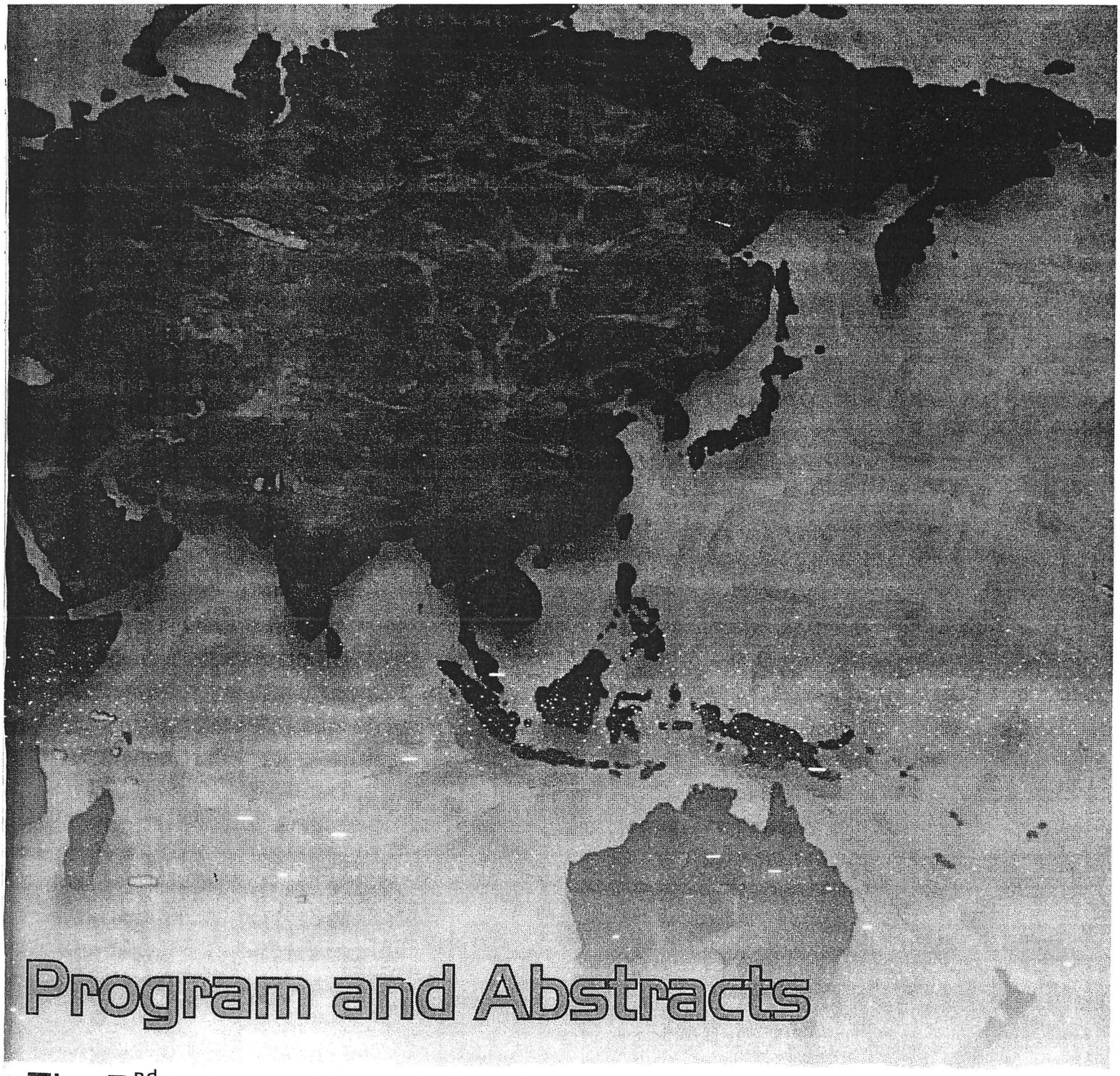

Program and Abstracts

The 2nd International Conference of the Indonesian Chemical Society 2013

ICICS 2013

Research in Chemistry for Better Quality of Environmental

Universitas Islam Indonesia, Yogyakarta, Indonesia
October, 22 - 23th 2013

Abdul Kahar Muzakkir, Conference Hall
Universitas Islam Indonesia (UII), Yogyakarta.
Kampus Terpadu, Jl. Kaliurang KM 14,5 Sleman, Yogyakarta.

