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EFFECT OF LONG-TERM ADMINISTRATION OF DEPOT MEDROXYPROGESTERONE ACETATE (DMPA) ON PLASMA ENDOTHELIN 1 (ET-1) LEVELS

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Abstract

Estrogen is known to increase nitric oxide and endothelial function, decrease endothelin 1 levels and improve lipid profiles. Estrogen suppression by DMPA use may alter endothelial function and other biomarkers of vascular health. This study used a cross sectional study design. Samples were selected using purposive sampling technique. Samples were subjects who met the exclusion and inclusion criteria. There are a total of 88 samples divided into 2 groups; DMPA acceptor group and control group. The collected data will be analysed Univariate and Bivariate. Univariate data analysis aims to describe the dermographic data of respondents by showing the characteristics of the research subjects. Bivariate data analysis is carried out to see the relationship between the dependent variable and the independent variable using the Independent T' Test statistical test if the data is normally distributed, but if the data is not normally distributed then the Mann-Whitney Test is used. The results of this study indicate that in the Body Mass Index (BMI) category, the mean BMI in the DMPA acceptor group is 25 ± 3.60 kg/m2 or Obesity I, while in the control group the mean BMI is 23.25 ± 3.5 kg/m2 or overweight. The mean systolic blood pressure in the DMPA acceptor group was 110 ± 15 mmHg and the mean diastolic blood pressure was 73 ± 9.2, while in the control group the mean systolic blood pressure was 110 ± 9.8 mmHg and the mean diastolic blood pressure was 73 ± 7.4 mmHg. The menstrual cycle category in the DMPA acceptor group found 32 subjects or 72.7% complained of never menstruating during the use of DMPA, while in the control group found 44 subjects or 100% regular menstrual cycles. Kolmogorov-Smirnov normality test of endothelin-1 levels in the DMPA acceptor group and the control group obtained a p value <0.05, namely 0.02 and 0.01 respectively, so it is stated if the data is not normally distributed. From the statistical test using the Mann-Whitney test, the value of p=0.214 was obtained. Because the value of p>α (α=0.05), so it can be concluded if there is no significant difference between plasma endothelin-1 levels in the DMPA acceptor group and the control group. There is an effect of Depot Medroxyprogesterone contraceptive use on increasing Body Mass Index (BMI) and effects on the menstrual cycle, namely amenorrhea, spotting / spotting and irregular / irregular menstruation. There is no significant effect of using Depot Medroxyprogesterone acetate on Endothelin 1 levels.

Keywords: Depot Medroxyprogesterone Acetate (DMPA), Endothelin-1.

INTRODUCTION

Depot medroxyprogesterone is a contraceptive that has been authorised by the US Food and Drug Administration (FDA) since 1992 [1]. Depot medroxyprogesterone acetate (DMPA) is a progesterone-containing contraceptive available since 1960. Progesterone injectable contraceptives are highly effective, reversible and safe for most women [2]. Depot medroxyprogesterone acetate (DMPA) also known as depo provera is a safe and effective contraceptive. Depo provera is a synthetic derivative of progesterone at a dose of 150 mg/1.0 ml per intramuscular injection in the upper arm

or buttocks [3]. After administration of DMPA at a dose of 150mg/IM, it takes about three weeks to reach peak plasma contraction of 1-7 ng/ml. Then the concentration drops esponentially until it becomes undetectable (<1 ng/ml) between 120-200 days after injection [4]. Interval of injections given every 3 months [5].

Medroxyprogesterone acetate is a synthetic progesterone that has a chemical structure of 17-hydroxy-6-alpha-methyl progesterone. The intramuscular absorption of DMPA varies with each individual. Depot Medroxyprogesterone Acetate (DMPA) is metabolised in the liver with the rate of metabolism varying across individuals. The main metabolite is a modification of the side chain at C (17) and C (21) of medroxyprogesterone. Medroxyprogesterone is bound to plasma proteins with total protein binding of approximately 90%. It is mainly bound to albumin, not to sex-hormone-binding globulin (SHBG).

Like other progesterones, DMPA diffuses freely into the nucleus of target cells and binds to progesterone receptors, influencing the transcription of selected genes and eventually resulting in protein synthesis. Progesterone receptors are mainly found in the female reproductive tract. The contraceptive mechanism of medroxyprogesterone is believed to result from inhibition of ovulation (by blocking the LH surge), thickening of cervical mucus, formation of endometrial atrophy, and premature luteolysis [1].

