

Correlation of Equol (7-Hydroxy-3-(4-Hydroxyphenyl)Chroman) in Woman Urine with the Symptoms of Menopause

Rima Yulia Effriyanti, Tita Husnitawati Madjid, Hanom Husni Syam

Department of Obstetrics and Gynecology, Faculty of Medicine, Universitas Padjadjaran/Dr. Hasan Sadikin
General Hospital, Bandung, Indonesia
Email: ryeffriyanti@yahoo.com

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Abstract

Objective: In order to identify the correlation between equol excreted through human urine and the symptom of menopause. **Methods:** The method used is cross sectional study examined to 99 postmenopausal women fulfilling the inclusion criteria from February 2014 to July 2014. This research was taken in the Endocrinology Clinic, Department of Obstetrics and Gynecology, Hasan Sadikin Hospital and Unpad's Pharmacokinetic Laboratory. All objects were interviewed using menopause rating scale (MRS) questionnaire. Their urine samples were analyzed using high performance liquid chromatography (HPLC). Then the results of questionnaire and HPLC test were evaluated. **Results:** The result is 97 objects (98%) detected having menopausal symptoms, produced equol. There is significant correlation between the level of equol in post-menopausal women that showed correlation (-0.71) which mean the higher the equol level, the lower the MRS score ($p < 0.001$) that mean the symptom is milder. **Conclusion:** Equol level in menopause women urine is a good predictor to identify the level of menopause symptoms.

Keywords

Equol, Menopause Symptoms, Menopause Rating Scale

1. Introduction

At this time, life expectancy of women gets increased. At the end of the 19th century, only 30% of women could

go through menopause. Now, the average of life expectancy in the world is almost 80 years. Along with the increase of health status and life expectancy of the Indonesia's population, the number and proportion of women over 50 years old entering the menopause age also continuously get increased. According to the population census in 2000, the number of such women was 15.5 million people. Then, it is estimated to be 30.3 million or around 11.5% of the total population of Indonesia in 2020. The life expectancy of women in Indonesia is 73.38 years old, while the British is 80 years and United States is 80.93 years old [1] [2]. They will spend a third of their age at menopause and it is conceivable that their lives are haunted with metabolic problems.

Estrogen decreasing at menopause not only changes the pattern of menstruation but also has an impact in general health, such as the loss of skin tissue elasticity, dyspareunia due to vaginal atrophy, dysuria, palpitations, migraine, hot flushes, night sweating, insomnia, and sexual disorders known as climacteric syndrome [3]. In collaborative research, it is stated that 75% of menopausal women experiences hot flushes for one year and 25% of them continues up to five years. Having entered the menopause, associated with levels of estrogen decreasing, women have an increasing risk for chronic disorders in long term, such as coronary heart disease, osteoporosis, cognitive function decreasing and malignancy. Approximately 35% of menopausal women experiences osteoporosis [3] [4]. The incidence of climacteric syndrome in West reaches 50% - 75%, while in Asia is only around 20% - 25% [5]-[7]. Immunological data show that the number of chronic and degenerative diseases incidence is significantly lower in Asia population who consume food containing soy rather than the population in Europe and America. The consumption of isoflavon, one of the phytoestrogen types, can increase the production of cytokines, whereas daidzein can strengthen the activity of *natural killer* cell [8].

Phytoestrogen is a natural substance from plants consisting of different chemical formulas compared to estrogen, but having very similar properties and behavior to estrogen, so-called estrogen-like substance and known as herbal estrogen. In humans there are two important effects: estrogenic and antiestrogenic which depend on endogenous estrogen level and estrogen receptor. Therefore, phytoestrogen is also known as *selective estrogen receptor modulator* (SERM) which has the agonists and antagonist effects [9]. Phytoestrogen may reduce the symptoms of hot flushes significantly, improve the plasma lipid profile, as well as inhibit the development of atherosclerosis; therefore it can reduce the risk of cardiovascular disease. In addition phytoestrogen also inhibits the growth of tumor cells/breast cancer, a very favorable effect for long-term treatment of menopause. It is also reported that phytoestrogen can recover the memory and reduce depression. Some researchers say that the weakness of phytoestrogen is no efficacies in the prevention of osteoporosis and treatment of vaginal dryness [10]-[13].

Isoflavone is a type of phytoestrogen and some researches indicate that isoflavone is the best type of phytoestrogen which is found in soybeans widely consumed by the people in Asia, including Indonesia. Soy isoflavone is often found in fruits, green tea, soybeans such as tempeh, tofu, and tauco. These meals are daily food in Indonesia and their prices are affordable. Daidzein is one of several potentially-known isoflavones and daidzein glycosides is the second largest one after genistein found in soybeans and their products [14]. Equol is the only metabolite that is active daidzein biotransformation and excreted together with urine [3]-[5] [14].