Preface

The international conference is an annual conference of the Indonesian Chemical Society (Himpunan Kimia Indonesia, HKI). In the year 2013, the mandate of the organizing committee was given to the HKI Yogyakarta branch and also supported by Department of Chemistry of Universitas Negeri Yogyakarta (UNY), Department of Chemistry of Universitas Gadjah Mada (UGM), Department of Chemistry of Universitas Islam Negeri Sunan Kalijaga (UIN Suka), National Nuclear Energy Agency (BATAN Yogyakarta), and Balai Penyelidikan dan Pengembangan Kegunungapian (BPPTK Yogyakarta).
For the year 2013, ICICS 2013 is hosted by Department of Chemistry, Faculty of Mathematics and Natural Sciences, Islamic University of Indonesia, Yogyakarta from October 22 – 23, 2013. This conference was also prepared to celebrate 70th anniversary of Universitas Islam Indonesia.

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The Sicentific Programme of ICICS2013 comprises the following:

1. Invited Speaker — 11 papers
2. A total 256 paper for parallel session
   a. Organic Chemistry — 32 papers
   b. Inorganic Chemistry — 43 papers
   c. Physical Chemistry — 37 papers
   d. Analytical Chemistry — 68 papers
   e. Education Chemistry — 23 papers
   f. Biochemistry — 43 papers

The breakdown of the presentation is as follows:

Session	Oral	Poster	Total
Invited Speaker	11	0	11
Organic Chemistry	25	7	32
Inorganic Chemistry	38	5	43
Physical Chemistry	31	6	37
Analytical Chemistry	61	7	68
Education Chemistry	22	1	23
Biochemistry	34	8	43
Total	**222**	**34**	**256**

Content

B-12

Biochem 014
Oral

Prevalence of Estrogen Receptor Alpha (*ESRα*) Gene Polymorphism in Javenese postmenopausal Women with DM type 2

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Abstract

Polymorphisms of estrogen receptor alpha (*ESR-α*) gene may cause deleterious effect to metabolic action, particularly in the glucose homeostasis due to insulin resistance. Polymorphisms in the ESR-α gene have been associated with the occurrence of breast cancer, menopausal osteoporosis, recurrent abortions, arterial hypertension, T2DM, dyslipidemia, cardiovascular complications such as coronary heart diseases and stroke. It is understood that in postmenopausal women the incidence of T2DM as well as its cardiovascular complications will increase. So far there are two polymorphisms in ER-α that are the most widely studied as risk factor for many diseases, namely the *PvuII* and *XbaI* (*rs2234693* and *rs9340799*). Both polymorphisms have been identified as markers of T2DM in postmenopausal women. The distributions of *PvuII* and *XbaI* polymorphisms among the postmenopausal women in the world have been studied, and therefore it is interesting to know their distributions in the Javanese postmenopausal population with T2DM. In this cross-sectional study as many as 121 postmenopausal women especially with T2DM, with age ranges from 45 to 70 years, were consecutively entered. *PvuII and XbaI* polymorphisms were determined by the PCR-restriction fragment length polymorphism (RFLP). The alleles for *PvuII* and *XbaI* were denoted as P and X for absence of restriction site and denoted as p and x respectively for presence of restriction site. The results disclosed *PvuII* genotypes distribution as follows, PP 26.4 %, Pp 46.3 %, and pp 27.3 %, and as for *XbaI* genotypes were XX 42.1 %, Xx 32.2 % and xx 25.6 %. Four haplotypes were recognized, and their distributions were PX 24.4 %, Px 23.1 %, pX 28.9 % and px 23.6 %. In conclusion, the prevalent gene for ER-α *PvuII* was heterozygote Pp (46,3 %) and as for *XbaI* was homozygote XX (41,3 %), and the prevalent for the haplotype was pX (28,9%).

