THE ROLE OF SURVIVIN AND RAF-1 KINASE AGAINST ENHANCEMENT OF PANCREATIC BETA-CELL APOPTOSIS IN PATIENTS WITH TYPE 2 DIABETES MELLITUS

EVA DECROLI[1]*, ASMAN MANAF[1], SYAFRIL SYAHBUDDIN[1], SARWONO WASPADJI[2], DWISARI DILLASAMOLA[3]

[1]Endocrinology and Metabolic Subdivision, Division of Internal Medicine, Dr. M. Djamil General Hospital/Medical Faculty, Andalas University, Padang, Indonesia. [2]Endocrinology and Metabolic Subdivision, Division of Internal Medicine, Medical Faculty, Indonesia University, Jakarta, Indonesia. [3]Department of Medicine, Division of Endocrinology and Metabolism, Faculty of Medicine Universitas Indonesia, Jakarta, Indonesia. Email: evadecroli@med.unand.ac.id

ABSTRACT

Objective: This study aimed to reveal differences in levels of survivin and Raf-1 kinase in prediabetes, controlled Type 2 diabetes mellitus (T2DM), uncontrolled T2DM, and their relationship with hemoglobin A1c (HbA1c) levels and serum triglyceride levels.

Methods: This study was an observational study with a cross-sectional design. The study involved 60 people with T2DM who visited the endocrine and metabolic clinic and 30 prediabetes patients. The variables were survivin levels and Raf-1 kinase enzymes that examined using enzyme-linked immunosorbent assay techniques. HbA1c values are measured by high-performance liquid chromatography and triglyceride levels measured by enzymatic method.

Results: Average levels of Raf-1 kinase were significantly higher in the prediabetes group, controlled T2DM, and uncontrolled T2DM (11.6 ± 1.4 pg mL, 9.9 ± 1.1 pg/mL, and 9.1 ± 1.5 pg/mL). Survivin was significantly higher in the prediabetes group, controlled T2DM, and uncontrolled T2DM (5.4 ± 0.4 pg mL, 5.0 ± 0.2 pg/mL, and 4.7 ± 0.1 pg/mL). There was no correlation between HbA1c with Raf-1 kinase levels (R=−0.215, p=0.250), but there was a correlation between HbA1c with serum survivin levels (R=−0.6, *p<0.05). There was a correlation between the levels of triglycerides with survivin but not with Raf-1 kinase (R=−0.267, *p=0.039).

Conclusion: Survivin and Raf-1 kinase levels are lower in uncontrolled T2DM. This explained the role of survivin and Raf-1 kinase against enhancement of pancreatic beta-cell apoptosis in patients with T2DM.

Keywords: Survivin, Raf-1 kinase, Hemoglobin A1c, Type 2 diabetes mellitus.

INTRODUCTION

Type 2 diabetes mellitus (T2DM) is a metabolic endocrine disorder that started with prediabetes. T2DM is a disease that is being a major threat to human health in the 21st century. Based on estimation made by the World Health Organization that, in 2000, the number of people with diabetes over the age of 20 years is up to 150 million people and over a period of 25 years later, in 2025, this number will increase to 300 million people [1-5].

Pancreatic beta cells defect and insulin resistance factor play a role in the pathogenesis of T2DM insulin resistance factor. Pancreatic beta cells function decreased by glucotoxicity because of the imbalance between proliferation and apoptosis of pancreatic beta cells. Beside lipotoxicity and glucotoxicity which affect pancreatic beta cells, it is noted also that in molecular basis there is some specific protein named survivin and the Raf-1 kinase that contribute the life of the beta cell. Survivin has the power to preserve beta cells from apoptosis whereas the Raf-1 kinase has the power to proliferate pancreatic beta cells [2,6-8].

Survivin is a protein that regulates the replication and survival of pancreatic beta cells, expressed during embryogenesis endocrine cells that undergo proliferation. The main function of the protein is to inhibit apoptosis and also regulate cell division. This protein contains a baculovirus inhibitor of apoptosis protein (IAP) repeat domain (BIR domain) that makes survivin is grouped into the IAP [8].

Raf 1 kinase is part of serine protein group/threonine kinase consisting of A-Raf, B-Raf, and C-Raf. Raf-1 kinase is also known as C-Raf that plays a role in signal transduction RAS-RAF-MEK-ERK. Activation of Raf-1 kinase signal transduction pathways lead mitogen-activated protein kinase (MAPK), and MAP3K become more active. These protein work in the downstream area of Ras proteins [7].