Equol (7-hydroxy-3-(4-hydroxyphenyl)chroman) is the isoflavone produced by intestinal bacteria responding to isoflavone intake but not in all human groups. They are diastereoisomers S-(-)equol; and R-(+)equol. The intestinal bacteria only produce S-(-)equol, which has a selective affinity to the estrogen receptor type (ER)- β . The existing evidence raises a debate whether there are advantages for the production of S-(-)equol in response to the intake of soy isoflavone or not. However, the ability to synthesize this type of diastereoisomers opens clinical trials for the future in order to analyze the existing potential directly [15]-[22].

Hormone therapy, estrogen or combination, is the type of therapy most widely prescribed for postmenopausal women in developed countries. Hormone therapy is used to treat the symptoms of menopause (climacteric syndrome), to prevent the occurrence of chronic diseases such as osteoporosis, coronary heart disease, hyperlipidemia, and alzheimer [23].

According to the recommendation of the American Society Reproductive Medicine (ASRM), an indication of hormone therapy is menopausal complaints such as vasomotor symptoms e.g. hot flushes or urogenital symptom [10]. In Indonesia and generally Asian countries, the use of hormone therapy is still very limited and only given to patients with complaints related to disturbing menopause estrogen deficiency or osteoporosis threat [14]. In the research conducted by the Women's Health Initiative (WHI), after five year observation, the study was stopped, according to a report that replacement therapy (HRT) increased the prevalence of breast cancer, myocardial infarction, stroke, and pulmonary embolism [6] [13] [24].

This research is to prove whether the habits and lifestyle of Indonesian people who are fond of consuming soy (isoflavones) in their daily food have a tendency of higher equol levels or not if it is related to milder menopausal symptoms. This research aims to analyze the correlation between equol excreted through human urine and the symptom of menopause. If it is proven, there is the potential development of equol supplementation, in that case women get benefit from consumed soy which can reduce the symptoms of menopause affecting the quality of life certainly. Although the evaluation in this preliminary research is using menopause rating scale instruments, measuring symptoms of menopause-related to quality of life is only performed at one time without the provision of treatment.

2. Methods

This research employs a cross-sectional analysis to identify whether women with a certain level of equol have milder menopausal symptoms or not compared to them having low levels of equol. This cross-cutting analysis research is the study to find the relationship between the independent variables (risk factors) with the dependent variable (effects) by conducting short measurement. The cross-cutting analysis research is an observational type to identify the correlation or influence between risk factors and disease. The research activity is conducted at the Clinic of Endocrinology, the Department of Obstetrics and Gynecology, Dr. Hasan Sadikin general hospital from February 2014 to July 2014. HPLC examination is conducted at the Laboratory of Pharmacokinetics, Faculty of Medicine, Universitas Padjadjaran.

The target population of this study is 40 - 60 year old woman experiencing postmenopausal. The objects are RSHS's employees experiencing postmenopausal. The inclusion criteria are:

- 1) Healthy women experiencing post-menopause who are able to carry out daily activities independently as well as to communicate well;
- 2) Not having hormonal therapy on menopausal problems within the last 3 months.

Interviews were conducted by using a questionnaire with menopause rating scale (MRS) and the urine test is also processed by using high performance liquid chromatography mass spectrophotometry (HPLC). Then the results of the questionnaire were compared to the results of the HPLC to evaluate the symptoms of menopause.

Statistical analysis is according to research objectives and hypotheses, namely to examine the relationship of equol (7-Hydroxy-3-(4-hydroxyphenyl)Chroman) in the urine of postmenopausal women with menopausal symptoms and to prove whether people with high levels of equol have milder menopausal symptoms or not. Statistical analysis is using the Kruskal Wallis test to determine the levels of equol between the various symptoms of menopause. If meaningful, then it is followed by Mann Whitney test. To analyze the correlation between equol and MRS, Spearman Correlation Test is used.

3. Results

Research on the correlation of equol and menopausal symptoms conducted to 99 postmenopausal women who came to the Endocrinology Clinic Obgyn/RSHS Bandung met the study criteria and was ready to be the objects of study by completing the informed consent of research. Towards the object of research, the data recording related to age, parity, and body measurements related to height and weight are conducted. The examination of menopausal disorders based on a questionnaire measuring devices menopause rating scale (MRS) and more research results are presented in **Tables 1-4**.

Correlation between equol level and menopause symptom based on MRS in Post-menopause women urine is illustrated in **Figure 1**.