Keywords: Prevalence- ESRα Polymorphism-Javanese Postmenopausal Women.

Himpunan Kimia Indonesia

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Present to

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The 2nd International Conference of the Indonesian Chemical Society 2013 (ICICS 2013)

at

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Yogyakarta, 22 - 23th October 2013

Dr. Muhammad A. Martoprawiro
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Yandi Syukri, M.Si., Apt.
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ICICS 2013

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Organizing Committee

Prevalence of Estrogen Receptor Alpha (*ESRα*) Gene Polymorphism in Javenese Postmenopausal Women with DM type 2

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Polymorphisms of estrogen receptor alpha (*ESR-α*) gene may cause deleterious effect to metabolic action, particularly in the glucose homeostasis due to insulin resistance. Polymorphisms in the ESR-α gene have been associated with the occurrence of breast cancer, menopausal osteoporosis, recurrent abortions, arterial hypertension, type 2 diabetes mellitus (T2DM), dyslipidemia, cardiovascular complications such as coronary heart diseases and stroke. It is understood that in postmenopausal women the incidence of T2DM as well as its cardiovascular complications will increase. So far there are two polymorphisms in ER-α that are the most widely studied as risk factor for many diseases, namely the *PvuII* and *XbaI* (*rs2234693* and *rs9340799*). Both polymorphisms have been identified as markers of T2DM in postmenopausal women. The distributions of *PvuII* and *XbaI* polymorphisms among the postmenopausal women in the world have been studied, and therefore it is interesting to know their distributions in the Javanese postmenopausal population with T2DM. In this cross-sectional study as many as 121 postmenopausal women especially with T2DM, with age ranges from 45 to 70 years, were consecutively entered. *PvuII and XbaI* polymorphisms were determined by the PCR-restriction fragment length polymorphism (RFLP). The alleles for *PvuII* and *XbaI* were denoted as P and X for absence of restriction site and denoted as p and x respectively for presence of restriction site. The results disclosed *PvuII* genotypes distribution as follows, PP 26.4 %, Pp 46.3 %, and pp 27.3 %, and as for *XbaI* genotypes were XX 42.1 %, Xx 32.2 % and xx 25.6 %. Four haplotypes were recognized, and their distributions were PX 24.4 %, Px 23.1 %, pX 28.9 % and px 23.6 %. In conclusion, the prevalent gene for ESR-α *Pvu*II was heterozygote Pp (46, 3 %) and as for *XbaI* was homozygote XX (42, 2 %), and the prevalent for the haplotype was pX (28, 9%).

Keyword: Prevalence- ESRα Polymorphism-Javanese Postmenopausal Women

I. INTRODUCTION

Type 2 Diabetes Mellitus (T2DM) is a disease caused mainly by disrupted homeostasis of glucose with deleterious consequences to many organ including heart, kidney, nervous system, eyes, and vasculare (1). The etiology of T2DM is combination of genetic factors such as polymorphism and mutation and enviromental factors such as diet and life style of low exercises (2,3).

The number of diabetic patients among women are increasing with the increasing age of women especially in menopause and postmenopausal women which known as low level estrogen-associated diabetes type 2 (4). In menopause and postmenopausal women, low level estrogen have associated with several condition or symptom such as vasomotor symptom (hot flash), mood disturbances (loss of control), sleep disorders (insomnia), sexual disfunction and urinary symptom (incontinence), vaginal dryness, cancer, osteoporosis and cardiovascular disease (hypertension) (5,6,7). This condition due to changes of aging itself and changes in endocrine system accompanying natural menopause.

Estrogen with estrogen receptor are becoming new players in diabetes mellitus especially in glucose hemostasis and insulin resistance (8). Estrogen can stimulate liver fatty acid metabolism, suppress hepatic glucose produc- tion, reduce both hyperglycemia and plasma in- sulin levels, protect pancreatic β-cell

function/survival, and increase GLUT-4 expression and glucose uptake. Estrogen modulates GLUT- 4 expression in tissues through its receptors (9). Estrogen modulates insulin secretion, regulates ATP-sensitive potassium channel (K- ATP channel) activity, and regulates calcium signals via plasma membrane estrogen receptors (10).

