The Difference Plasma Levels of Endotelin-1 in Type 2 Diabetes Mellitus Women with and without Hypertension

Enita Rahmawati Kurniatmaja[1], Hemi Sinorita[2] Luthfan Budi Purnomo[2]

[1]Speciality Training Program, Department of Internal Medicine, Faculty of Medicine, Universitas Gadjah Mada, Dr. Sardjito General Hospital
[2]Department of Internal Medicine, Faculty of Medicine, Universitas Gadjah Mada, Dr. Sardjito General Hospital

ABSTRAK

Latar belakang. Diabetes Melitus (DM) dan hipertensi merupakan penyakit yang saling berkaitan satu sama lain dan mempunyai predisposisi yang kuat terhadap kejadian aterosklerosis kardiovaskular. Kejadian hipertensi 2 kali lebih besar pada penderita DM dibandingkan non DM. Disfungsi endotel mendasari semua kejadian ini. Marker endotelial yaitu endotelin-1 diketahui dapat mempengaruhi tekanan darah.
Tujuan. Penelitian ini bertujuan untuk mengetahui perbedaan rerata kadar endotelin-1 plasma pada wanita DM tipe 2 dengan dan tanpa hipertensi.
Metode. Penelitian dilakukan secara potong lintang. Penelitian dilakukan di poliklinik Endokrin dan Metabolik unit Penyakit Dalam RSUP Dr. Sardjito Yogyakarta mulai bulan Juli 2010 sampai jumlah sampel terpenuhi. Pemeriksaan kadar endotelin-1 diukur menggunakan metode ELISA sandwich. Karakteristik subyek penelitian disajikan dalam bentuk rerata dan simpang baku. Perbedaan antara dua kelompok dianalisa dengan uji t tidak berpasangan dan uji Mann-Whitney. Dianggap bermakna secara statistik bila $p<0,05$ dengan interval kepercayaan 95%.
Hasil. Didapatkan rerata kadar endotelin-1 plasma pada wanita DM tipe 2 dengan hipertensi (n = 32) dan tanpa hipertensi (n = 32) berturut-turut 19,17 ± 7,53 ng/ml dan 13,75 ± 6,19 ng/dl, dan perbedaan ini bermakna secara statistik dengan p = 0,003 dengan RK 95% -8,87 sd -1,97.
Kesimpulan. Kadar endotelin-1 pada wanita DM tipe 2 dengan hipertensi lebih tinggi dibandingkan kelompok tanpa hipertensi

Kata kunci: *endotelin-1, hipertensi, diabetes melitus tipe 2*

ABSTRACT

Background. Diabetes and hypertension are related to each other and have a strong pre-disposition on the incidence of atherosclerosis. The incidence of hypertension 2 times greater in patients with DM than non DM. Endothelial dysfunction underlies these events. Endothelial markers, endothelin-1 is known to affect blood pressure.
Aim. The aim of this study is to determine the difference plasma levels of endothelin-1 in type 2 diabetes mellitus in women with and without hypertension.
Methods. The cross sectional design was conducted on women with type 2 diabetes mellitus with and without hypertension at metabolic and endocrinology clinic, Dr. Sardjito General Hospital Yogyakarta from July 2010 until the sample met the criterias. Subjects were divided into two groups, type 2 DM woman with hypertension groups and without hypertension. ELISA sandwich's method was used to measure plasma levels of endothelin-1. Characteristics of the study are presented in the form of mean

and standard deviations. The differences between groups were compared by student's unpaired t-test an Mann Whitney test. The P value was considered significant if p<0.05 with 95% confidence interval.

Results. In this research, the plasma levels of endothelin-1 in in type 2 diabetes mellitus in women with hypertension (n=32) and without hypertension (n=32) respectively 19.17±7.53 ng/ml and 13.75±6.19 ng/dl, and this difference was stastistically significant with p= 0.003 CI 95% -8.87 to -1.97.

