Effects of Depo-medroxyprogesterone Acetate (DMPA) on Lipid Profile, Body Weight and Blood Pressure among Women in Tekele Hymanot and Lomeda Health Centers, Addis Ababa, Ethiopia

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This is to certify that thesis prepared by Muluken Fekadie, entitled: Effects of Depo-medroxy progesterone Acetate (DMPA) on Lipid Profile, Body Weight and Blood Pressure among Women in Tekele Hymanot and Lomeda Health Centers, Addis Ababa, Ethiopia and submitted in partial fulfillment of the requirement for degree of Master's Science in Medical Biochemistry complies with regulation of the University and meets the accepted standards with respect to originality and quality.

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Declaration

I, the undersigned, declare that this thesis is my original work, has not been presented for a degree in any other university and that all sources of materials used for the thesis have been duly acknowledged.

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Abstract

Back ground: Depo-medroxyprogesterone Acetate (DMPA) is a long acting, injectable progesterone derivative contraceptive which is currently used by more than 90 million women worldwide, including Ethiopia. This contraceptive is suggested to induce changes in lipid profile, body weight and blood pressure among various populations and ethnic group with different patterns, similar to those associated with an increased risk of coronary heart disease (CHD).

Objective: To investigate the effects of use of DMPA on lipid profile, body weight and blood pressure of women attending family planning unit in Tekele Hymanot and Lomeda Health Centers, Addis Ababa, Ethiopia.

Methods: Institutional based cross-sectional study design was followed on 50 healthy women who had been using DMPA attending the family planning unit and another 50 age-matched healthy controls who were not using any hormonal contraceptives, attending the family planning and other unit in Health Centers between 14 February, 2015 and 13 March, 2015. Fasting blood samples were collected from the study participants for the estimation of total cholesterol (TC), triglyceride (TG), high-density lipoprotein cholesterol (HDL-c) and low-density lipoprotein cholesterol (LDL-c) levels, and the weight and blood pressure were measured during their visit. The data obtained was entered and analyzed using SPSS version 21 software packages. During analysis descriptive statistics of variables was done. Student's independent t-test, student's paired t-test, Pearson's correlation-test and one-way ANOVA analysis of variance were used to evaluate the presence of mean difference, correlation between variables and relationship between changes in variables and duration of use of DMPA.

Results: Serum TC and LDL-c levels in DMPA users were significantly increased compared to controls (P=.003 and P =.001, respectively), on the other hand, serum HDL-c level in DMPA users was significantly decreased (P =.001) compared to controls. Serum TG level in DMPA users was higher than control group, however, the difference was not statistically significant (P=.24). The mean weight and body mass index (BMI) of DMPA users were increased significantly (P =.02 and P =.019, respectively). There were no significant difference in mean arterial blood pressure (MAP) of DMPA users compared to controls or compared to their respective pretreatment value (P=.85, P=.67, respectively). Changes in variables of DMPA users were independent (P>.05) to the duration of use of DMPA (6-24, 27-48 and 51-96 months).

Conclusions: Use of DMPA induces marked changes in lipid metabolism which included development of high serum TC, LDL-c, TC to HDL-c and LDL-c to HDL-c ratio levels and a marked decrease in serum HDL-c level compared to controls. DMPA users showed weight gain and an increased BMI. All these changes appeared to be independent to the duration of use of DMPA. But, DMPA use didn't exert significant change in MAP of users.

Key words: *DMPA, lipid profiles, weight gain, body mass index, blood pressure*

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Abbreviations and Acronyms

ApoB	Apolipoprotein B
BMI	Body Mass Index
CHD	Coronary Heart Disease
CL	Corpus Luteum
COC	Combination Oral Contraception
DBP	Diastolic Blood Pressure
DMPA	Depo Medroxyprogesterone Acetate
ENG	Etonogestrel
ETB	Ethiopian Birr
FSH	Follicle Stimulating Hormone
GnIH	Gonadotropin Inhibiting Hormone
GnRH	Gonadotropin Releasing Hormone
HDL-c	High Density Lipoprotein Cholesterol
HPG-Axis	Hypothalamic Pituitary Gonadal Axis
IM	Intramuscular
IUCD	Intra-Uterine Contraceptive Device
LDL-c	Low Density Lipoprotein Cholesterol
LH	Luteinizing Hormone
LNG-IUS	Levonorgestrel Intrauterine System
MAP	Mean Arterial Blood Pressure

MPA	Medroxyprogesterone Acetate
NET-EN	Norethisterone Oenanthate
NGO	None Governmental Organization
NHC	Non Hormonal Contraceptive
NO	Nitric Oxide
P450ssc	P450-linked Side Chain Cleaving Enzyme
PEG	Polyethylene Glycol
RPM	Rotation per Minute
SBP	Systolic Blood Pressure
TC	Total Cholesterol
TCu 380A IUD	Copper Intrauterine Contraceptive Device
TG	Triglyceride
US FDA	United Stets Food and Drug Administration
USP	United States of America Pharmacopeia
vLDL-c	Very Low Density Lipoprotein Cholesterol

Operational Definitions

Atherosclerosis: a common arterial disease in which cholesterol deposits are formed on the inner surfaces of arteries obstructing blood flow.

Blood Pressure: the force exerted against blood vessel walls as the heart pumps blood through those vessels.

Contraceptives: Various devices, drugs, agents, sexual practices, or surgical procedures to prevent conception or pregnancy.

Coronary Heart Disease: a condition and especially one caused by atherosclerosis that reduces blood flow through the coronary arteries to the heart and typically results in chest pain or heart damage

Follicular Phase: is the phase of the estrous cycle, during which follicles in the ovary mature.

Lipid Profile: is a panel of blood tests that serves as an initial broad medical screening tool for abnormalities in lipids, such as cholesterol and triglycerides.

Luteal Phase: is the part of the estrous cycle that starts at ovulation and ends the day before the next menstruation.

Menopause: It is the time in a woman's life when the function of the ovaries ceases in which a woman is characterized by absence of menstrual periods for 12 months which can happen in their 40s or 50s.

Myocardial infarction: is a medical emergency that occurs when a portion of the heart is deprived of oxygen because of blockage of one of the coronary arteries, which supply the heart muscle (myocardium) with blood.

Ovarian Follicle: is a roughly spheroid cellular aggregation set found in the ovaries.

Thromboembolic Disorder: is a condition in which a blood vessel is obstructed by an embolus carried in the bloodstream from the site of formation.

1. INTRODUCTION

1.1. Background of the study

Irregular population growth is considered as a serious threat to the international community and is also a major obstacle for nations' social and economic development. In 2011, world population stood at 7 billion (Mohammed *et al*, 2014). Africa accounts more than 1 billion of world population of which, Ethiopia is the second populous country in Africa accounting to 98.1 million people with high fertility and fast population growth rates (World population data sheet, 2015).The average total fertility rate worldwide ranges from 1.7 children per woman in more developed countries to 4.6 in the least developed countries (Mohammed *et al*, 2014). Total fertility rate in Ethiopia is 4.1 children per woman (Central Statistical Agency, 2014; World population data sheet, 2015).This puts Ethiopia among countries with highest total fertility rates in the world. For fertilities to fall to those low levels, increased use of family planning methods plays a significant role especially in less developed countries including Ethiopia (World population data sheet, 2015). The family planning service which is subsidized of cost is provided in both governmental and NGO health facilities in Ethiopia, including hospitals, clinics, health centers, and health stations (United Nations, 2007). Among the different methods of family planning, injectable hormonal contraceptives; Depo-medroxyprogesterone acetate (DMPA) is the most popular contraceptive method in Sub-Saharan Africa including Ethiopia, contributing 40% of total method mix in the region (United Nations, 2013).