The physiological effect of estrogen is mediated mainly through estrogen receptors. Currently there are three estrogen receptors, i.e. ESRα, ESRβ and the G protein-coupled ER (GPER) (11,12,13). GPER have been identified in rodent and human β cells. Activation of ESR-α enhances glucose-stimulated insulin biosynthesis, reduces islet toxic lipid accumulation and promotes β-cell survival from proapoptotic stimuli, while activation of ESRβ increases glucose-stimulated insulin secretion, whereas activation of GPER protects β cells from apoptosis, raises glucose-stimulated insulin secretion and lipid homeostasis without affecting insulin biosynthesis (14).

Estrogen receptor alpha (ESRα) function is determined by ESRa gene with various polymorphism such as *PvuII* and *XbaI* (Fig.1) in the first intron. The *ESR-α* gene is large approximately 140 kb of DNA, encompassing 8 exons, encodes protein consisting of 595 amino acids with molecular weight of 66 kDa. The ESRα is located on chromosome 6q25.1 [15].

The aim of this study was to determine the distribution of polymorphism in *ESR α* gene in diabetics postmenopausal women population. We addressed this question in diabetics Javenese postmenopausal women-based studies.

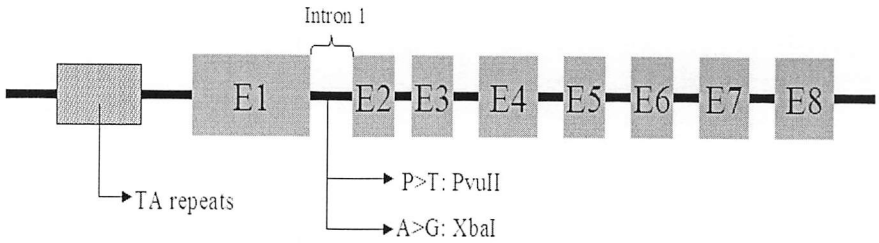

Figure 1. Location of ESRα PvuII and XbaI Polymorphism

II. MATERIALS & METHODS

Study Subjects

A total of 121 diabetic subjects with T2DM were recruited in this study by cross sectional design. All the patients were attended during public service program of Faculty of Medicine Islamic University of Indonesia in several places of Kalasan, Condong Catur and Prambanan sub district from April to December 2012. The subjects were selected under the inclusion and exclusion criteria. Diagnosis of type 2 diabetes mellitus was based on WHO 1999 criteria (16). Individuals with fasting blood glucose levels are equal to or greater than 126 mg/dl. Group of individuals are selected as subject of this research in line with

requirements specifically have no history of taking HRT, do not suffer from liver failure and kidney failure and age over 45 years and under 65 years old.

The subjects were recruited only from Javanese ethnic. Then questionnaire were prepared to assess the socio-demographic factors, history and the duration of T2DM.

We have performed physical examination to all subject encompassed blood pressure and *anthropometry* measurements such as waist circumference, height, weight, BMI, hip circumference and waist circumference.

Sample Collection and Biochemical Analysis

Four-five milliliters of peripheral blood samples were collected and transferred into an EDTA tube by a qualified phlebotomist. Blood samples were collected from the subjects after a 10-12-h fasting. Total cholesterol, HDL cholesterol, and triglyceride levels were determined by standard methods using commercial kits conducted in Kalasan Islamic Hospital Jogjakarta as certified laboratory. Plasma was separated from the blood by centrifuge at 4500 rpm and stored at -20°C until further analysis. Individual weight and height were obtained to calculate body mass index (BMI) using the formula, weight (kg)/ [height (m2)]. To determine the levels of triglycerides (TG), high density lipoprotein cholesterol (HDL-C) and total cholesterol (TC), plasma samples was analyzed using *Metrolab 2300* Autoanalyser (Diatron, Argentina) with kits supplied by Clona-test Diagnostics (Linear Chemical, Spain). Low density lipoprotein cholesterol (LDL-C) was calculated using Friedewald formula (17). We have used *vectube* (Shanghai, China) for blood collection container containing EDTA anticoagulant. In this study triacylgliserol (TAG) examination should be evaluated with serum examination to do check and make certain the result of plasma examination within 10 % of sample. Some literature explained the higher result of TAG in serum sample than plasma sample in range 2.5-4.5%. TAG in non fasting subjects is higher up to 40% than in fasting subjects but for HDL and cholesterol total are not significantly different between non fasting and fasting subjects (18).