Raf-1 kinase has a major role to proliferate in some cells including pancreatic beta cells. When raf-1 kinase was blocked by Raf-1 inhibitor, it would increase cell death due to proliferation inhibition in pancreatic beta cells. Raf-1 kinase specifically works to control cell proliferation to maintain the quantity of pancreatic beta cells [7].

On the progression of prediabetes and T2DM, pancreatic beta cells condition was unknown, whereas pancreatic beta cells play an important role in the etiopathogenesis of prediabetes and T2DM. Therefore, the study of survivin and Raf-1 kinase against enhancement of pancreatic beta cells apoptosis in patients with T2DM is important to understand the pathophysiology of diabetes.

METHODS

This study is an observational study with a cross-sectional design. This study involved 60 patients who visited the clinic of T2DM metabolic Endocrinology Department of Dr. M. Djamil Hospital Padang and 30 people with prediabetes are obtained from family or children of persons with T2DM. Patients were divided into groups of uncontrolled T2DM, controlled T2DM, and prediabetes. Patients' blood was collected for laboratory examination. Some of the analyzed variables were levels of survivin and Raf-1 kinase levels that represent the pancreatic beta-cell defects. These two variables were associated with blood sugar

control reflected by hemoglobin A1c (HbA1c) and triglyceride levels. All patients have provided signed consent. Ethical approval was granted by Medical Faculty University of Andalas Research Ethics Committee.

Examination methods

Survivin levels and Raf-1 kinase enzymes were examined using enzyme-linked immunosorbent assay techniques. HbA1c was checked using the method of high-performance liquid chromatography, whereas triglyceride was examined using enzymatic methods.

Statistical analysis

Categorical scale data were written in frequencies and percentages while interval data or ratio scale was written in mean ± standard deviation. Difference in the two averages was normally tested using t test, while the data that were not normal tested using Mann–Whitney. A correlation test was performed using the Spearman test. p<0.05 was considered significant.

RESULTS

Observational studies had been conducted with a cross-sectional design in patients with T2DM and prediabetes patients. In Table 1, it could be seen that the subjects were as many as 90 subjects, consisted of 30 prediabetes subjects, and 60 T2DM subjects. On the subjects of prediabetes, fasting blood glucose was <126 mg/dL, and 2-h oral glucose tolerance blood glucose was <200 mg/dL. In the diabetic group either controlled or uncontrolled, fasting blood glucose levels were >126 mg/dL, and 2-h oral glucose tolerance blood glucose was over 200 mg/dL. Research's subjects characteristics can be seen in Table 1.

Differences in levels of survivin and Raf-1 kinase between prediabetes, controlled diabetes, and uncontrolled diabetes groups

In this study, both survivin and Raf-1 kinase had differences in groups of prediabetes, controlled T2DM, and uncontrolled T2DM. Survivin was significantly higher in the prediabetes group compared with the controlled T2DM group. Survivin was also higher in the controlled T2DM than uncontrolled T2DM. The same thing was found in Raf-1 kinase (Figs. 1 and 2).

Correlation between survivin and Raf-1 kinase with HbA1c

To assess the relationship of HbA1c with survivin protein and Raf-1 kinase, we tested two variables with the Spearman correlation test. In the correlation test, we found a negative correlation between survivin with HbA1c (p<0.05) and Raf-1 kinase with HbA1c (p=0.250) (Figs. 3 and 4).

The correlation between serum triglycerides with survivin and Raf-1 kinase

It is only to describe whether blood lipid levels affect survivin levels and Raf-1 kinase. In this study, a negative correlation was found between serum triglycerides with survivin, but not with the Raf-1 kinase (Table 2).