In Africa, restrictive laws have limited DMPA provision to clinics until very recently (Welsh *et al*, 2006; Malkin, 2011). However, studies from Uganda, Madagascar, Malawi and Ethiopia have demonstrated that with sufficient training of community health workers DMPA injections can be provided with comparable safety, acceptability, and continuation rates as clinic based providers, which increases DMPA users in Africa (Stanback *et al*, 2007; Prata *et al*, 2011; Malarcher *et al*, 2011; Hoke *et al*, 2012).

In Ethiopia, the contraceptive prevalence rate is 29 % for all women and 42 % for currently married women. The vast majority of women use modern methods than traditional methods.

Forty percent of currently married women are using a modern method compared with just 2 % using a traditional method. Use of any contraceptive method varies notably by region, ranging from 64 % in Addis Ababa to 3 % in the Somali region.

Similarly, use of any modern contraceptive method is highest in Addis Ababa (57 %) and lowest in the Somali region (2 %). The most common modern method used by each group of women is injectable hormonal contraceptive (DMPA). In Ethiopia, use of DMPA increased from 3 % in 2000 to 31 % in 2014 (Central Statistical Agency, 2014).

Depo-medroxyprogesterone acetate (DMPA, Depo-Provera) is among a highly effective, convenient non-daily injectable hormonal contraceptive option with a very low failure rate that has been available worldwide for many years (Al-Youzbaki, 2011; Prata *et al*, 2013). DMPA is approved by the US FDA since 1992 (Al-Youzbaki, 2011). It is used by more than 90 million women worldwide (Bakry *et al*, 2008), which is designed to suppress the secretion of pituitary gonadotropins which, in turn, prevents follicular maturation and ovulation, therefore help to prevent pregnancy (Anonymous, Pfizer New Zealand Ltd, Data Sheet, 2013). In addition to its role as conceptive (prevention of pregnancy) it has been used with benefit to manage dysfunctional uterine bleeding, endometrial hyperplasia and carcinoma, premenstrual tension, and endometriosis. Anti-cancer activity of DMPA at pharmacologic doses may be dependent on its effect on the hypothalamic-pituitary-gonadal axis estrogen receptors and the metabolism of steroids at the tissue level (Anonymous, Pfizer New Zealand Ltd, Data Sheet, 2013).

DMPA is the most popular, long acting and highly effective contraceptive and one of the major means of family planning, yet its use is not without side effects including alteration in lipid profile (Bakry *et al*, 2010; Yadav *et al*, 2011; Asare *et al*, 2014), weight gain (Lopez *et al*, 2013; Asare *et al*, 2014; Bonny *et al*, 2014; Dal'Ava *et al*, 2014; Lange *et al*, 2015) and increase in blood pressure (Al-Youzbaki, 2011; Asare *et al*, 2014). Therefore, the present study was designed to evaluate the effect of DMPA on changes of lipid profile, body weight and blood pressure among Ethiopian women. The effect of DMPA on various biochemical parameters has not been studied on Ethiopian women who mostly use such drugs as a major contraceptive method. So, this study may help to obtain a variety of evidences and can provide base line data for further investigations to researchers who want to do more on the effect of DMPA on varies biochemical parameters.

1.2. Literature review

1.2.1. Endocrine regulation of female reproductive system

Female reproductive system is controlled by the hypothalamic-pituitary-gonadal (HPG) axis consists of an intercommunicating set of neural and endocrine tissues that function as a highly integrated unit in the regulation of fertility (Constantine, 2012). The gonadotropin-releasing hormone (GnRH) neurons of the hypothalamus control the release of the gonadotropic hormones, luteinizing hormone (LH) and follicle-stimulating hormone (FSH), from the pituitary gland (Herbison, 2009).

Activation of HPG axis commences with the pulsatile secretion of GnRH from the hypothalamus. The hypothalamic decapeptide, GnRH, stimulates the gonadotrope subpopulation of the anterior pituitary gland to synthesize and secrete the gonadotropins, LH and FSH (Hiller-Sturmhöfel and Bartke, 1998). The gonadotropins are dimeric glycoprotein hormones composed of distinct hormone-specific β subunits paired with a common α subunit (αGSU) (Zheng *et al*, 2014). The gonadotropins have three primary effects on the ovaries: 1) stimulation of oogenesis (formation of ova), 2) stimulation of ovarian hormone (estrogen and progesterone) secretion and 3) maintenance of the structure of the gonads (the gonads atrophy if the pituitary gland is removed (Fox, 2011). LH stimulates the production of androgens by the thecal cells that surround the growing ovarian follicle. During the terminal stages of follicular growth, LH also drives the production of progesterone from the granulosa cells of the preovulatory follicle. FSH binds to receptors on the surface of ovarian granulosa cells stimulating the expression of aromatase enzymes that convert thecal androgens to estradiol (Bliss *et al*, 2010).

The HPG axis is subject to both positive feed-forward and negative feed-back regulation at several levels (Bliss *et al*, 2010). Estrogen and progesterone with short and long negative feedback loops regulate the secretion of FSH and LH by the pituitary and GnRH by the hypothalamus (Herbison, 2009).

Low levels of circulating sex hormone (estrogen and progesterone) reduce feedback inhibition on GnRH synthesis (the long loop), leading to elevated FSH and LH. The latter peptide hormones bind to gonadal tissue and stimulate P450ssc activity, resulting in sex hormone production via cyclic AMP (cAMP) and protein kinase-A (PKA) mediated pathways and adenylate cyclase activation is coupled to the binding of LH to plasma membrane receptors (Sherwood, 2010; King, 2013).