Genotyping Methods

Genomic DNA was extracted from peripheral blood using Genomic DNA isolation kit (Nucleo Spin, Macherry Nagel, Hamburg, Germany). The concentration and the purity of extracted DNA were quantified by micro-volume spectrophotometer, using *MaestroNano* (Maestrogen, China). Isolation of DNA carried out with Nucleo Spin kit method. DNA was isolated from peripheral blood leukocyte cells. Examination of the presence of *PvuII* and *XbaI*

polymorphisms in the ESR alpha gene has been performed by PCR-RFLP. By using the forward primer 5'CTGCCACCCTATCTGTATCTTTTCCTATTCTCC-3' and reverse primer 5'TCTTTCTCTGCCACCCTGGCGTCGATTATCTGA3'1. PCR reaction was performed by using a *Biometra* PCR thermo cycler and reagents *intron*. PCR reactions using the total volume of 30 µl PCR reaction consisted of 2 µl DNA, 15 µl PCR master mix containing 1 × PCR buffer, 150 mM dNTP and U Tag DNA polymerase, 2 µl primer and 1 µl of each forward and reverse, 11 µl distilled water. Temperature conditions of PCR cycles that initial denaturation for 5 min at 95^0C, followed by 35 cycles of PCR with denaturation at 95^0C 30 sec, annealing at 62^0C 30 sec, extension at 72^0C for 30 sec, 2-minute final extension at 72^0C and cooling at 4^0C (4,9,10).

Polymorphism Analysis

PCR products have been digested by using restriction enzyme of *PvuII* and *XbaI*. For each volume 8 µl of PCR products have incubated with 1 unit of *XbaI* for detecting polymorphism of X/x and with 1 unit *PvuII* for detecting polymorphism of P/p. The codes of P/p and X/x means that upper case lettering is signifying the absence and the lower case lettering is indicating the presence of the restriction site. Then we have added 1.5 µl of NE buffer 1x, 1.5 µl BSA10x, 3µl distilled water up to 15 µl for each reaction of PvuII and XbaI enzyme. Incubation has conducted for 8 hours at 60°C for both. To distinguish c.454-397 T > C (*PvuII*) and c.454-351 A>G (*XbaI*) polymorphism, the amplified PCR fragment of 1372 bp was digested with restriction enzyme *XbaI* and *PvuII* separately, followed by electrophoresis on 2% agarose gel. For PvuII, the mutated homozygous variant pp (TT) produced two fragments 982 and 390 bp when heterozygote Pp (CT) produced three fragments of, 1372 and 982 bp and 390. Wild-type P (CC) produced one fragment of 1372 bp. For XbaI the mutated homozygous variant xx (AA) produced two fragments 936 and 436 bp when heterozygote Xx (AG) produced three fragments of 1372, 936, and 436 bp and XX (GG) wild-type produced one fragment of 1372 bp (4,9,10).

Statistics

Statistical analysis was performed with SPSS version 17.0 and THESIAS software version 3.1. Genotype distribution was tested for Hardy-Weinberg equilibrium by χ^2 to analyze haplotype in Javanese population based on maximum likelihood model and the SEM algorithm (19). A p-value less than 0.05 were considered significant.

Ethics

The study protocol was approved by the Ethical Committee of the Faculty of Medicine and Health Sciences, Universitas Muhammadiyah Yogyakarta (UMY). Ethical committee have issued the Ethical Clearance for this study. Written informed consent was obtained from all the subjects.

III. RESULT AND DISCUSSION

All characteristics are presented in table 1 as general infomation of all subjects. The criterion of T2DM is based on fasting glucose levels according to WHO criteria using ≥ 126 mg. For all variables are normal condition in average except glucose.