Side effects of Depo-Provera contraceptive use reported by 1-5% of subjects were decreased libido or anorgasmia, back pain, leg cramps, depression, nausea, insomnia, leucorrhoea, acne, vaginitis, pelvic pain, breast pain, alopecia, rash and oedema [1]. There are FDA warnings that DMPA use may lead to low estrogen and decreased bone mineral density. Estrogen is known to increase Nitric Oxide and endothelial function, decrease endothelin 1 levels and improve lipid profiles. Estrogen suppression by DMPA use may alter endothelial function and other biomarkers of vascular health [6].

Vascular endothelium dysfunction is an early finding in the development of cardiovascular disease and is closely related to clinical events in patients with atherosclerosis and hypertension. Endothelial dysfunction often refers to the situation of decreased bioavailability and consequently impaired vasodilator effects of endothelium-derived relaxing factors such as nitric oxide (NO), prostacyclin or endothelium-derived hyperpolarising factor. One of the other important changes in endothelial dysfunction is the increased production and bilogical activity of potent vasoconstrictor and proinflammatory peptide endothelin (ET)-1 [7]. Endothelin-1 binds to two different receptors, the ETA (ETAR) and ETB (ETBR) receptors.

Endothelin receptors are found in many cells and tissues, one of which is in the blood vessels, which is found in smooth muscle cells that mediate vasoconstrictor effects. ETBRs (Endothelin B receptors) are also found on vascular endothelial cells, where their activation results in vasodilation mediated mainly by Nitride Oxide (NO) [8]. In the cardiovascular system, endothelin-1 activity is tightly regulated by several oppositely acting autocrine mechanisms.

For example, physical factors or vasoconstrictor agents including norepinephrine (NE), thrombin, angiotensin II and vasopressin are all capable of regulating endothelin-1 biosynthesis. In addition, several other different stimuli such as growth factors, cytokines, hormones and adhesion molecules can also increase endothelin-1 production.

Increased endothelin-1 synthesis is associated with vaso-occlusive disorders and estradiol inhibits endothelin-1 synthesis probably through an estrogen receptor (ER)-dependent pathway. Thus, inhibition of ET-1 production by estradiol may contribute to its cardioprotective effects. Endothelin-1 synthesis is regulated by mitogen activated protein kinase (MAPK) pathway [9]. Vascular smooth muscle cells, cardiomyocytes, fibroblasts, hepatocytes and neurons all express ETA receptors that show higher affinity to ET-1 than ET-2 and ET-3.

ET-1 binds to the ETA receptor activating phospholipase C (PLC) which will form the second messenger inositol triphosphate (IP3) and diacylglycerol (DAG) which can stimulate the release of intracellular calcium and activation of protein kinase C (PKC) thus providing a vasoconstrictor effect [10].

Plasma ET-1 levels are elevated in various abnormal human conditions such as pulmonary hypertension, acute myocardial infarction, essential hypertension, subarachnoid haemorrhage and diabetes mellitus. Plasma ET-1 levels increase with the severity of congestive heart failure (CHF) [11].

METHOD

Research Design

This research is a cross sectional study design.

Location and Time of Research

The location of blood sampling for the measurement of Endothelin 1 levels was carried out at the Moncobalang Health Centre while the measurement of its levels was carried out at the Hasanuddin University Medical Research Center (HUM-RC) Laboratory, Hasanuddin University Hospital in July 2024.

Population and Sample

The population in research is a subject (e.g. human: Client) that fulfils predetermined criteria. The population of this study were acceptors who received Depot Medroxyprogesterone acetate (DMPA) injections. The sample of this study was contraceptive acceptors of Depot Medroxyprogesterone acetate (DMPA) injections taken with purposive sampling technique that met the inclusion and exclusion criteria as follows; Inclusion criteria: Female participants who received DMPA injections for ≥ 3 years. willing to become research respondents, participants aged 25 years - 45 years; Exclusion criteria: Birth control acceptors with a history of other comorbidities such as diabetes mellitus and heart disease, patients taking drugs, patients smoking. The sample size estimate for this study used the unpaired numerical analytical research sample size formula, as follows:

$n_1 = n_2 = 2(\frac{(Z\alpha+Z\beta)S}{X1-X2})^2$

Z_α = Alpha Standard Deviation, type 1 error set at 5% = 1,64

Z_β = Standardised Deviation of Beta, type 2 error of 10% = 0,84

S = Combined Default Intersection = 6,31

$X_1 – X_2$ = Minimum difference in mean considered significant = 3,5

$$(Sg)^2 = \frac{[S_1^2\ x\ (n_1-1) + S_2^2\ x\ (n_2-1)]}{n_1+n_2\ -2}$$

$$= \frac{[6{,}1^2\ x\ (45-1) + 6{,}5^2\ x\ (51-1)]}{45+\ 51\ -2}$$

$$= \frac{[37{,}21\ x\ 44 + 42{,}5\ x\ 50]}{94}$$

$$= \frac{[3749{,}74]}{94}$$

$$Sg = \sqrt{39{,}89} = 6{,}31$$

Based on this formula, the sample size is obtained as follows:

$$n_1 = n_2 = 2\left(\frac{(Z\alpha+Z\beta)S}{X1-X2}\right)$$

$$= 2\ (\frac{(1{,}64+0{,}84)6{,}31}{3{,}5})^2$$

$$= 2\ (\frac{(2{,}48)6{,}31}{3{,}5})^2$$

$$= 2\ (4{,}471)^2$$

$$= 2\ x\ 19{,}989$$

$$= 39{,}97$$

$$= 40\ \text{sample}$$

Based on the calculation of the formula above, the number of samples for each group is 40 samples. To anticipate respondents who drop out, a correction is made to the sample size by adding 10% of the calculated sample size, so that the number of samples for each group becomes 44 samples.

Sampling Technique

Samples were selected randomly using purposive sampling technique. This study was conducted after obtaining ethical approval from the Research Ethics Committee of the Faculty of Medicine, Hasanuddin University with ethical number 521/UN4.6.4.5.31/PP36/2024.

Plasma Endothelin-1 levels were examined using the Human Endothelin-1 Elisa Kit from Assay Genie (Ireland Factory), using the sandwich Enzymed Linked Immunosorbent Assay (ELISA) method. The test was conducted at Hasanuddin University Medical Research Centre (HUM-RC) Laboratory, Hasanuddin University Hospital.

Data Analysis

The collected data will be analysed univariately and bivariately. Univariate data analysis aims to describe the dermographic data of respondents by showing the characteristics of the research subjects.

Bivariate data analysis is carried out to see the relationship between the dependent variable and the independent variable using the Independent T' Test statistical test if the data is normally distributed, but if the data is not normally distributed, the Mann-Whitney Test is used.

RESULT

Research Characteristics

Table 1: Characteristics of Research Subjects

Characteristics	DMPA Acceptor Group			Control Group		
	Total (n)	Percentage (%)	Mean ± SD	Total (n)	Percentage (%)	Mean ± SD
Age (Years)	44	100	34,70 ± 5,6	44	100	29,59 ± 4,3
25-30	10	22,7		29	65,9	
31-35	19	43,2		9	20,5	
35-40	8	18,2		5	11,4	
41-45	7	15,9		1	2,3	
Duration of DMPA Use	44	100	5,13 ± 2,4	-	-	-
Kategori IMT (kg/m^2)	44	100	25 ± 3,60	44	100	23,25 ± 3,5
< 18,5 = BB Less	1	2,3		4	9,1	
18,5 – 22,9 = Normal	10	22,7		20	45,5	
23 – 24,9 = BB Lebih	15	34,1		20	45,5	
25–29,9 = Obesity I	15	34,1		-	-	-
≥ 30 = Obesity II	3	6,8		-	-	-
Blood Pressure Systolic	44	100	110 ± 15	44	100	110±9,8
Blood Pressure Diastolic	44	100	73 ± 9,2	44	100	73±7,4
Menstrual Cycle	44	100		44	100	
Regular menstruation every month	5	11,4		44	100	
Irregular menstruation	7	15,9		-	-	
Never Menstruating	32	72,7		-	-	

Source: Primary Data

Based on the sample characteristics table above, the mean age of the research subjects for the DMPA acceptor group was 34.70 ± 5.6 years with the highest age category of 31-35 years as many as 19 subjects or 43.2% with a mean duration of DMPA use of 5.13 ± 2.4 years, while in the control group the mean age was 29.59 ± 4.3 with the highest category of 25-30 years as many as 29 subjects or 65.9%. In the category of Body Mass Index (BMI), the mean BMI in the DMPA acceptor group was 25 ± 3.60 kg/m2 or Obesity I with the most BMI categories being 23 - 24.9 kg/m2 or overweight and 25 - 29.9 or Obesity I, each as many as 15 subjects or 34, 1%, while in the control group the mean BMI was 23.25 ± 3.5 kg/m2 or overweight with the most categories being 18.5 - 22.9 or normal weight and 23 - 24.9 kg/m2 or overweight each as many as 20 subjects or 45.5%.

The mean systolic blood pressure in the DMPA acceptor group was 110 ± 15 mmHg and the mean diastolic blood pressure was 73 ± 9.2, while in the control group the mean systolic blood pressure was 110 ± 9.8 mmHg and the mean diastolic blood pressure was 73 ± 7.4 mmHg. The menstrual cycle category in the DMPA acceptor group found 32 subjects or 72.7% complained of never menstruating during the use of DMPA, while in the control group found 44 subjects or 100% regular menstrual cycles.