DISCUSSIONS

Results of statistical tests in this study find a significant difference between the levels of survivin in the prediabetes group and the controlled

T2DM group with an uncontrolled T2DM group (p<0.05). This suggests differences in the level of destruction of pancreatic beta cells in these three groups. Wang defined a mechanism of survivin regulation by EGF through the Raf-1/MEK/ERK pathway in pancreatic beta cells, through prolongation of survivin protein half-life and inhibition of the ubiquitin-mediated proteasomal degradation pathway. In prediabetes, survivin levels are higher than uncontrolled T2DM. The lower levels of survivin in uncontrolled T2DM can impair pancreatic beta cells ability to defend from damage because of glucotoxicity. The high levels of survivin in prediabetes are expected to prevent the pancreatic beta cells damage. Prediabetes is a condition that can reverse to normal, still, survive as prediabetes or progress to T2DM. In people with prediabetes, it is found that beta-cell dysfunction is still reversible [9,10].
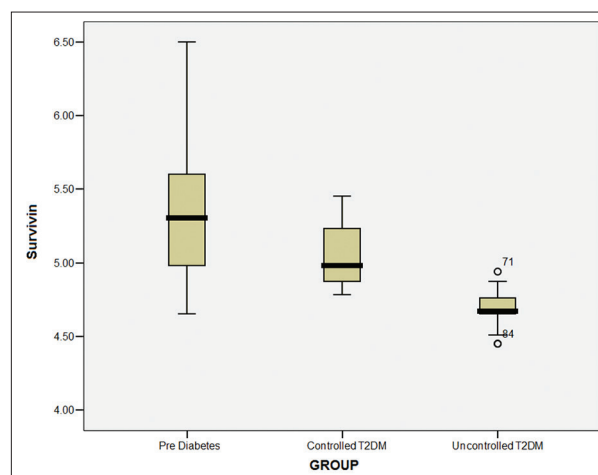

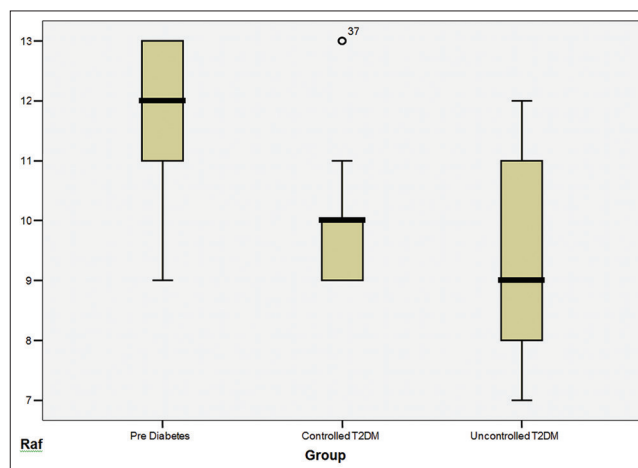**Fig. 1: Mean levels of survivin in the prediabetes, controlled, and uncontrolled Type 2 diabetes mellitus group**

Fig. 2: Average levels of Raf-1 kinase in the prediabetes, controlled, and uncontrolled Type 2 diabetes mellitus group

Table 1: Characteristics of the sample and the mean levels of survivin and Raf-1 kinase in the prediabetes, controlled, and uncontrolled T2DM group

Characteristics	Prediabetes (n=30)	Controlled T2DM (n=30)	Uncontrolled T2DM (n=30)
Age (years)	52.9±10.1	58.2±10.7	55.3±8.1
FBG (mg/dL)	105.7±8.5	140.8±15.9	211.1±15.6
BG-2hrOGTT (mg/dL)	175.9±15.8	279.4±41.1	282.9±30.8
HbA1c (%)	-	6.4±0.3	9.2±2.1
Triglyceride (mg/dL)	-	131.3±56.5	156.7±99.9
Raf (pg/mL)	11.6±1.4	9.9±1.1	9.1±1.5
Survivin (pg/mL)	5.4±0.4	5.0±0.2	4.7±0.1

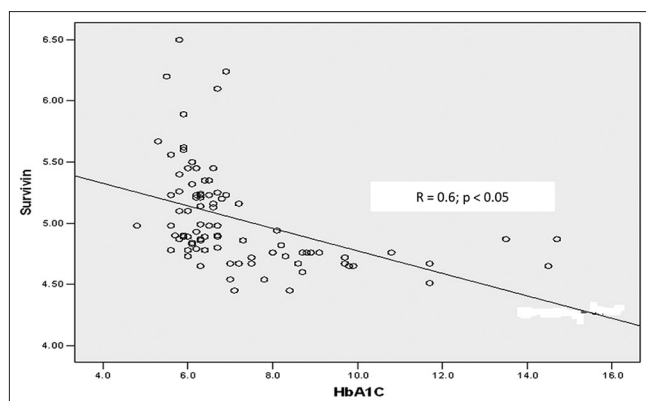HbA1c: Hemoglobin A1c, T2DM: Type 2 diabetes mellitus