Conclusion. Plasma levels of endothelin-1 in type 2 diabetes woman with hypertension higher than without hypertension

Keywords: *endothelin-1, hypertension, diabetes mellitus type 2*

INTRODUCTION

Diabetes mellitus (DM) is a major threat to human health in the 21st century. In Asia-Africa, especially the western Pacific region, estimated more than 30 million people suffer from diabetes. Databases from World Health Organization (WHO) in the year 2000 the diabetes frequencies over the age of 25 years amounted to 150 million people and by 2025 that number estimated will be 300 million people.[1,2]

Hypertension and diabetes are related and have a strong predisposition to atherosclerotic cardiovascular events. The incidence of hypertension 2 times greater in patients with DM than non DM. Lifestyle and genetic factors plays an important role in hypertension and DM. About 35-75% cardiovascular and renal complication in patients with DM associated with hypertension. Its also contribute to the incidence of retinopathy DM. For all this reason, hypertension and DM should recognize and treated early and aggressively. [3,4]

Chronic diabetic complications constitute a group of diseases responsible for substantial morbidity and mortality, thus preventing complications of diabetes management is important. Several studies have identified several factors that play key role in the pathogenesis of diabetic complications such as protein kinase C (PKC) activation, non-enzymatic glycation, oxidative stress, and alterations in growth factor and vasoactive factor expression. Several of these factors may subsequently lead to further endothelin (ET) activation in diabetic subjects.[5]

In some DM complications found increased levels of ET-1. Endothelin-1 levels affect blood pressure in various ways, increasing vasoconstriction, affecting the inflammatory response that contributes to vascular disorders remodeling and endothelial dysfunction.[6]

Increased levels of ET have an important role in the occurrence of hypertension in diabetic subject. The changes in plasma levels of ET can be found in several diseases associated with endothelial dysfunction, such as diabetes, hypertension and atherosclerosis.[5]

METHODS

The cross sectional design was conducted on women with type 2 diabetes mellitus with and without hypertension at metabolic and endocrinology clinic, Dr. Sardjito General Hospital Yogyakarta from July 2010 until the sample met the criterias. Subjects were divided into two groups, type 2 DM woman with hypertension groups and without hypertension. Inclusion criteria were women with type 2 diabetes who attend at the Endocrine Clinic Dr. Sardjito aged ≥ 22

years to ≤ 60 years, signed informed consent. Exclusion criteria were patients with chronic renal failure, chronic heart failure, malignancy, liver cirrhosis, sepsis, under steroid treatment, using hormonal contraceptives, pregnancy or breastfeeding.

Subjects who met the inclusion and exclusion criteria were invited to attend the appointed day. Inclusion and exclusion criteria established by anamnesis, physical examination and medical records.

The subjects were asked to perform daily activities and have diet as usual. Hypertension medications commonly taken by subjects with hypertension are not taken within the previous 3 days. On the appointed day, the subject came in a fasting state for at least 8-10 hours. The subject's identity was recorded in the form of research conducted anamnesis, physical examination (blood pressure, height, respiratory frequency, temperature, weight and waist circumference). We also put personal data in the form of research, such as age, sex, history of hypertension, the treatment of hypertension, hypertension drug, and family history of hypertension. The blood pressure was measured twice. After lying for 10 minutes, and then performed taking as much as 20 ml of venous blood for examination fasting glucose, postprandial glucose levels, lipid profile and levels of endothelin-1. Examination of blood samples carried out in biochemistry and laboratory in Medical Faculty of Gadjah Mada University. Figure 1 shown the scheme of this research.
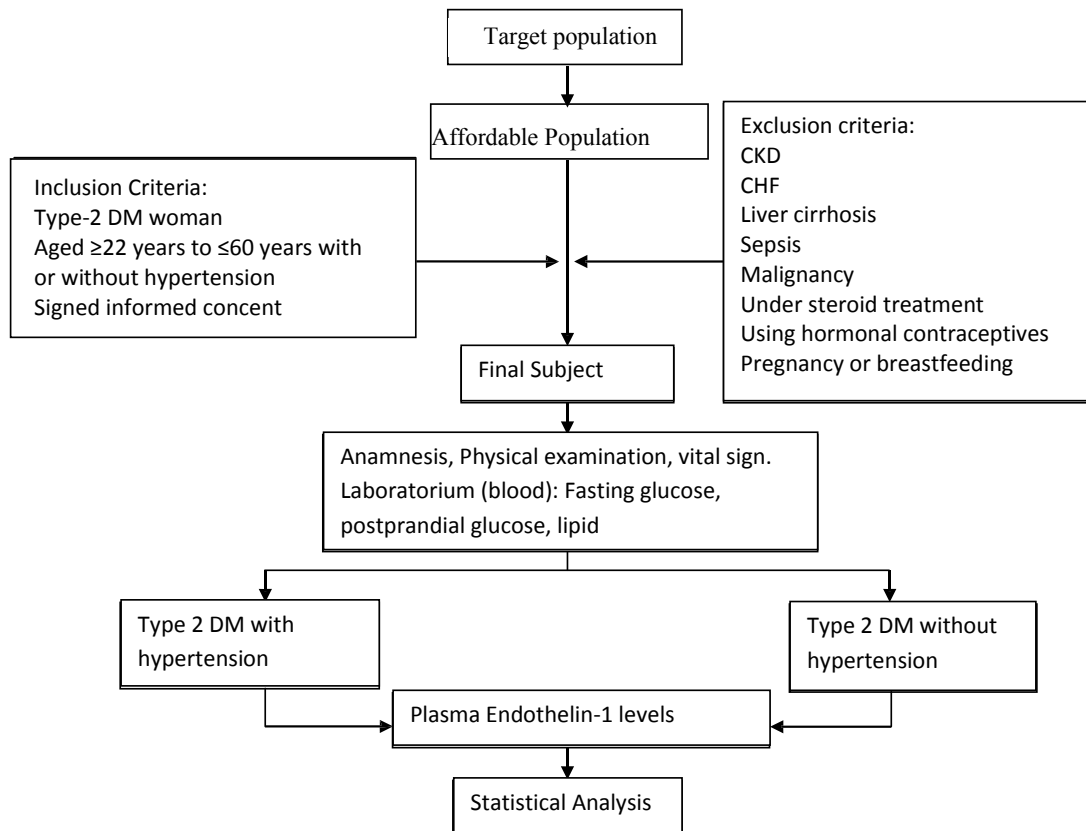