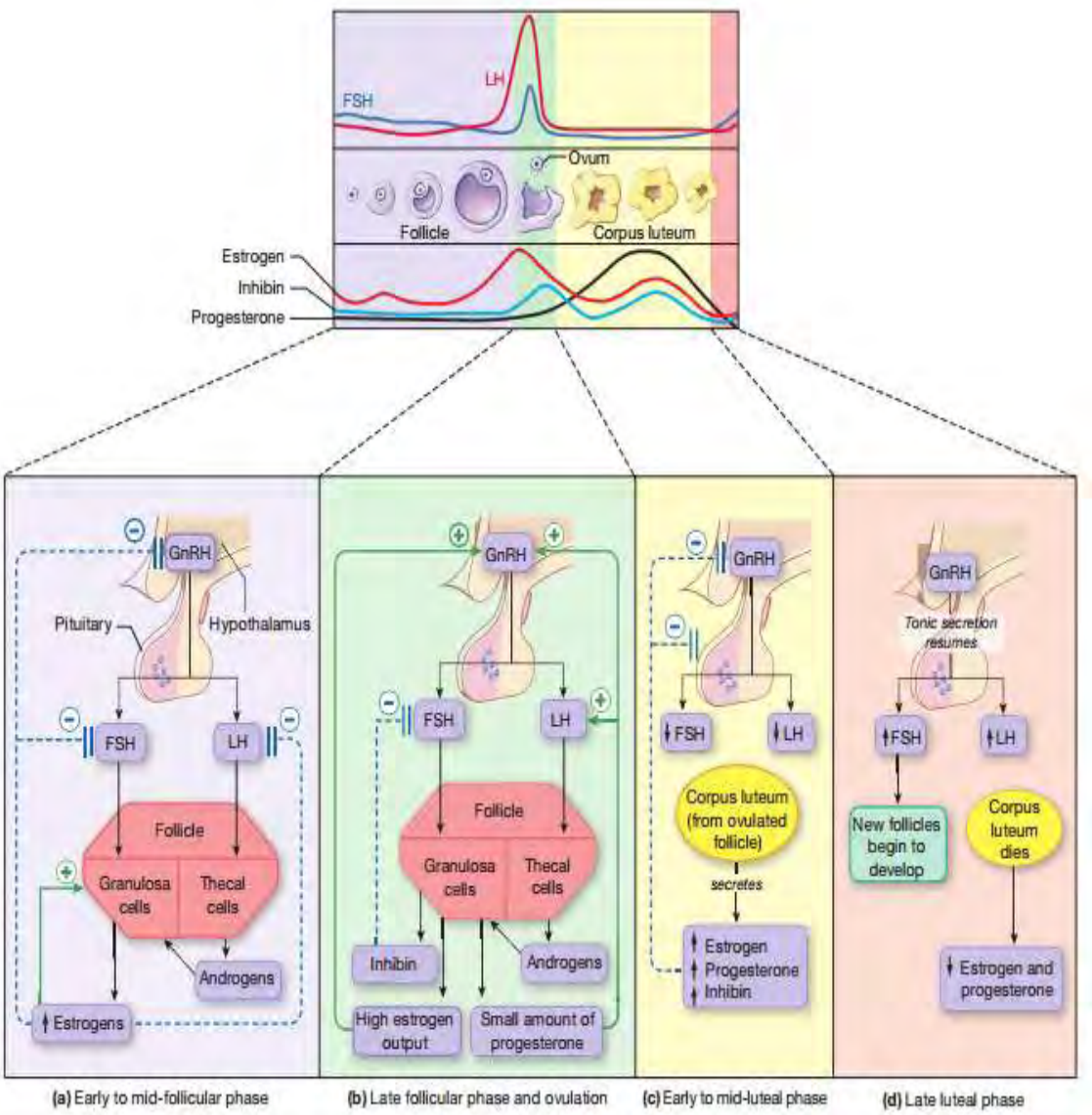


Figure 1.1 General Pattern for hormonal control of female reproduction (Silverthorn, 2010).

Where: GnRH=gonadotropin releasing hormone, FSH=follicle stimulating hormone, LH=luteinizing hormone, (+) = stimulation, (-) = inhibition.

1.2.1.1. Estrogen and its effect

Estrogen is a steroid hormone, derived from cholesterol, and synthesized by theca and granulosa cells of the follicles which exerts critical homeostatic feedback effects upon GnRH neurons to maintain fertility (Herbison, 2009). In adult women, the primary functions of estrogens include regulating the menstrual cycle, contributing to the hormonal regulation of pregnancy and lactation, and maintaining female libido (Hiller-Sturmhöfel and Bartke, 1998).

Estrogen has both negative and positive feedback actions to suppress and stimulate GnRH neuron activity at different times of the ovarian cycle (Herbison, 2009; Fox, 2011). The rising, moderate level of estrogen early in the follicular phase inhibits LH secretion, the high level of estrogen that occurs during peak estrogen secretion late in the follicular phase stimulates LH secretion and initiates the LH surge. Thus, LH enhances estrogen production by the follicle, and the resultant peak estrogen concentration stimulates LH secretion (Fox, 2011). The high plasma concentration of estrogen acts directly on the hypothalamus to increase GnRH, thereby increasing both LH and FSH secretion. It also acts directly on the anterior pituitary to specifically increase LH secretion by the gonadotropes. The latter effect largely accounts for the much greater surge in LH secretion compared to FSH secretion at mid-cycle (Sherwood, 2010). The LH surge begins about 24 hours before ovulation and reaches its peak about 16 hours before ovulation. It is this surge that acts to trigger ovulation (Guyton, 2011).

During menopause, estrogen production in the ovaries ceases (Silverthorn , 2010).The resulting reduction in estrogen levels leads to symptoms such as hot flashes, sweating, pounding of the heart (palpitations), increased irritability, anxiety, depression, and brittle bones (osteoporosis). The administration of estrogens (hormone replacement therapy) can alleviate those symptoms and reduce the risk of osteoporosis and coronary heart disease in postmenopausal women (Hiller-Sturmhöfel and Bartke, 1998).

1.2.1.2. Progesterone and its effect

Progesterone is also a steroid hormone derived from cholesterol (Silverthorn , 2010). The corpus luteum (CL) is the principal source of progesterone and its main function is maintenance of pregnancy. During the estrous cycle, blood progesterone concentrations influence several other hormones.

On day 5 (day 0 =ovulation), plasma concentrations of progesterone increase (due to release from the CL). Progesterone is the dominant ovarian steroid present in the peripheral circulation during luteal phase of the mammalian reproductive cycle and serves a number of important regulatory roles (Skinner, 1998).

Even though a high level of estrogen stimulates LH secretion, progesterone, which dominates the luteal phase, powerfully inhibits LH secretion, as well as FSH secretion by acting at both the hypothalamus and the anterior pituitary (Ferin, 2008). Inhibition of FSH and LH by progesterone prevents new follicular maturation and ovulation and cause progesterone and estrogen levels sharply decrease when the corpus luteum degenerates during the luteal phase (Sherwood, 2010). Progesterone has differential effects on LH and FSH. An increase in FSH is enable in the presence of elevated progesterone to initiate a new follicular wave; however, LH pulsatility does not reach a peak under a high progesterone environment, suppressing ovulation of the dominant follicle (Ferin, 2008).

1.2.2. Depo-medroxyprogesterone acetate (DMPA)

DMPA is a long-acting form of the synthetic progestin, medroxyprogesterone acetate (MPA), as its active ingredient (Anonymous, RxList the internet drug index, 2014), administered by intramuscular (IM) injection every 3 months for contraception (Torgrimson *et al*, 2011; Yadav *et al*, 2011). DMPA is a white to off-white; odorless crystalline powder that is stable in air and that melts between 200°C and 210°C. It is freely soluble in chloroform, acetone and dioxane, sparingly soluble in alcohol and methanol, slightly soluble in ether, and insoluble in water (Anonymous , RxList the internet drug index, 2014). The principal metabolite of MPA that has been identified is a 6-alphamethyl-6-beta 17alpha, 21-trihydroxy-4-pregnene-3, 20-dione-17-acetate which is excreted in the urine (Anonymous, Pfizer New Zealand Ltd, Data Sheet, 2013). DMPA injectable suspension, for IM injection is available in vials and prefilled syringes, each containing 1 mL of MPA sterile aqueous suspension 150 mg/mL (Anonymous, RxList the internet drug index, 2014).