Fig. 3: The correlation between hemoglobin A1c and the levels of survivin
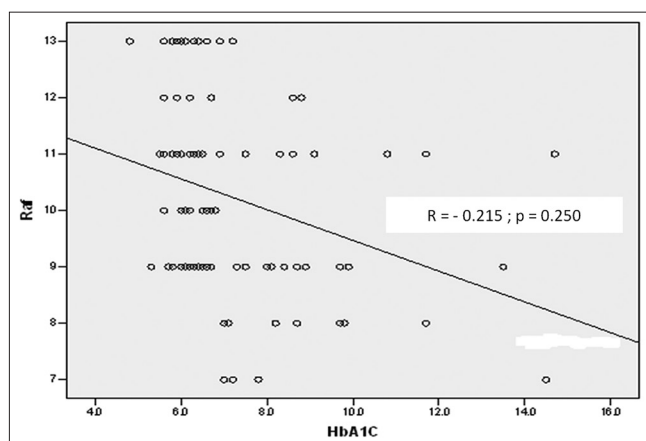

Fig. 4: The correlation between hemoglobin A1c and the levels of Raf-1 kinase

Table 2: The correlation between the serum triglyceride levels of survivin and Raf-1 kinase

Level (pg/ml)	T2DM (n=60)	
	Serum triglyceride (mg/dL)	
	R	p
Survivin	−0.267	0.039
Raf-1 kinase	−0.041	0.760

At T2DM, chronic hyperglycemia has occurred that is very crucial for pancreatic beta-cell function deterioration, which can lead to beta-cell failure. Beta-cell failure is such a worst result of glycemic control in patients with T2DM [11]. Defective pancreatic beta cells are then reflected by the worsening effort in regenerating cells, as reflected in low levels of survivin and Raf-1 kinase [12,13].

This study finds a significant inverse relationship between survivin levels with HbA1c levels in the group of patients with T2DM. In patients with T2DM with HbA1c levels >7% which means bad glycemic control, long glucotoxicity process that can damage pancreatic beta cells by increasing apoptosis, which is characterized by low levels of survivin. Wu *et al.* indicated that survivin is essential for beta-cell mass expansion and appeared to exhibit a preferential requirement for proliferation of preexisting beta cells. The lower levels of survivin in this study may cause by pancreatic beta cells mitochondria damage because of glucotoxicity. It shows that in patients with uncontrolled T2DM, destruction of pancreatic beta cells will be increasing, characterized by low levels of survivin. In the management of diabetes, HbA1C levels

can be lowered, but the impact of previous glucotoxicity process is irreversible, so whenever HbA1c levels are reduced, survivin levels will not increase [13,14].

Results of statistical tests in this study find a significant difference between the levels of Raf-1 kinase in the prediabetes group and the controlled T2DM group with uncontrolled T2DM group (p<0.05). In this study, there is no significant correlation between HbA1c with Raf-1 kinase in T2DM group, mean that the increase in HbA1C levels is not necessarily degrading Raf-1 kinase. It is suspected that chronic hyperglycemia is a factor that could destroy pancreatic beta cells, that does not show a direct effect on Raf-1 kinase but work in conjunction with other PKs such as PI3 kinase and survivin PKC. In Raf-1 kinase against HbA1C correlation, it is shown that chronic hyperglycemia impairs survivin earlier than Raf-1 kinase. Beith found that overexpression of Raf-1 was sufficient to increase proliferation in the absence of insulin, whereas a dominant-negative Raf-1 reduced proliferation in the presence of insulin. In this study, the lower levels of Raf-1 kinase in uncontrolled T2DM can impair the pancreatic beta cells ability to proliferate [7,12,13,15].

Statistically, in this study, there is a significant negative correlation with the weak correlation between serum triglyceride levels with serum survivin levels at the T2DM group. Statistical test with statistical methods of linear regression shows that high triglyceride levels in patients with T2DM relate with the low levels of survivin. This assumes that survivin is more easily influenced by triglycerides, but it still does not find a certain explanation. Ju *et al.* found that survivin attenuated DNA damage-related stress responses suggesting that survivin may facilitate adipocyte maintenance in response to inflammatory stimuli [8,12,16]. Fatty acid is a contributing factor in the growth and survival of mature pancreatic beta cells with the effect of reducing proliferation and lowering survival [17,18].

