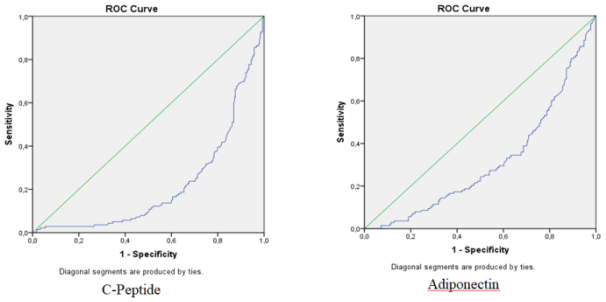


Figure 2. Roc Curve Of C-Peptide And Adiponectin For Predicting The Incidence Of Diabetes. There Are No Cut Off Point, AUC<0,5 Can Not Be Used To Predict Diabetes Incidence.

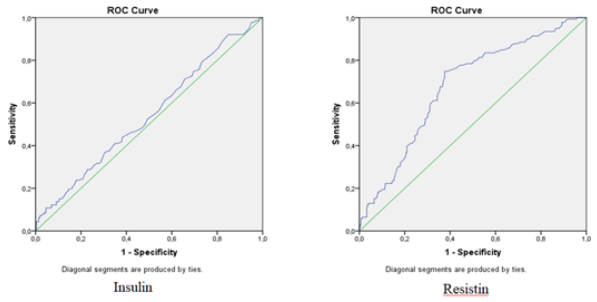


Figure 3. Roc Curve Of Insulin And Resistin For Predicting The Incidence Of Diabetes. The Cutoff Of Insulin Level Is 10.16 Uiu/Ml But P-Value>0,05, and The Cutoff of Resistin is 5.9 Ng/Ml P-Value<0,01.

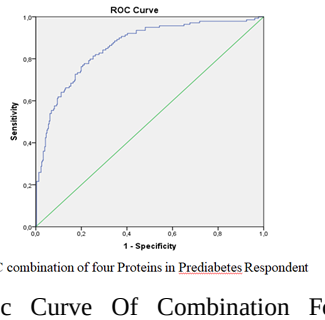


Figure 4. Roc Curve Of Combination Four Protein (Resistin, Adiponectin, C-Peptide And Insulin) For Predicting Diabetes, The Curve Show Better Results, Sensitifity Dan Specificity Are Higher.

Table 1. The Area Under The Curves (Auc) of Prediabetes Respondent Proteins In Predicting Diabetes

	AUC (95% Confidence Interval)		P-value	Sensitivity	Specificity
Adiponectin	0.313	(0.261 – 0.365)	0.000	-	-
C-peptide	0.216	(0.171 – 0.260)	0.000	-	-
Insulin*	0.541	(0.483 – 0.599)	0.167	0.921	0.150
Resistin**	0.677	(0.625 – 0.730)	0.000	0.748	0.624
Insulin+Resistin	0.701	(0.651 – 0.750)	0.000	0.806	0.581
Combination***	0.857	(0.819 – 0.895)	0.000	0.777	0.788

* AUC equal and above 0.5 but not significant
** AUC equal and above 0.5 and significant
*** AUC Combination ratio of 4 Proteins are significantly better than AUC resistin and resistin+insulin ratio
P<0.05 significant

Area under the curve of adiponectin and c-peptide are below than 0.5 ($p<0.05$) cannot be a marker to predict the diabetes, it is not sensitive and not specific. Area under the curve of insulin is more than 0.5 ($p>0.05$) but the specificity is not worthy enough to be a predictor unless they are combined with resistin.

Adiponectin is a specific cytokine hormone in adipose tissue that is able to influence insulin sensitivity because adiponectin is able to increase tyrosine phosphorylation in insulin receptors and insulin receptor substrates in muscle cells. Adiponectin levels will increase if the individual loses weight. But in individuals who are visceral obesity with symptoms of type 2 diabetes adiponectin expression will decrease significantly. Adiponectin also plays a role in insulin resistance [8].

Resistin is a hormone produced by adiposity and is said to cause obesity and type 2 diabetes. In this case resistin also plays a role in causing insulin resistance, resistin is also called 'insulin resistant'. The results of the examination showed the results in the pre-diabetic group had higher levels than other groups. Resistin is a hormone that involved in obesity and type 2 diabetes mellitus. High resistin level in pre-DM respondents with obesity will cause insulin resistance [9].

Increased protein levels associated with type 2 DM are adiponectin and resistin. The examination results showed that the pre-diabetic group had the lowest levels of adiponectin so it can be concluded that there had been a decrease in sensitivity to insulin (insulin resistance).[10] Adiponectin levels, lipid and proinsulin profiles between obese and normal adolescents. From these studies it is known that obese adolescents have adiponectin which is up to 50% lower compared to teens with normal weight. This adiponectin level is positively related to hepatic and peripheral insulin sensitivity and negatively related to proinsulin levels and proinsulin-insulin ratio. So it can be concluded that hypoadiponectinemia found in adolescents is related to obesity (especially visceral obesity), insulin resistance, pancreatic β cell dysfunction and several components of metabolic syndrome. Adiponectin can be used as an early marker in obese adolescents as a risk for the emergence of diabetes and atherosclerosis [8].

In predicting non-communicable diseases what is needed is that these markers are very specific to the disease. So that its emergence in the future can be anticipated. The problem that exists for non-communicable diseases is that if not immediately known within a certain period will be fatal because when exposed to diabetes then a lifetime will depend on medication and must always maintain blood glucose in normal levels. ROC curve analysis will help in increasing specificity in predicting the occurrence of a disease.

ROC analysis is used as an initial screening to predict chronic diseases precisely and accurately to look for high specificity values. The cutoff point results of pre-DM Resistin levels of respondents compared to normal respondents were 5.9 ng / ml with AUC (Area Under Curve) 0.68 (0.63-0.73), ($P<0.001$), sensitivity 74.8% and specificity 62.4%. While the Area Under Curve in the combined four proteins (resistin, insulin, adiponectin and c-peptide) increased to 0.86 (0.82-0.9) ($P<0.001$), sensitivity 77.7% and specificity 78.8%.

In chronic diseases that are not life-threatening if immediately help is not given, the aspect of specificity takes precedence over the aspect of sensitivity. Based on ROC analysis, the combined use of four proteins resulted in a significant enhancement of diabetes prediction. Even though the combined results of resistin+insulin and the combination of 4 proteins are better, it will increase the cost of the examination. Resistin in predicting diabetes deserve attention in the future. Resistin has been implicated in variety of disease processes besides obesity and diabetes and have possible role in the development of endothelial dysfunction, thrombosis, angiogenesis, inflammation and smooth muscle cell dysfunction [5].

4. CONCLUSION

The conclusion, resistin can be used as a biomarker to predict the prediabetes respondents to become diabetes. As our study is limited by the public health data, we have not performed model validation in the current report. In addition, laboratory measurements for Resistin or Resistin+insulin ratio or Resistin combined with four proteins ratio are still not readily available in clinical practice. However, our postulated diabetes prediction model involved only single fasting blood sample for identifying patients at increased risk of developing diabetes type 2.

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