Figure 1. Research scheme

Table 1. Baseline characteristics of the subjects

Variable	Mean±SD Median (Min-Max)	Frequencies (%)
Hypertension status		
• With hypertension		32 (50)
• Without hypertension		32 (50)
DM duration (years)	7(2-17)	
Ages (years)	55 (44-59)	
Height (m)	1.55±0.05	
Weight (kg)	59.22±8.36	
Body Mass Index (kg/m²)	24.65±3.27	
Systolic blood pressure (mmHg)	130 (110-150)	
Waist circumference (cm)	80 (70-90)	
Fasting glucose (mg/dl)	131 (70-286)	
Postprandial glucose (mg/dl)	177.33±38.66	
HbA1C (%)	8.19±1.88	
Endotelin-1 levels (ng/ml)	16.46±7.37	

Measurement

Levels of endothelin-1 were examined by using Quantikinine Human endothelin Immunoassay. Results are expressed in ng/ml.

Statistical Analysis

Characteristics of the subjects are presented in the form of mean and standard deviations. Mean difference between the levels of an ET-1 group of type 2 diabetes woman with and without hypertension were analyzed by independent t-test if normally distributed or the non-parametric test Mann-Whitney U when the data are not normally distributed. Normality test using the Kolmogorov-Smirnov analysis (for samples > 50) or the Shapiro-Wilk analysis (for samples <50). Differences were considered statistically significant, defined by p <0.05 with a confidence interval (CI) 95%. [7]

RESULTS

This study was conducted from July to December 2010. During the study period, we found 64 female subjects with type 2 diabetes who met the inclusion criteria consisting of 32 subjects with hypertension (50%) and 32 subjects without hypertension (50%). Baseline characteristics of the subjects are shown in Table 1.

In this study, the median age of women with type 2 diabetes was 55 years, 10 subjects with aged 41-50 years (16.13%) and aged between 51-60 years 52 subjects (83.87%), with median duration of diabetes for 7 years. This suggests that increased endothelial damage as a result of microvascular complications in line with the long-suffering DM.

In this study, the mean of HbA1C levels about 8.19 ± 1.88%. In accordance with PERKENI[8] consensus, HbA1C values in these subjects including poor control criteria. Overall the mean plasma levels of ET-1 at 16.46 ± 7.37 ng/dl. Normal endothelin levels is 1-2 pg/ml.[9] This is consistent with previous studies, the levels of endothelin in patients with diabetes are higher than those who did not suffer from diabetes.