Normality Test

The normality test of the research results of Endothelin-1 levels in the presence of Depo Medroxyprogesterone contraceptive use was carried out in determining whether the data distribution was normal or not. The test used is the Kolmogorov-Smirnov test (Table 2).

Table 2: Normality test of endothelin-1 levels

	Endothelin-1 levels		
	Statistics	**n**	**P**
DMPA Acceptor Group	0,171	44	0,02
Control Group	0,177	44	0,01

Source: Primary Data

Based on table 2 Kolmogorov-Smirnov normality test of endothelin-1 levels in the DMPA acceptor group and the control group obtained a p value <0.05, namely 0.02 and 0.01 respectively, so it is stated that the data is not normally distributed

Endothelin 1 Level Comparison Test between Depot Medroxyprogesterone acetate (DMPA) Acceptor Group and Control Group

Based on the normality test of plasma Endothelin-1 levels and the conclusion that the data is not normally distributed, the Mann-Whitney Test was used to determine the comparison of plasma Endothelin-1 levels in the DMPA acceptor group and the control group.

Table 3: Comparison Test of Endothelin 1 Level between Depot Medroxyprogesterone acetate (DMPA) Acceptor Group and Control Group

Group	**N**	***Endothelin-1* (pg/mL)**		**p**
		Range	**Mean±SD**	
DMPA acceptor	44	0,65-068	0,659±0	0,214
Control	44	0,65-0,67	0,658±0	

Mann-Whitney Test

Source: Primary Data

Based on table 3, the endothelin level in the DMPA acceptor group was found to be an average of 0.659 pg/ml and the control group had an average endothelin level of 0.658 pg/ml. From the statistical test using the Mann-Whitney test, the value of p=0.214 was obtained. Because the value of p>α (α=0.05), so it can be concluded if there is no significant difference between plasma endothelin-1 levels in the DMPA acceptor group and the control group.

DISCUSSION

According to any dictionary, contraception is defined as the prevention of conception or intentional pregnancy by some means such as drugs, techniques or devices. Hormonal contraceptives, which have been around for the last 50 years, consist of female sex hormones with estrogen, progesterone or both. One of the most common methods of contraception is an injectable hormonal contraceptive called Depo-Provera or also known as Depot-medroxyprogesterone acetate (DMPA) which is injected intramuscularly every 3 months [1]. According to Indonesian Health Statistics Data in 2023, the most widely used contraceptive method by couples of childbearing age in Indonesia was contraceptive injections at 53.34% followed by pills 18.74%, and implants 10.75%. Depo Provera was developed by the Upjohn Company in 1954 for the treatment of endometriosis and miscarriage. However, in the early 1960s, it was recognised that women who received it subsequently experienced a delay in the return of fertility and it was developed into a fertility-regulating agent. DMPA is effective with failure rates ranging from 0.1 to 2 per 100 women per year. It is also affordable,

reversible, long-acting and demands less compliance. It also does not require special storage and is suitable for use in the tropics. Depo Provera is available in two preparations; 150mg/ml for intramuscular injection and 104 mg/0.65 ml for subcutaneous injection. These injections are given every three months with contraceptive protection continuing the following week. Depo Provera works by primarily inhibiting gonadotropin secretion thereby inhibiting follicular maturation and ovulation, thickening cervical mucus to prevent spermatozoa motility into the uterus and causing endometrial atrophy thereby making implantation of the fertilised ovum difficult. DMPA is beneficial in breastfeeding mothers because it does not affect the quality, quantity and composition of breast milk [3].

Based on the sample characteristics table above, the mean age of the research subjects for the DMPA acceptor group was 34.70 ± 5.6 years with the highest age category of 31-35 years as many as 19 subjects or 43.2%. These age characteristics are in line with research in Nigeria by Nonye et al 2020, which shows the mean age characteristics of DMPA acceptors of 33.1 years. Research at the University of Uyo Teaching Hospital on 166 DMPA acceptors obtained the highest mean age characteristics of 34.4 years (35%) [12]. Adolescents or young adults are discouraged from using progesterone-only contraceptives due to their effect on bone mineral density which can lead to osteoporosis. Prospective studies found mean bone mineral density (BMD) loss at the lumbar spine between 0.87% and 3.52% which appears to be proportional to duration of DMPA use [12]. In the largest clinical trial on DMPA, more than 3900 women treated for up to 7 years, reported adverse reactions that may or may not be related to the use of Depo-Provera injectable contraceptives. More than 5% of subjects experienced menstrual cycle disruption (bleeding or amenorrhoea, or both), weight gain, headache, abdominal pain, dizziness and asthenia (fatigue) [1].