The initial injection should be given during the first 5 days after the onset of a normal menstrual period; within 5 days post-partum if not breast-feeding; or if exclusively breast-feeding at or after six weeks post-partum in the gluteal or deltoid muscle (Anonymous, Pfizer New Zealand Ltd, Data Sheet, 2013).

Table 1.1 Content of MPA per ml of aqueous suspension (Anonymous, RxList the internet drugindex, 2014).

Content	**Amount (mg/ml)**
Medroxyprogesterone acetate	150
Polyethylene glycol 4000	29
Polysorbate 80	2
Sodium benzoate	2.35
Sodium citrate dehydrate	0.2
Water for injection	

DMPA is the depot form of progestin which is derived from the natural progesterone hormone (Bakry and Abdullah, 2009). The contraceptive appears to be a potent inhibitor of gonadotrophins (Yadav *et al*, 2011). Progestin in DMPA prevents pregnancy in different ways: blocking LH secretion and preventing ovulation, and /or maintaining a powerful barrier against the entry of sperm into the uterus by keeping the cervical mucus thick and sticky (Anonymous ,The New York Times **,** 2008; Anonymous, Pfizer New Zealand Ltd, Data Sheet, 2013). DMPA is classified as sex hormone binding globulin (SHBG) (Bakry *et al*, 2010),which suppresses natural cyclic fluctuations of female sex hormones, lowering endogenous estradiol levels to those seen in the early follicular phase of a menstrual cycle or postmenopause (Torgrimson *et al*, 2011).

The drug is contraindicated for those women with: thrombophlebitis, thromboembolic disorders, known or suspected pregnancy, missed abortion, undiagnosed vaginal bleeding, known or suspected malignancy of the breast (when used for ovulation suppression or gynaecology indications), undiagnosed breast pathology, undiagnosed urinary tract bleeding , severe uncontrolled hypertension, severe liver dysfunction, coronary heart disease (CHD), known hypersensitivity to MPA or any component of the drug (Anonymous, Pfizer New Zealand Ltd, Data Sheet, 2013).

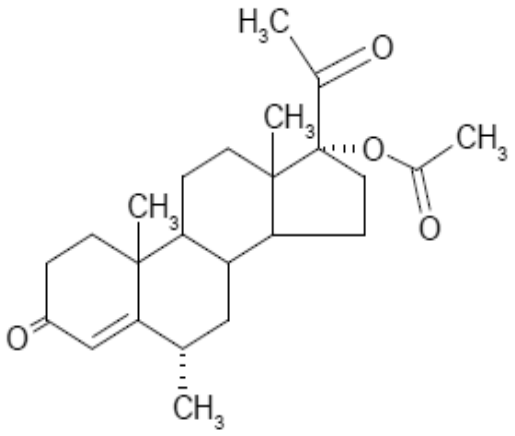


Figure 1.2 The structure of MPA (Anonymous, Rx List the internet drug index, 2014).

Following a single 150 mg IM dose of MPA, its concentrations, measured by an extracted radioimmunoassay procedure, increase for approximately 3 weeks to reach peak plasma concentrations of 1 to 7 ng/mL. The levels then decrease exponentially until they become undetectable (<100 pg/mL) between 120 to 200 days following a single injection. Using an unextracted radioimmunoassay procedure for the assay of MPA in serum, the apparent half-life for MPA following IM administration is approximately 50 days (Anonymous, Pfizer New Zealand Ltd, Data Sheet, 2013).

1.2.3. Effects of DMPA on lipid metabolism

Injectable hormonal contraceptives are convenient and highly effective methods for fertility regulation, being simple to administer and long acting. Various synthetic progestins including DMPA that are used as contraceptives have been reported to influence lipid profile, weight and blood pressure. Although, a number of investigations have been conducted on the relationship between DMPA and lipid profile, body weight (BMI) and blood pressure, results are not consistent between studies.

The study of effects of long-term use of DMPA on lipid metabolism was conducted among 60 DMPA users and 100 control groups of Nepalese women. They found that the LDL-c and TC levels in DMPA users were significantly increased (P=.001, P=.001, respectively) compared to controls, but, neither HDL-c nor TG levels were affected by DMPA use (Yadav *et al*, 2011).

In a clinical trial performed by Kongsayreepong *et al, (*1993) aimed to assess the effects of long-term use of DMPA on lipid metabolism, including women who had been using DMPA at a dose of 150 mg every 3 months for 3 to 9 years. Their TC, LDL-c, HDL-c and TG levels were compared to a control group of IUCD users. The author found that long-term use of DMPA induces moderate changes in lipid metabolism, which are unfavorable in terms of risk for atherosclerosis including higher LDL-c levels, lower HDL-c.

Healthy postmenopausal women, aged 43–57 years, and premenopausal women with regular cycles, aged 31– 40 years were received conjugated estrogen and MPA for 10 days, to evaluate the effects of hormone replacement therapy (HRT) on oxidized LDL (oxLDL) activity in menopausal status. An increase in oxidative activity and increased oxLDL was observed in women treated with MPA which leads to initiation of atherosclerosis (Topcuoglu *et al*, 2005).

In one study to investigate the effects of DMPA on 80 average Egyptian women by Faddah *et al*, (2005) showed DMPA induces gradual increase (but not significant) in the LDL-c to HDL-c ratio in comparison to control group, however, neither TC nor TG were affected by the drug. The lipid peroxide product malondialdehyde (MDA) was significantly elevated in a gradual manner without any change in blood nitric oxide (NO), which was defined as the endothelium derived relaxation factor (EDRF) having a powerful vasodilator action through its effect on blood vessel's smooth muscles leading to modulation of blood flow.

In Al-Youzbaki, (2011) study, conducted on DMPA users illustrated that significant increase in serum TG level after 6 months of DMPA use. But, there was no significant change in mean serum TC, HDL-c, LDL-c among DMPA users in comparison to the non-users.

The study conducted by Berenson *et al*, (2009) on 805 non-Hispanic black, non-Hispanic white and Hispanic women between 16 and 33 years of age, demonstrated that DMPA users were at increased risk of developing an abnormally low HDL-c level as well as an abnormally high LDL-c level. They also reported an increase in LDL-c to HDL-c ratio compared to non-hormonal contraceptive (NHC) users, but, no differences were observed in TG, TC, LDL-c, or v-LDL-c levels between DMPA and NHC users.

DMPA were given to two women groups seeking contraceptive advice. Before treatment and after 1, 6, 7, 12 and 13 months, blood samples were taken. At the end of the study, they revealed that DMPA induced a decrease in all lipid components of the HDL approximately 30% and tended to increase LDL-c. DMPA also decreased HDL by approximately 15% (Enk *et al*, 1992).