Table 2. Mean difference variables of type 2 DM subjects by hypertension status

Variable	Type 2 DM With Hypertension	Type 2 DM Without Hypertension	P	IK 95%
Age (years)	55.53 ± 2.69	52.56 ± 3.81	0.002*	
Height (m)	1.56±0.04	1.55±0.06	0.335	-0.03 sd 0.01
Weight (kg)	61.81±6.72	56.63±9.12	0.012	-9.19 sd -1.19
Body Mass Index (kg/m²)	25.43±2.61	22.86±3.69	0.034*	
Systolic blood Pressure (mmHg)	137.50±8.03	118.75±7.93	<0.001*	
Diastolic blood pressure (mmHg)	88.75±3.36	74.69±5.07	<0.001*	
Fasting glucose (mg/dl)	136.56±43.56	136.50±30.52	<0.545*	
Postprandial glucose (mg/dl)	167.25±39.46	187.41±35.66	0.036	1.36 sd 38.95
HbA1C (%)	7.80±2.13	8.58±1.53	0.09	-0.15 sd 1.69
Endothelin-1 levels (ng/ml)	19.17±7.53	13.75±6.19	0.003	-8.87 sd -1.97

Table 2 also shown the analysis of the subject variable of type 2 diabetes based on hypertension status. The mean age of the subjects with type 2 diabetes with hypertension (55.53 ± 2.69) were older compared with type 2 diabetes without hypertension (52.56 ± 3.81), p = 0.002.

The mean body mass index (BMI) in subjects with type 2 diabetes with hypertension were significantly higher than those with type 2 diabetes without hypertension, respectively 25.43 ± 2.61 kg/m² and 22.86 ± 3.69 kg/m², and the Mann Whitney test statistically significant (p = 0.034).

From blood pressure, the mean systolic and diastolic blood pressure in subjects with type 2 diabetes women with hypertension at 137.50 ± 8.03 and 88.75 ± 3.36 mmHg, and this difference was statistically significant (p = <0.001).

The mean fasting blood glucose in subjects with type 2 diabetes with hypertension than those without hypertension, respectively 136.56 ± 43.56 mg/dl and 136.50 ± 30.52 mg/dl. These results were not statistically significant. The mean of the postprandial glucose levels in subjects with type 2 diabetes

with hypertension also lower than without hypertension with mean value of each 167.25 ± 39.46 mg/dl and 187.41 ± 35.66. The mean of HbA1c (%) was lower in the group with type 2 diabetes with hypertension (7.80 ± 2.13%) compared with the group without hypertension (8.58 ± 1.53%).

In this study, the levels of plasma endothelin-1 levels in patients with type 2 diabetes with and without hypertension respectively 19.17 ± 7.53 ng/ml and 13.75 ± 6.19 ng/dl. The levels of endothelin-1 in type 2 diabetes with hypertension than those with type 2 diabetes without hypertension is different and the difference was statistically significant with p = 0.003 (CI 95% -8.87 to -1.97).

DISCUSSION

In this study, the subjects with age 51-60 years are more than age 41-50 year groups. These results are consistent with studies that reported by Yang et al[10] and Ferrara et al[11] the prevalence of diabetes increases with age (20-39 years age group increased 3.2%, the group increased 11.5% 40-59 years, and ≥ 60 years increased 20.4%). In older people there

is a decrease in physical activity and muscle mass and increased body fat that contributes to worsening insulin sensitivity with age in both men and women. Type 2 diabetes is usually diagnosed in people aged 40 years or older. AkG et al[12] showed that patients with diabetes duration > 10 years had higher levels of ET-1 than patients with diabetes duration <10 years (p = 0.02). In this study, the median duration suffered from diabetes is 7 years. This suggests that increased endothelial damage as a result of microvascular complications in line with the long-suffering DM.

At baseline characteristics, the mean body mass index (BMI) of the subjects is 24.65 ± 3.27 kg/m². Based on WHO[13] criteria for the Asia, it is include pre-classification of obesity (BMI 23.0 to 24.9 kg/m²). The increased risk was further increased comorbid in individuals with increased waist circumference. Based on PERKENI[8], the mean of BMI in the subjects of this study included a moderate diabetes control (BMI 23-25 kg/m²).