TABLE 1.
SUBJECT CHARACTERISTICS

Subject Characteristics	Postmenopausal Women (n= 121)
	Mean ± SD
Age (years)	55.50±6.342
Systole (mmHg)	126.78±18.57
Diastole (mmHg)	79.82±9.132
Height (cm)	152.26±6.12
Weight (Kg)	54.87±9.67
BMI	23.12±3.72
Abdominal Circumference (cm)	85.96±10.60
Cholesterol (mg/dl)	197.12±48.73
LDL (mg/dl)	120.43±38.12
HDL (mg/dl)	55.20±20.01
TGA (mg/dl)	144.12±73.04
Fasting-Glucose (GLU) mg/dl	133.39±65.78

Then the subjects have been examined for the genotype to determine the *XbaI* and *PvuII* polymorphism. The results of genotype examination have been presented in table 2. In the population genotyped for PvuII, this polymorphism was distributed as follows: PP 26.3% (n=32), Pp 46,3% (n=56), pp 27.3% (n=33). The frequency of XbaI genotype in the population was XX 42.2% (n=51), Xx 32.2% (n=39), xx 25,6% (n=31). The distribution of genotypes was in Hardy-Weinberg equilibrium for *PvuII* ($\chi 2$ p=0.413651) and *XbaI* ($\chi 2$ p= 0.091688) calculated by using THESIAS software. Table 2 shows the genotype and alelle frequencies of the ESRα intronic polymorphisms *PvuII* and *XbaI* in the studied population.

TABLE 2.

DISTRIBUTION OF ESR α *PvuII* & *XbaI* POLYMORPHISMS IN JAVANESE POSTMENOPAUSAL WOMEN WITH T2DM

Genotype and Alelle	Subjects	%
PP	32	26.4
Pp	56	46.3
pp	33	27.3
XX	51	42.2
Xx	39	32.2
xx	31	25.6
P	120	49.6
p	122	50.4
X	141	58.3
x	101	41.7

The study result in Javanese diabetics women have been supported by the result study in Iran presenting the prevalence of ESR polymorhism for PP, Pp, pp and for XX, Xx, xx as follows; 21%, 53%, 26%, 27%, 46%, 27% respectively (Table 3 & 4). Alelle frequencies for P and p in Iranian population reported by Golkhu et al are 48% and 52% in diabetics postmenopusal women respectively. Alelle frequencies for X and x are 50% each alelle (9).

TABLE 3. DISTRIBUTION OF *PvuII* GENOTYPE IN DIABETICS IN SEVERAL COUNTRIES

No	Researchers	Ethnics	pp	Pp	PP
1	Qin *et al* (10)	China	12%	57,6%	30,4%
2	Jakimiuk *et al* (11)	Poland	17,2%	50%	32,8%
3	Golkhu *et al* (9)	Iran	26%	53%	21%
4	Yoshihara *et al* (20)	Japan	31,74%	55,5%	12,69%
5	Ganasyam S.R. *et al* (21)	India	39 %	43%	18%
6	Akhmad, S.A *et al* (this study)	Indonesia	26,4%	46,3%	27,3%

The result study from China population had different distribution with Javanese population. In comparison, genotype frequencies for PP, Pp and pp in China are 12.1%, 30.4%, 57.5 % and for XX, Xx, xx are 11.1%, 39.1% , 49.8% respectively whereas alelle frequencies are 40.8.%, 59.2% , 36%, 64% for P, p, X, x respectively (10). According to Ganansyam *et all.* the genotype frequencies for PP, Pp, pp in India postmenopausal women population with diabetics are 18 %, 43%, 39% whereas the genotype frequencies for XX, Xx, xx are 75 %, 20%, 5 % respectively. Alelle frequencies for P and p are 41. 5 % and 63.5%. whereas alelle frequencies for X and x are 85% and 15% (21). The result of our study Javanese women is not similar with study in China, Iran and India (p=0.000) after evaluating with Chai Square.