In an experimental study, adult female rats (*Rattus norvigcus*) were treated with a single dose of DMPA the result showed that statistical significant increase ($P < .01$) in TC, TG, as well as LDL-c and vLDL-c. They also observed a marked decrease of HDL-c in DMPA-treated groups compared to control (Bakry and Abdullah, 2009). It was also suggested DMPA induces several alterations in lipid profile in the injected mice which were dose and duration dependant and manifested by increase in the concentration of TC, TG, LDL-c and dose-related increase in vLDL-c. While, it causes a decrease in serum HDL-c level (Bakry *et al*, 2010).

On the other hand, a study performed by Lizarelli *et al*, (2009) aimed to determine whether the use of a combination oral contraception (COC) or DMPA interferes with endothelial function, indicated the beneficial effects of DMPA, in which the DMPA group had lower values of TC and LDL-c than COC users and the control group (TC: DMPA=139.9±21.5 mg/dL vs. COC=168.2±37.5 mg/dL vs. controls =167.1±29.2 mg/dL, P =.001; LDL-c: DMPA=85.3±20.1 mg/dL vs. COC=106.7±33.3 mg/dL vs. controls=102±24.5 mg/dL, P =.01). But, DMPA group had lower HDL-c level compared to controls and COC groups (DMPA=42.2±7.2 mg/dL vs. controls=52.4±14.1 mg/dL vs.COC=45.4±9.1 mg/dL, P=.001).

Similarly, in a cross sectional study performed on 54 young Pakistan females of age ranging 26-32 years, to compare the extent of cardiovascular atherosclerotic risk associated with the lipid metabolism in women using hormonal contraceptives: oral contraceptives, DMPA, norethisterone oenanthate (NET-EN), implant and non-hormonal intra-uterine contraceptive device (IUCD). It was suggested that beneficial effects of DMPA, where DMPA group poses the lowest TC, TG and LDL-c values (next to implant), compared to other method of contraceptive. The DMPA group also showed the highest HDL- c value compared to other method (next to oral contraceptives) (Jamil and Siddiq, 2012).

Women using DMPA contraception have low circulating estrogen and elevated synthetic progestin. Estrogen is known to enhance NO and endothelial function, decrease endothelin levels, and improve lipid profiles, the suppression of estrogen by DMPA may modify lipid profiles and endothelial function (Torgrimson *et al*, 2012).

1.2.4. Effects of DMPA on body weight

Prior studies have demonstrated the association between DMPA use and weight gain. Weight gain is a commonly perceived side effect of hormonal contraception and may cause women to avoid or discontinue contraceptive methods and is highly variable among women on DMPA (Bonny *et al*, 2014; Vickery *et al*, 2014).

Studies reported a mean weight gain of 2.5 kg at the end of 1 year, but only 2% of women discontinued treatment due to excessive weight gain and also indicate that weight gain occurs mainly in the first year of use and some 20 to 40 % of DMPA users actually lose weight during treatment (Anonymous, Pfizer Canada, Product Monograph, 2013).

There was a tendency for women to gain weight while on DMPA. From an initial average body weight of 61.8 kg, women who completed 1, 2, 4 and 6 years of therapy with DMPA gained an average of 2.45, 3.68, 6.3 and 7.5 kg, respectively (Anonymous, Pfizer New Zealand Ltd, Data Sheet, 2013).

In a longitudinal study, on 703 contracepting women for 36-month, found that DMPA users had a 5.1 kg weight gain compared to 1.5 kg gain among oral contraceptive users (P <.001) and 2.1 kg gain among non-hormonal contraceptives users (P<.001). In addition, DMPA users had a greater increase in total body fat and percent body fat (Berenson *et al*, 2009).

Study on adolescents (N=40; age 12 – 21) initiating DMPA illustrated that excessive weight gain (BMI gain ≥10%) was observed in 11 (27.5%) study participants, where excessive and non-excessive weight gainers did not significantly differ at baseline with regards to mean chronologic/gynecologic age, race, or BMI (Bonny *et al*, 2014).

In an experimental study, performed on female mice that were daily injected DMPA intramuscularlly (doses of 0.39 & 0.78 mg), and sacrificed after 4 or 6 weeks. The data indicated statistically significant increase (P <.01) in body weight of the DMPA treated groups compared to corresponding control, belonging to 4 and 6 weeks and these increases were dose and duration dependant (Bakry *et al*, 2010).

Another study, performed by Bakry and Abdullah (2009) on adult female rats (*Rattus norvigcus*) treated with a single dose of DMPA that is comparable to the weight corrected dose in humans (2.7 mg/rat or 5.4 mg/rat). They found that treating group with 2.7 mg/rat or 5.4 mg/rat of DMPA for 10 and 15 days led to a dose-related increase in body weight (maximum ~ 24.77%).

Weight change at 12 months in users of three Progestin-Only Contraceptive methods was studied on 427 women who had been using the etonogestrel (ENG) implant, the levonorgestrel intrauterine system (LNG-IUS), or DMPA with women using the copper IUCD continuously for at least 11 months. The mean weight change at 12 months was greater among ENG implant and DMPA users compared to copper IUCD users; +2.1 kg among ENG users and +2.2 kg among DMPA users.

Weight gain (kg) was greater for the DMPA group than the group using a non-hormonal IUCD in years 1 through 3 [MD: (2.28 to 2.77), (2.71 to 3.30), and (3.17 to 3.83), respectively. Adolescents using DMPA had a greater increase in body fat compared to a group not using a hormonal method (11.00 to 19.36). The DMPA group also had a greater decrease in lean body mass (-4.00 to -1.07) (Lopez *et al*, 2013).

In a comparative study done to assess weight variations among Brazilian users of LNG-IUS compared with users of the copper intrauterine contraceptive device (TCu 380A IUD) and DMPA users. The result showed a significant association between weight increase and length of use of DMPA (Yela *et al*, 2006).

The study by Xiang *et al*, (2007), to investigate the effect of NHC, COC, and DMPA on lipid metabolism and blood pressure in women with recent gestational diabetes mellitus (GDM), showed that DMPA users gained significantly more weight (4.3 ± 6.9 kg/year) compared with NHC and COC users (1.2 ± 4.7 and 0.7 ± 6.0 kg/year, respectively; $P < .0001$).

On the other hand, study done by Al-Youzbaki, (2011) reported DMPA injections didn't cause a significant increase in body weight among the DMPA users compared with non-users after 6 and 12 months. But DMPA caused a significant increase in BMI after 12 months compared to non-users.

There was significant variability in weight change among contraceptive method groups ranging from −16.3 to +32.7 kg for ENG implant users, −15.9 to +19.1kg for LNG-IUS users, −7.7 to +21.8 for DMPA users, and −16.3 to +16.3 kg for copper IUCD users. Because, race was associated with weight change, the study was also stratified weight change by race and showed black women had greater mean weight gain across all of the contraceptive methods compared to white or other women although, this was not statistically significant (Vickery *et al*, 2014).

1.2.5. Effects of DMPA on blood pressure

Concerning the association between DMPA and blood pressure, different scholars suggested none or small effects of use DMPA. A study by Taneepanichskul *et al*, (1999), to investigate the body weight and blood pressure changes on total of 50 healthy women who had been using DMPA for 120 months were compared with 50 IUCD acceptors who had been using an IUCD for 120 months. They found no difference in blood pressure between DMPA users and IUCD users, which indicates long term DMPA use does not have unfavorable effects on blood pressure.