The mean of HbA1C levels in this study is 8.19 ± 1.88%. In accordance with PERKENI[8], HbA1C levels in these studies, including poor control criteria. This is consistent with Sanchez et al1[4] study, that showed a correlation between the levels of ET-1 with glycemic control. Diabetic patients with poor glycemic control showed elevated levels of ET-1 were higher than those with good glycemic control (13.03 ± 7.57 v.s. 7.64 ± 1.40 pg/ml, p <0.01).

In this study, overall the mean plasma levels of ET-1 at 16.46 ± 7.37 ng/dl. This is consistent with previous studies; the levels of endothelin in patients with diabetes are higher than those who did not suffer from diabetes.

The study involved two groups of female subjects with type 2 diabetes were divided based on the status of hypertension,

the hypertensive group included 32 subjects (50%) and without hypertension were 32 subjects (50%). The relationship between hypertension and diabetes is very strong because there are several criteria that often exist in patients with hypertension such as the increased of blood pressure, obesity, dyslipidemia, and increased of blood glucose. Hypertension in diabetes incidence associated with major cardiovascular diseases. Incidence of hypertension are 2 times greater in patients with DM than non DM. The presence of hypertension in patients with diabetes will accelerate the microvascular complications, and increased the mortality of 4 to 5 times.[15]

From the blood pressure parameters, we have known the mean systolic and diastolic blood pressure in subjects with type 2 diabetes women with hypertension at 137.50 ± 8.03 and 88.75 ± 3.36 mmHg. These results are consistent with the Aziza et al[6], which states that the expression of ET-1 in hypertension than those with pre-hypertension and ET-1 in the pre-hypertension higher than normotensive (p =0.007). DM is closely linked to hypertension, about 75% of diabetic patients develop into hypertension. Some of the mechanisms leading to the occurrence of hypertension in patients with diabetes include activation of the renin-angiotensin-aldosterone system, presence of intravascular volume expansion caused by hyperglycemia, decreased baroreceptor response, autonomic dysfunction, endothelial dysfunction and oxidative stress vascular. [16]

The levels of endothelin-1 in patients with type 2 diabetes with and without hypertension respectively 19.17 ± 7.53 ng/ml and 13.75 ± 6.19 ng/dl. Levels of endothelin-1 in type 2 diabetes with hypertension than those with type 2 diabetes without hypertension are

higher and this difference was statistically significant with p = 0.003 (CI 95% -8.87 to -1.97). These results are consistent with Singh et al1[7] study, reported a significant relationship between levels of ET-1 and hypertension, elevated levels of endothelin in patients with type 2 diabetes with hypertension compared with controls with average levels of endothelin respectively 4.94 ± 1.42 and 4.21 ± 0.84 pg/ml (p = 0.046).

Another study conducted by Skalska, et al1,[8] reported in subjects with hypertension the levels of ET-1 were higher (1.35 ± 0.51 VS1, 12 ± 0.46 pg/ml, p = 0.04) compared controls. ET-1 levels are increased is associated with low plasma antioxidant status, oxidative stress resulting from an increase in diabetes and hypertension.

Schneider et al[19] also reported patients with diabetes or hypertension have elevated levels of ET-1 compared with controls, but the elevated levels of ET-1 do not cause hypertension in diabetes. These findings suggest that plasma levels of ET-1 are not a marker of endothelial dysfunction, but the changes of plasma levels of ET-1 may precede vascular complications associated with hypertension and diabetes.

In this research, we have done in women with type 2 diabetes with and without hypertension, between the two groups there are significant differences on several variables, age, body mass index, postprandial blood glucose and ET-1 levels.

REFERENCES

1. Cockram, C. S. , 2000. The epidemiology of diabetes mellitus in the Asia- Pacific region. HKMJ; 6: 43-52. Schneider, J. G., Tilly, N., Hierl, T., Sommer, U., Hamann, A., Dugi, K., Leidig-Bruckner, G., Kasperk, C. 2002. Elevated P lasma E ndothelin-1 L evels in D iabetes M ellitus. Am J Hyperten s; 15 (11): 967-72.

2. Suyono, S. 200 9. Diabetes Mellitus in Indonesia. In: Aru, W, S., Bambang, S., K., A., Marcellus, SK, Siti, S., (eds). Textbook of Medicine, Volume III. Issue 5. Faculty of medicine, University of Indonesia: 187 3-79.