4. Discussion

From the equol measurement results using high-performance liquid chromatography (HPLC), 97 objects are successfully detected (98.0%). Based on **Table 1**, the characteristics of postmenopausal women age is 51.23 years old with a deviation standard *i.e.* 7.03 years old with the youngest age is 40 years old and the oldest is 61 years old, and mostly in the age group of 50 - 54 years old is 45 women (45.5%), the highest parity, 2 - 3, is 58 women (58.6%) and the average value of BMI is 23.61 kg/m² with a deviation standard of 3.00 kg/m² in the range of 17.8 kg/m² up to 31.63 kg/m² and mostly with a BMI of 18.5 to 24.99 kg/m² is 66 people (66.7%).

Weight gain that occurs during menopause is suspected as slowed metabolic rate due to changes in endocrine

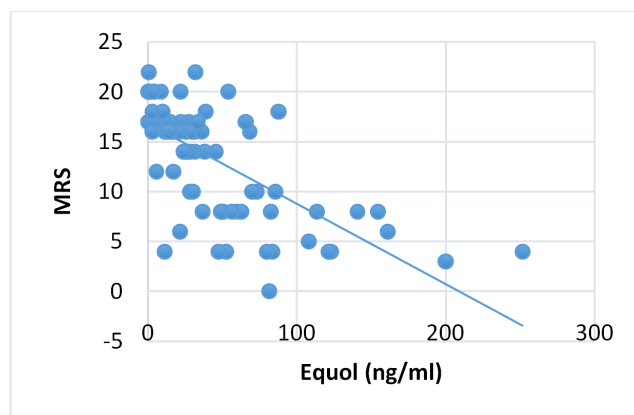


Figure 1. Correlation between equol level and menopause symptoms based on MRS.

Table 1. Characteristics of research objects.

Characteristics	N	%	Mean (SD)	Median	Range
1. Age (year):			51.23 (7.03)	52	40 - 61
<50	22	22.2			
50 - 54	45	45.5			
≥55	32	32.3			
2. Parity:			3 (2)	2	0 - 8
0 - 1	24	24.2			
2 - 3	58	58.6			
≥4	17	17.2			
3. Body mass index (kg/m ²):			23.61 (3.00)	22.94	17.80 - 31.63
<18.5	4	4.0			
18.5 - 24.99	66	66.7			
25 - 29.99	25	25.3			
≥30	4	4.0			
Total	99	100.0			

system during menopause *i.e.* the hypothalamus and the pituitary glands that have to adapt to the already sluggish ovary to secrete estrogen. Changes in hormonal levels will disrupt the hunger-satiety center in the brain.

Aging will cause the activities of the body get decreased. This effects the body movement getting reduced and causing fat accumulation. Whether the influence of BMI is associated with the production of equol or not is not yet identified. In this study, it is found no significant relationship between BMI with high levels of equol in the urine of postmenopausal women. Research conducted by Baohua L. *et al.* 25 also said the similar ideas. The study used 200 objects of adults in Beijing who produce equol only 26%, then given isoflavone supplement for 3 days and seen their equol levels afterwards. Equol level got increased to 60.4% but the increase is not associated with BMI or serum lipid contained in the blood plasma.

In **Table 2**, it is obtained equol levels of 36.23 ng/mL with a deviation standard of 35.98 ng/mL, a median of 21.34 ng/mL with a range of 251.54 ng/mL. For menopause rating scale, it is obtained the mean 13.92 with a deviation standard of 5.25 with a range of 0 - 22 and the most with severe menopausal symptoms as many as 45 women (45.5%) and only 10 women (10.1%) without symptoms of menopause. Similar to a research by Ishiwata *et al.* 16 which was conducted for 12 weeks using 134 Japanese women in the age 40 - 59 years with a double-blind randomization, the women who produce equol (34.3%), in fact, had lower symptoms of menopause than the non-equol producer.

Overall it appears that post-menopausal women age at various menopausal symptoms are almost similar and statistics test results using Kruskal Wallis test at 95% interval confidence, show that there are no significant age

differences based on the symptoms of menopause in the urine of postmenopausal women with $p = 0.259$ (p -value > 0.05) and postmenopausal women BMI at various menopausal symptoms which are almost identical. There are no significant differences in BMI based on the symptoms of menopause in the urine of postmenopausal women with $p = 0.408$ (p -value > 0.05).

Table 3 shows that the median of equol level, without menopause symptoms, in the urine of postmenopausal women is 82.22 ng/mL with the lowest levels of equol is 11.00 ng/mL and the highest is 251.54 ng/mL, while the median levels of equol with mild menopause symptoms in the urine of postmenopausal women is lower than the one without symptoms *i.e.* 60.92 ng/mL with the lowest levels of equol is 21.35 ng/mL and the highest is 160.79 ng/mL.