CONCLUSION

Survivin and Raf-1 kinase levels are lower in uncontrolled T2DM. This explained the role of survivin and Raf-1 kinase against enhancement of pancreatic beta-cell apoptosis in patients with T2DM.

AUTHOR'S CONTRIBUTION

ED contributed to study design, data collection, and manuscript writing.

AM, SS, and SW contributed to study design, final review, and approval. ED and DD contributed in statistical data analysis.

CONFLICTS OF INTEREST

All the authors have no conflicts of interest.

REFERENCES

1. Wild S, Roglic G, Green A, Sicree R, King H. Global prevalence of diabetes: Estimates for the year 2000 and projections for 2030. Diabetes Care 2004;27:1047-53.
2. Stumvoll M, Goldstein BJ, van Haeften TW. Type 2 diabetes: Pathogenesis and treatment. Lancet 2008;371:2153-6.
3. Dussa KN, Sahay RK, Ndararajan PM, Hanarao MV. Adaptation of diabetes knowledge questionnaire for South Asian countries context. Int J Pharm Pharm Sci 2017;9:252-6.
4. Hussain M, Naqvi SB, Khan MA, Rizvi M, Alam S, Abbas A, *et al*. Direct cost of treatment of diabetes mellitus Type 2 in Pakistan. Int J Pharm Pharm Sci 2014;6:261-4.
5. Presetiawati I, Andrajati R, Sauriasari R. Effectiveness of a medication booklet and counseling on treatment adherence in Type 2 diabetes mellitus patients. Int J App Pharm 2017;9:27-31.
6. Dharma S, Macson J, Tobat SR, Dillasamola D. Effect of giving white egg chicken embryo and green beans (*Phaseolus* radiates) to the histopathology of pancreatic beta-cell from diabetic rats (*Rattus novergicus*). Res J Pharm Biol Chem Sci 2016;7:2059-67.
7. Roskoski R Jr. RAF protein-serine/threonine kinases: Structure and regulation. Biochem Biophys Res Commun 2010;399:313-7.

8. Chiou SK, Jones MK, Tarnawski AS. Survivin-an anti-apoptosis protein: Its biological roles and implications for cancer and beyond. Med Sci Monit 2003;9:PI25-9.

9. Bacha F, Lee S, Gungor N, Arslanian SA. From pre-diabetes to Type 2 diabetes in obese youth: Pathophysiological characteristics along the spectrum of glucose dysregulation. Diabetes Care 2010;33:2225-31.

10. Wang H, Gambosova K, Cooper ZA, Holloway MP, Kassai A, Izquierdo D, *et al.* EGF regulates survivin stability through the Raf-1/ERK pathway in insulin-secreting pancreatic β-cells. BMC Mol Biol 2010;11:66.

11. Bonora E. Protection of pancreatic beta-cells: Is it feasible? Nutr Metab Cardivasc Dis 2008;18:74-83.

12. Butler AE, Janson J, Bonner-Weir S, Ritzel R, Rizza RA, Butler PC, *et al.* Beta-cell deficit and increased beta-cell apoptosis in humans with Type 2 diabetes. Diabetes 2003;52:102-10.

13. Marchetti P, Del Prato S, Lupi R, Del Guerra S. The pancreatic beta-cell in human Type 2 diabetes. Nutr Metab Cardiovasc Dis 2006;16 Suppl 1:S3-6.

14. Wu X, Zhang Q, Wang X, Zhu J, Xu K, Okada H, *et al.* Survivin is required for beta-cell mass expansion in the pancreatic duct-ligated mouse model. PLoS One 2012;7:e41976.

15. Beith JL, Alejandro EU, Johnson JD. Insulin stimulates primary beta-cell proliferation via Raf-1 kinase. Endocrinology 2008;149:2251-60.

16. Ju L, Zhang X, Deng Y, Han J, Yang J, Chen S, *et al.* Enhanced expression of survivin has distinct roles in adipocyte homeostasis. Cell Death Dis 2017;8:e2533.

17. Evans JL, Goldfine ID, Maddux BA, Grodsky GM. Oxidative stress and stress-activated signaling pathways: A unifying hypothesis of Type 2 diabetes. Endocr Rev 2002;23:599-622.

18. Lingohr MK, Buettner R, Rhodes CJ. Pancreatic beta-cell growth and survival – a role in obesity-linked Type 2 diabetes? Trends Mol Med 2002;8:375-84.