Based on the table of characteristics of research subjects in the category of Body Mass Index (BMI) in the DMPA acceptor group who have used DMPA contraceptives with an average duration of use of 5.13 ± 2.4 years, the mean BMI is 25 ± 3.60 kg/m2 or Obesity I with the most BMI categories being 23 - 24.9 kg/m2 or overweight and 25 - 29, 9 or Obesity I each as many as 15 subjects or 34.1%, while in the control group the mean BMI was 23.25 ± 3.5 kg/m2 or overweight with the most categories being 18.5 - 22.9 or normal weight and 23 - 24.9 kg/m2 or overweight each as many as 20 subjects or 45.5%. These results illustrate that subjects who use DMPA have excessive body weight and tend to be obese. Depot Medroxyprogesterone acetate induces weight gain by increasing fat deposits, rather than through anabolic properties or fluid retention. Approximately 25% of 12 non-breastfeeding women taking DMPA injections gained approximately 6 kg of weight over a 1-year period. None of the subjects showed changes in fluid compartment size, creatinine excretion rate or nitrogen metabolism [1]. Another study conducted by Espey et al 2000, found that significant weight gain was associated with DMPA use in Navajo women [13]. In a study to evaluate weight changes between groups of women using different contraceptive methods, namely DMPA and IUD for 5 years, it was found that DMPA users experienced significantly higher weight gain compared to IUD users. In the second to fifth year of observation, DMPA users gained 4.3kg over 5 years and IUD users gained 1.8kg [14]. Based on this study, it was found that DMPA acceptors experienced a tendency to change the menstrual cycle. In the DMPA acceptor group, 32 subjects or 72.7% complained that they never menstruated during the use of DMPA, while in the control group, 44 subjects or 100% had a regular menstrual cycle.

This is in accordance with the theory that one of the effects of DMPA use is changes in the menstrual cycle including amenorrhea, spotting or spotting, irregular menstruation / irregular menstruation every month and longer menstrual duration [15]. This is in line with the research of Samal and Das 2021, which found that 83.33% of the research subjects experienced amenorrhea [16]. Depot medroxyprogesterone acetate works by inhibiting the secretion of gonadotropins in the pituitary gland which causes no ovulation and decreased oestrogen production [17]. Estrogen has many biological effects on the cardiovascular system, as it is known to increase nitric oxide and endothelial function, decrease endothelin 1 levels, and improve lipid profiles. Estrogen suppression by DMPA may also alter endothelial function and other vascular biomarkers [6].

In this study, a comparison of endothelin 1 levels in the DMPA acceptor subject group and the control group showed that the mean endothelin level in the DMPA acceptor group was 0.659 pg/ml and the control group had a mean endothelin level of 0.658 pg/ml. From the statistical test using the Mann-Whitney test, the value of p=0.214 was obtained. Because the value of p>α (α=0.05), so it can be concluded if there is no significant difference between plasma endothelin-1 levels in the DMPA acceptor group and the control group. This is in line with research conducted by Akbar and Husnah 2020, on Wistar rats to see the effect of DMPA administration on ET-1 levels, where the results showed a tendency to increase ET-1 levels but not significantly so that there was no significant difference in ET-1 levels between the treatment group and the control group. Another study by Torgrimson et al 2011 on DMPA acceptors who have used DMPA contraceptives for more than 9 months found that ET-1 levels are not significantly different at week 3, 6 and week 9 measurements. Depot Medroxyprogesterone acetate is not known to directly affect ET-1 secretion or production, but progesterone has a decreasing effect on estrogen [18]. Estrogen has been shown to increase gene expression and plasma angiotensinoge levels. Estrogen also has an effect on circulating renin levels. Estrogen deficiency has been shown to increase Ang II receptor subtype 1 (AT1) mRNA levels,. The downregulation of AT1 receptor mRNA and protein expression by oestrogen occurs through ER activation and is mediated by a nitric oxide-dependent pathway [19].

CONCLUSIONS

Based on the results of research on the effect of long-term administration of Depot Medroxyprogesterone acetate on Plasma Endothelin 1 levels, the conclusions obtained are: There is an effect of using Depot Medroxyprogesterone contraceptives on increasing Body Mass Index. Depot Medroxyprogesterone acetate has an effect on the menstrual cycle, namely amenorrhoea, spotting and irregular menstruation. There is no significant effect of using Depot Medroxyprogesterone acetate on Endothelin 1 levels.

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