In one study, injection of DMPA was associated with very small effects on blood pressure; SBP change differed only between COC and DMPA groups ($P = .01$), but, DBP was not significantly different among groups (Xiang *et al*, 2007).

While another study by Al-Youzbaki (2011) reported no significant increase in systolic blood pressure (SBP) and diastolic blood pressure (DBP) among DMPA users in comparison with the non-users after 6 and 12 months. Increase in mean SBP was not significant. But, a significant increase was observed in the mean DBP of DMPA users compared to changes with their respective pretreatment values and it was found to be duration dependent.

Low estrogen and certain progestins have been shown to impact endothelial function even in young healthy women. Because estrogen is known to enhance NO and endothelial function, decrease endothelin-1 levels (ET-1) the suppression of estrogen by DMPA may also modify endothelial function and other biomarkers of vascular health. DMPA antagonizes beneficial effects of estrogen on arterial vasodilation in the forearm, brachial artery, and aorta of younger and older women (Torgrimson *et al*, 2012).

1.3. Significance of the study

Although a number of investigations have been conducted on the relationship between DMPA and lipid profiles, results are not consistent between studies. For example, some have demonstrated that DMPA does not affect serum lipids (Faddah *et al*, 2005; Berenson *et al*, 2009; Al-Youzbaki, 2011). While others have shown an adverse relationship (Enk *et al*, 1992; Bakry and Abdullah, 2009; Bakry *et al*, 2010; Yadav *et al*, 2011; Asare *et al*, 2014) and others reported beneficial effect of DMPA (Lizarelli *et al*, 2009; Jamil and Siddiq, 2012).

Weight gain and increased blood pressure are also associated with the contraceptive DMPA, however, this issue remains controversial (Tneepanichskul *et al*, 1999; Xiang *et al*, 2007; Al-Youzbaki, 2011; Dal'Ava *et al*, 2014). A recent systematic review concluded that there are limitations of studies on DMPA use and weight gain, and that this issue still remains to be clarified (Lopez *et al*, 2013). Furthermore, studies performed among different ethnic groups showed diverse results (Al-Youzbaki, 2011). Available data on this topic are mostly from developed countries and frequently on Caucasians. But, no published data is available concerning the effects of DMPA on different biochemical parameters among Ethiopian women. The purpose of this research was to investigate the effect of use of DMPA on lipid profile, body weight and blood pressure of women who attend family planning unit in selected health centers found in Addis Ababa, Ethiopia.

1.4. Hypothesis of the study

The null hypothesis (Ho) assumes that use of DMPA has no effect on lipid profile, body weight and blood pressure. The alternative hypothesis (H_A) supposes that use of DMPA affect lipid profile, body weight and blood pressure.

2. OBJECTIVES

2.1. General objective

- ❖ The aim of this study was to investigate the effects of use of DMPA on lipid profile (TC, TG, LDL-c, and HDL-c), body weight and blood pressure of women attending family planning unit in Tekele Hymanot and Lomeda Health Centers.

2.2. Specific objectives

- To compare the lipid profiles (TC, TG, LDL-c, and HDL-c) of DMPA users with controls.
- To determine the change in body weight and BMI of DMPA users from their respective pretreatment value.
- To determine the change in MAP of DMPA users from their respective pretreatment value.
- To evaluate the association between lipid profiles, changes in body weight, BMI and MAP and duration of use of DMPA.

3. METHODOLOGY

3.1. Study area

The study was conducted at Tekele Haymanot Health Center, found in Lideta Sub City and Lomeda Health Center, found in Kolfe Keranyo Sub City, Addis Ababa, Ethiopia. Tekele Hymanot Health Center is one of the public health center, established in 1975, which provides basic medical service including family planning and reproductive health care for more than 84,000 people of Lideta Sub City. Eight thousand two hundred eighteen clients had family planning service, of which 2,676 were DMPA users in 2013. Lomeda Health Center is another public health center which was established in 2012 and it provides public health care for more than 47,000 residents of Kolfe Keranyo Sub city. In 2013, 6,342 women visited the family planning unit from which 2,520 women were DMPA users.

3.2. Study design and period

Institutional based cross sectional and patient card review was conducted among DMPA users attending family planning unit in the health centers. The study was done between months of February, 2015 and July, 2015.

3.3. Source and study population

The source population were all women who visited Tekele Haymanot and Lomeda Health Centers. The study population comprises all healthy women between 18-45 years of ages who had voluntarily consented to participate, and who were on DMPA, attending the family planning unit in the health centers and non-users of hormonal contraceptive who visited the health centers during the study period (controls).

3.4. Study subjects

Study subjects were 50 healthy women who had been using DMPA and 50 healthy age-matched (±2 years) controls who were not using any hormonal contraceptive.

3.5. Inclusion and exclusion criteria

3.5.1. Inclusion criteria

- Women between the ages of 18-45 years
- Women with no risk factors for coronary CHD, hypertension : Chronic alcohol and/or tobacco use
- Women who had been using DMPA for at least 6 months
- Another 50 apparently healthy volunteer women who have the same inclusion criteria as the DMPA users except that they were not using any hormonal contraceptives were used as age-matched controls

3.5.2. Exclusion criteria

Exclusion criteria included the following:

- DMPA use within the past 12 months (for controls)
- Other hormonal contraceptive use within the past 3 months (for controls)
- Known or suspected pregnancy
- Breastfeeding
- Chronic disease and medication known to affect lipid profile, body weight and blood pressure were excluded from the study

3.6. Sample size determination

Sample size is determined based on double-population formula with the following assumption. The specific standard deviation for each sub groups in Ethiopia was not obtained, the standard deviation of LDL-c for DMPA users, 1.1 and for non-hormonal contraceptive users, 0.7 is taken from Nepal study. Assuming 95% confidence interval and 80% power, the minimal sample size in each group is calculated by using the formula:

$$n_1 = \frac{r+1}{r}\frac{(Z_\alpha + Z_\beta)^2 \sigma^2}{\Delta^2}$$

Where: n_1= sample size for each group

r=Proportion of DMPA users and controls

Zα= Z-score for two-tailed test based on α-level

Zβ= Z-score based on β level of 80% power

σ^2= Standard deviation

Δ^2 =Change in Standard deviation

Sample size was determined by using the highest standard deviation, adding 5% sampling error to be 50, total of 100 (50 DMPA users and 50 controls) study participants were included in this study.

3.7. Sampling technique

Systematic random sampling technique to select 50 DMPA users and convenient sampling technique were used to recruit 50 age-matched controls. Since there were about 223 and 210 DMPA users per month in Tekele Hymanot and Lomeda Health Centers, respectively, every 4th women were taken from the study population during the study period.

3.7. 1. Methods of sample and data collection

All women were screened for eligibility based on a medical interview and they also completed a written questionnaire on their age, marital status, economic status, alcohol use, smoking, suspected pregnancy, breast-feeding, physical exercise, health status, medication and other. Then, from the study participants who fulfill the inclusion criteria; blood pressure, height and body weight of each participants were measured. Five milliliter of venous blood was collected using sterile syringes and needles from each participant between 8:30 AM and 11:30 AM following an overnight fast. The blood samples were left for 30 to 40 minutes and then centrifuged at 3000 RPM for 5 minutes. Serum of blood samples were separated and stored at -80^{o}C. Estimation of lipid profiles (TC, TG, and HDL-c), were conducted using Bio-system kits on a mindray BS-200 Chemistry Analyzer (Shenzhen, China).