3. Epstein, M., Sowers, JR 1992. Diabetes Mellitus and Hypertension. Hypertension; 19 (5): 403-18. Iglarz, M., Schiffrin, EL 2003. Role of endothelin-1 in Hypertension. Current Hypertension Reports; 5: 144-148.

4. Chakrabarti, S., Khan, ZA, Cukiernik, M., Fukuda, G., Chen, S., Mukherjee, S., 2002. Alteration of Endothelins: A Common pathogenetic Mechanism in Chronic Diabetic Complications. Int. JNL. Experimental Diab. Res; 3: 217-231.

5. Aziza, L., Sja'bani, M., Haryana, SM, Soesatyo, M., Sahadev, AH 2011. Endothelin-1 relationship with Hypertension in Population Mlati, Sleman, Yogyakarta, Indonesia. Indon J Med Assoc; 61: 237-42.

6. Dahlan, SM 2006. Large Sample in Medical and Health Research. Jakarta. PT.ARKANS: 14-102.

7. Perkumpulan Endokrinologi Indonesia (PERKENI). Pengelolaan Diabetes Melitus Tipe 2. Dalam Soewondo, P (eds). Konsensus dan Pencegahan Diabetes Mellitus Tipe-2 di Indonesia. PB PERKENI. 2011: 6-47.

8. Takahashi, K., Ghatei, M.A., Lam, H.C., O'Halloran, D.J., Bloom, S.R. Elevated Plasma Endothelin in Patients with Diabetes mellitus. Diabetologia. 1990; 33: 306-310.

9. Yang, W., Lu, J., Weng, J., Jia, W., Ji, L., Xiao, J. Prevalence of Diabetes among Men and Women in China. New Engl J Med. 2010; 362: 12.

10. Ferrara, C. M., Golgberg, A.P., Nicklas, B.J., Sorkin, J.D., Ryan, A.S. Sex differences in insulin action and body fat distribution in overweight and obese middle-aged and older men and Women in Appl Physiol Nutr Metab. 2008;33(4): 784-790.

11. Ak, G., Buyukberber, S., Sevinc, A., Turk, H.M., Ates, M., Sari, R., et al. The relation between plasma endothelin-1 levels and metabolic control, risk factors, treatment modalities, and diabetic microangiopathy in patients with type 2 diabetes mellitus. J Diabs Compl. 2001;15(3):150

12. World Health Organization. Obesity : Preventing and Managing the Global Epidemic. World health organ tech rep ser. 2000: 894.

13. Sanchez, S.S., Aybar, M.J., Velarde, M.S., Prado, M.M.,Carrizo, T. Relationship between plasma endothelin-1 and Glycemic control in type 2 Diabetes mellitus. Horm Metab res. 2001; 33:748-51.

14. Chobanian, A.V., Bakris, G.L., Black, H.R., Cushman, W.C., Green, L.A., Izzo, Z.L. Jr., Jones, D.W., Materson, B.J., Oparil, S., Wright, J.T.Jr., Roccella, E.J. Joint National Committee on Prevention, Detection, Evaluation, and Treatment of High Blood Pressure, National Heart, Lung, and Blood Institute, National High Blood Pressure Education Program Coordinating Committee. Seventh report of the Joint National Committee on Prevention, Detection, Evaluation, and Treatment of High Blood Pressure. Hypertension. 2003; 42: 1206-52.

15. Bakris, G. Maximizing cardiorenal benefit in the management of hypertension: achieve blood pressure goal, J Clin Hypertens (Greenwich). 1999; 1:141-147.

16. Singh, U., Singh, R.G., Singh, S., Singh, S. Plasma Endothelin Level in Hypertension and Diabetes Mellitus. Med. Case Stud. 2011; 2(6) : 50-53.

17. Skalska, A.B., Pietrzyckab, A., Stepniewskib, M. Correlation of endothelin-1 plasma levels with plasma antioxidant capacity in elderly patients treated for hypertension. Clinical Biochemistry. 2009; 3: 42(4-5): 358-364

18. Schneider, J.G., Tilly, N., Hierl, T., Sommer, U., Hamann, A., Dugi, K., Leidig-Bruckner, G., Kasperk, C. Elevated Plasma Endothelin-1 Levels in Diabetes Mellitus. Am J Hypertens. 2002; 15(11) : 967-72.