TABLE 4. DISTRIBUTION OF *XbaI* GENOTYPE IN DIABETICS IN SEVERAL COUNTRIES

No	Researcher	Ethnics	XX	Xx	xx
1	Qin *et al* (10)	China	11,03%	49,83%	39,13%
2	Jakimiuk *et al* (11)	Poland	6,25%	34,4%	59,4%
3	Golkhu *et al* (9)	Iran	27%	46%	27%
4	Akhmad, S.A *et al* (this study)	Indonesia	32,2%	42,2%	25,6%

TABLE 5.
DISTRIBUTION OF HAPLOTYPE ESR α *XbaI & PvuII* POLYMORPHISM IN DIABETICS JAVANESE MENOPAUSAL WOMEN

Haplotype	Frequencies	%
PX	118	24.4
Px	112	23.1
pX	140	28.9
px	114	23.6

The frequencies of haplotype in diabetics Javeneses postmenopausal women presented in table 5. In comparison, the frequencies of haplotype in diabetics postmenopausal women is different from other ethnics in non-diabetics postmenopause women presented in table 6. Frequencies of pX is higher in diabetics subject than in no diabetics subject as comparison presented in table 3 and 4. This result is inconsistent with the prior study in Javanese population having frequencies 19.37%, 25.63%, 19.37%, 35.63% respectively for PX, Px, pX, px in more subjects of study (4). In fact, different ethnic populations may partly explain discrepancies among ESRα polymorphism with differential degree of linkage disequillibrum. Haplotype pX was observed in diabetics subject but not observed in the majority of studies in non diabetics subjects. This difference of haplotype frequencies may result from the disequilibrium which is not complete and may be due to recombination occured at these two polymorphic sites rather than multiple mutation. There are a lot of evidences that ESRα gene polymorphism influence many physiological processes in humans, women in particular, as well as may be the etiopathological factor of various diseases (11).

It is still not understood how the intronic polymorphism of the ESRa gene influences the receptor function. Polymorphisms with its positions in an intron, near the gene

promoter may suggest a possible role in either transcription regulation or mRNA processing and stability. It was recently shown that transition to P allele resulted in asite for myb binding, hence, the presence of this allele possible augments ESRa transcription. Another explanation to the observations of associations between these SNPs and human illness is a possible linkage disequilibrium between the *PvuI* and *XbaII* polymorphisms with other polymorphisms in the ESRa gene (15,22).

TABEL 6.

COMPARISON OF FREQUENCY OF *PvuII-XbaI* HAPLOTYPES OF THE HUMAN ESTROGEN RECEPTOR A GENE IN SEVERAL ETHNICS IN NON DIABETICS WOMEN

			PvuII and XbaI Haplotype			
Ethnics Group	Place of study	No.of Subject	Px	PX	Px	pX
Asians	Japan	238	54.9	18.7	26.5	0.3
Asians	Japan	2238	59.4	18.3	22.3	0
Asians	Korea	598	57.7	18.5	22.3	2.3
Africans-Americans	US	19	36.8	50	13.6	0
Caucasians	UK	206	56.1	33.5	9.2	1.2
Caucasians	Poland	64	47.4	17.3	24.4	10.9

Source; Jakimiuk *et al.* (11)

IV. CONCLUSIONS

In conclusion, the prevalent gene for ESR-α *Pvu*II was heterozygote Pp (46,3 %) and as for *XbaI* was homozygote XX (42,2 %), and the prevalent for the haplotype was pX (28,9%).We have found the different distribution of genotype frequencies *PvuII* and *XbaI* polymorphisms in diabetics postmenopausal women in Javanese population with other population.

Acknowledgement

We wish to express our appreciation to DIKTI (Directorate of Higher Education of Republic of Indonesia) for supporting this research by providing Compotitive Research Grant.

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at

Auditorium Abdul Kahar Muzakkir Universitas Islam Indonesia (UII)
Yogyakarta, 22 - 23th October 2013

Dr. Muhammad A. Martoprawiro
President
Indonesian Chemical Society

Yandi Syukri, M.Si., Apt.
Dean of Faculty of Mathematics and Natural Sciences

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Organizing Committee
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