Serum LDL-c was calculated by using Friedewald equation: LDL-c (mg/dL) = TC-[HDL-c + TG/5] (Friedewald *et al*, 1972). TC/HDL-c ratio (Castelli index I) and LDL-c/HDL-c (Castelli index II) were calculated to determine the CVD risk (Castelli, 1998).

The MAP was calculated by using the formula MAP = Pd + 1/3(Ps-Pd). BMI was computed by dividing weight (Kg) to the square of height (m).The change in body weight, BMI and MAP of DMPA users were calculated as the difference between initial body weight, BMI and MAP on the first day of injection and the final weight, BMI and MAP taken at their visit during study period.

3.8. Variables

Dependent variables: Lipid profiles (TC, TG, LDL-c, HDL-c, TC to HDL-c and LDL-c to HDL-c ratio), Weight, BMI and Blood pressure (MAP, SBP and DBP).

Independent variables: Use of DMPA, duration of use of DMPA, age, height, exercise, income, smoking cigarette and alcohol use.

3.9. Measurement of Blood Pressure

Blood pressure of study participants was measured by experienced nurses using a recently calibrated standard mercury sphygmomanometer. Preliminary, blood pressure measurement was done on both arms and the arm that had a consistently higher pressure was subsequently used for blood pressure measurement and interpretation.

The inflatable cuff was encircled on the upper arm and was inflated until it exerted pressure higher than the systolic pressure driving arterial blood.

When the cuff pressure exceeded arterial pressure and blood flow into the lower arm stopped, pressure on the cuff was gradually released. When the cuff pressure fell below systolic arterial blood pressure and blood squeezed through the still-compressed artery, a thumping noise called a Korotkoff sound was heard with each pressure wave through a stethoscope placed over the brachial pulse point. Once the cuff pressure no longer compresses the artery, the sounds disappeared (Lucas, 2015).

The pressure at which a Korotkoff sound is first heard was recorded as the systolic pressure by the manometer connected to the cuff. The point at which the Korotkoff sounds disappeared was recorded as the diastolic pressure.

3.10. Estimation of Serum Lipid Profiles

3.10.1 Determination of Total Cholesterol

Principles of the Method: Total cholesterol was measured enzymatically in serum in a series of coupled reactions that hydrolyze cholesteryl esters and oxidize the 3-OH group of cholesterol. Cholesterol esters are hydrolyzed to free cholesterol by cholesterol ester hydrolase. The free cholesterol produced is oxidized by cholesterol oxidase to cholest-4-en-3-on with the simultaneous production of hydrogen peroxide (H_2O_2), which oxidatively couples with 4-aminoantipyrine and phenol in the presence of peroxidase to yield Quinoneimine dye with maximum absorbtion between 500-550 nm (Roschlay *et al*, 1975).

The reaction sequence is as follow:

$$\text{Cholesteryl ester} + H_2O \xrightarrow{\text{Cholesteryl Ester Hydrolase}} \text{Cholesterol} + \text{Fatty acid}$$

$$\text{Cholesterol} + O_2 \xrightarrow{\text{Cholesterol oxidase}} \text{Cholest-4-en-3-one} + H_2O_2$$

$$2H_2O_2 + \text{4-aminophenazone} + \text{Phenol} \xrightarrow{\text{Peroxide}} \text{Quinoneimine} + 4H_2O$$

The test comes in the form of a commercial kit in which serum sample is incubated with enzymes and reagents from the kit and the change in absorption at 500nm is measured spectrophotometrically.

This change in absorption is proportional to the concentration of total cholesterol in serum sample and can be calculated by comparison with absorption changes that occur with standard solutions containing known cholesterol concentrations.

Procedure: Ten microliter (10µL) serum sample were added into the sample cups and put on the sample disk which rotates to bring the desire sample cup in to position next to the sample probe for specimen sampling. 1000µL reaction reagent (4-Aminophenazone, phenol, peroxidase, cholesterol esterase, cholesterol oxidase) were pipetted into reagent bottles leveled for TC and put on reagent disk and then on the screen menu of the machine TC was entered as a parameter to be tested. The sample probe was pipetted sample from the sample disk and transferred to the reaction disk which contains cuvettes. On the other side of the machine, the reagent probe was pipetted reagents from the reagent disk and transferred it into reaction disk which is a large rotatable disk holding reusable cuvettes with a stirring paddle to stir or mix thoroughly the sample and the reagents. The cuvettes were immersed into reaction water bath and incubated at 37^0C for 5 minutes. Next the reaction disk was rotated the cells to all reaction stations including the photometer light path. Finally, the light was passed through the cuvettes and absorbance of the sample was measured at 500nm.

$$\text{TC concentration } (mg/dL) = \frac{\Delta A_{sample}}{\Delta A_{standard}} \text{ x } C_{standard}$$

3.10.2. Determination of Triglyceride

Principles of the Method: The method is based on the enzymatic hydrolysis of triglycerides to glycerol and free fatty acids by lipoprotein lipase (LPL). Glycerol is converted to glycerol-3-phosphate and adenosine-5-phosphate (ADP) by glycerol kinase and ATP. Glycerol-3-phosphate is oxidized by glycerol phosphate oxidase to form dihydroxy acetone phosphate and H_2O_2. In the presence of peroxidase and H_2O_2, 4-aminoantipyrine couples with phenol to form a coloured product (quinoneimine) that can be measured spectrophotometrically at a wavelength of 500nm.

The reaction sequence is as follows:

$$\text{Triglycerides} + 3H_2O \xrightarrow{\text{Lipase}} \text{glycerol} + \text{fatty acid}$$

$$\text{Glycerol} + \text{ATP} \xrightarrow{\text{Glycerol kinase}} \text{glycerol-3-phosphate} + \text{ADP}$$

$$\text{Glycerol-3-phosphate} + O_2 \xrightarrow{\text{Glycerol phosphate oxidase}} \text{dihydroxyacetone phosphate} + H_2O_2$$

$$H_2O_2 + \text{4-aminoantipyrine} \xrightarrow{\text{Peroxidase}} \text{Quinoneimine} + 2H_2O$$

The triglyceride test comes in the form of a commercial kit containing the reagents, reactants and enzymes needed. Serum samples will be incubated with the kit reagents and enzymes for 5 minutes at 37^0C and absorbance measured at 500 nm against the reagent blank and against known concentrations of standard triglyceride concentrations. The change in absorbance is proportional to the concentration of triglyceride in the serum sample.

Procedure: Ten micro liter (10µL) serum samples were added into the sample cups and put on the sample disk which rotates to bring the desire sample cup into position next to the sample probe for specimen sampling. 1000µL buffer and 1000µL substrate were pipetted into reagent bottles leveled for TG and put on the reagent disk. Then on the screen menu of the machine TG was entered as a parameter to be tested. The sample probe was pipetted sample from the sample disk and transferred to the reaction disk which contains cuvettes.