Based on **Table 3**, it is also seen that the median of equol level, with menopausal symptoms, in postmenopausal women is lower than the one without symptoms *i.e.* 25.52 ng/mL with the lowest level of equol is 2.62 ng/mL and the highest is 85.45 ng/mL. Even the median of equol level, with the severe menopausal symptoms, in postmenopausal women is lower than the one without symptoms *i.e.* 3.93 ng/mL with the lowest level of equol *i.e.* 0 ng/mL and the highest is 87.53 ng/mL.

Overall it appears that postmenopausal women with higher level of equol, the menopausal symptoms are milder than the women with lower level equol. The statistics test results using Kruskal Wallist test at 95% trust level show that there is a significant relationship between equol level with the symptoms of menopause in the

Table 2. Statistical descriptive of equol and *menopause rating scale* (MRS).

Variables	Statistical measurement				
	n	%	Mean (SD)	Median	Range
1. Equol			36.23 (35.98)	21.34	0 - 251.54
2. MRS			13.92 (5.25)	16	0 - 22
3. Menopause symptom:					
No disorder	10	10.1			
Mild disorder	14	14.1			
Medium disorder	30	30.3			
Severe disorder	45	45.5			
Total	99	100.0			

Table 3. Correlation among Age, BMI, equol with menopause symptoms.

Variables	Menopause symptoms				Value p ^{a)}
	No disorder (n = 10)	Mild disorder (n = 14)	Medium disorder (n = 30)	Severe disorder (n = 45)	
1. Age (year):					0.259
Mean (SD)	50.70 (13.27)	51.36 (3.87)	51.03 (3.81)	51.44 (7.74)	
Median	54.5	51	51	53	
Range	40 - 61	44 - 57	40 - 56	40 - 61	
2. BMI (kg/m ²):					0.408
Mean (SD)	22.39 (2.48)	23.54 (1.10)	23.84 (3.393)	23.74 (3.24)	
Median	22.22	23.73	23.44	22.82	
Range	17.80 - 26.56	21.33 - 25.63	17.80 - 30.92	17.80 - 31.63	
3. Equol (μmol/L):					<0.001
Mean (SD)	104.98 (72.80)	81.64 (45.56)	29.30 (20.30)	11.41 (18.62)	
Median	82.22	60.92	25.52	3.93	
Range	11.00 - 251.54 (a)	21.35 - 160.79 (a)	2.62 - 85.45 (b)	0 - 87.53 (b)	

Note: ^{a)}based on Kruskal-Wallis Test. The median value followed with different letter in appointed line show significant value based on Mann-Whitney Test.

Table 4. Correlation between equol level and menopause symptoms based on MRS in postmenopause women urines.

Correlation	r_s	Value p
Equol level >> menopause symptom based on MRS	-0.71	<0.001

Note: r_s = rank Spearman correlation.

urine of postmenopausal women with $p < 0.001$ (p -value < 0.05) similar to research conducted by Uchiyama *et al.* 19 investigating the type of isoflavone in urinary excretion with menopausal symptoms found in higher level of equol which is obtained the lower menopausal symptoms. Aso T. dan Ishiwata *et al.* 16, conducting further research on equol, obtained that s-equol extract delivery is able to decrease the menopause symptoms, in that case it can be an alternative for menopause symptom therapy.

In detail, it seems that statistics test results using Mann Whitney test in the 95% trust level show that there is significant difference between middle menopause symptom compared to the ones without menopause symptom in post-menopause women urine with the value $p < 0.001$ (nilai $p \leq 0.05$). In addition there is significant difference of equol level between severe menopause symptom compared to the one without menopause symptom in postmenopause women urine with $p < 0.001$ (value $p < 0.05$). It is, however, not found significant difference in equol level between mild menopause symptom compared to the one without menopause symptom in postmenopause women urine with $p = 0.508$ (value $p > 0.05$).

Based on **Table 4**, it can be seen that analysis result of Spearman Correlation Test in the trust level 95% statistically show the significant correlation between equol level with menopause symptom based on MRS in postmenopause women urine with $p < 0.001$ (value $p < 0.05$) with the correlation strength 0.71 showing a very strong correlation in negative direction which means the higher the equol level, the lower the MRS score showing milder disorder level.

The limitation of this research is an Introduction Research detecting the existence of equol in postmenopause women urine dan the correlation between equol level and menopause symptom with menopause rating scale (MRS) instrument. Measurement is only conducted at one time, it is not given the treatment or similar condition in object population.

5. Conclusion

It is found the correlation between equol in postmenopause women urine with menopause symptom.

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