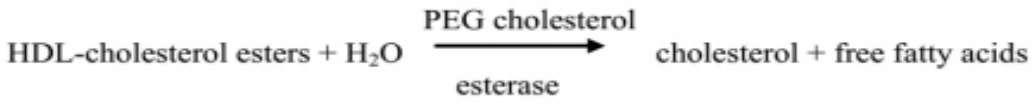
On the other side of the machine, the reagent probe was pipetted reagents from the reagent disk and transferred it into rotatable reaction disk holding reusable cuvettes with a stirring paddle to stir or mix thoroughly the sample and the reagents. The cuvettes were immersed in to reaction water bath and incubated at 37^0C for 5 minutes. Next the reaction disk was rotated the cells to all reaction stations including the photometer light path. Finally, the light was passed through the cuvettes and absorbance of the sample measured at 500nm.

$$\text{TG level concentration } (mg/dL) = \frac{\Delta A_{sample}}{\Delta A_{standard}} \text{ x } C_{standard}$$

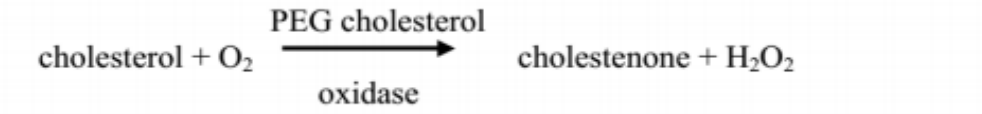
3.10.3. Determination of High Density Lipoprotein Cholesterol

Principles of the Method: The basic principle of the method is as follows. The apo-B containing lipoproteins in the specimen react with antibodies to apo-B that renders them nonreactive with the enzymatic cholesterol reagent under conditions of the assay. The enzymes used are also pegylated, and this allows them to react only with HDL-c and not with antibody-bound LDL-c, vLDL-c or chylomicrons. The apo-B containing 39 lipoproteins are thus effectively excluded from the assay and only HDL-c is detected under the assay conditions (Jacobs *et al*, 1990).

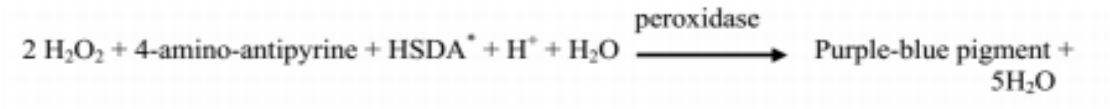
The HDL-c test is a two reagent homogenous system for the selective measurement of serum or plasma HDL-c in the presence of other lipoprotein particles. The assay is comprised of two distinct phases. In phase one, it is likely that in the presence of slightly alkaline buffer and magnesium sulfate and dextran sulfate selectively form water-soluble complexes with LDL-c, LDL-c, and chylomicrons, which are resistant to polyethylene glycol (PEG)-modified enzymes. In phase two the cholesterol concentration of HDL-c cholesterol is determined enzymatically by cholesterol esterase and cholesterol oxidase coupled with PEG to the amino groups (approx. 40%).



Cholesterol esters are broken down quantitatively into free cholesterol and fatty acids by cholesterol esterase.



In the presence of oxygen, cholesterol is oxidized by cholesterol oxidase to be converted in to cholestenone and hydrogen peroxide.



Where: HSDA= N-(2-hydroxy-3-sulfopropyl)-3, 5-dimethoxyaniline.

In the presence of peroxidase, the hydrogen peroxide generated reacts with 4-aminoantipyrine and HSDA to form a purple-blue dye. The color intensity of this dye is proportional to the cholesterol concentration and can be measured spectrophotometrically.

Procedure: Ten micro liter (10µL) serum samples were added into the sample cups and put on the sample disk which rotates to bring the desire sample cup into position next to the sample probe for specimen sampling. 1000µL buffer and 1000µL substrate were pipetted into reagent bottles leveled for HDL-c and put on the reagent disk. Then on the screen menu of the machine HDL-c was entered as a parameter to be tested. The sample probe was pipetted sample from the sample disk and transferred to the reaction disk which contains cuvettes.

On the other side of the machine, the reagent probe was pipetted reagents from the reagent disk and transferred it into rotatable reaction disk holding reusable cuvettes with a stirring paddle to stir or mix thoroughly the sample and the reagents. The cuvettes were immersed in to reaction water bath and incubated at 37^0C for 5 minutes. Next the reaction disk was rotated the cells to all reaction stations including the photometer light path. Finally, the light was passed through the cuvettes and absorbance of the sample was measured at 500nm.

3.10.4. Determination Low Density Lipoprotein Cholesterol

Most of the circulating cholesterol is found in three major lipoprotein fractions: vLDL, LDL and HDL. LDL-c is calculated from measured values of total cholesterol, triglycerides and HDL-c according to the Friedewald equation: LDL-c = TC-[HDL-c + TG/5)]. Where [TG]/5 is an estimate of vLDL-c, all values are expressed in mg/dL. The equation is derived from another equation, [Total Cholesterol] = [vLDL-c] + [LDL-c] + [HDL-c], but TG is easier to estimate than vLDL and [TG/5] is a good estimate of vLDL, although the Friedewald equation is not valid for calculating LDL-c if the serum TG is above 400 mg/dL.

3.11. Data Quality Assurance

Questionnaires and laboratory results were checked for completeness on daily basis by the immediate supervisor and the principal investigator. Missed questionnaire were sent back to respective data collector and laboratory technologist for correction. The completed questionnaires were rechecked repeatedly by the principal investigator to maintain the quality of data. Blood pressure was measured using recently calibrated standardized sphygmomanometer. The blood sample was collected with precaution using the right procedures of sample collection for lipid profile analysis and the lipid profile was determined by mindray BS-200 Chemistry Analyzer after it was calibrated to adequate temperature and appropriate wavelength to conduct the standard procedure and also indiscriminate results were repeated.

3.12. Ethical Considerations

The significance of the study and safety of the participants was evaluated by the ethical committee of Department of Biochemistry, School of medicine, Addis Ababa University and the ethical clearance was obtained from the Department with a protocol number of SOM/BCHM061/2006. Prior to data collection general agreement was asked to the health centers family planning unit focal person through a letter written from the Department of Biochemistry. The principal investigator has explained the purpose of the study for the concerned bodies and obtained permission from focal persons of the health centers. During distribution of questionnaires, subjects were informed that the information collected would kept anonymous to protect individual privacy and the objective of the study was explained to the study participants prior to obtaining their informed consent. The subjects were briefed about the confidentiality of their response and the importance of providing correct information. The selection of subjects and the collection of samples from the study participants were done after prior notice and approval for all protocols involved, and made clear that the participants under study have the right to refuse participation or quit participation at any point of the interview, data collecting, and laboratory test if they so wish.

3.13. Data Analysis

SPSS software (v21; IBM Corporation, Armonk, NY, USA) was used for data management and statistical analysis. Standard statistical methods were used to determine the mean, standard deviation (SD) and the range. Student's independent t-test was used to compare the results of lipid profiles and blood pressure of DMPA users and control group. Student's paired t-test was used to evaluate change in weight, BMI and blood pressure of DMPA users. One-way ANOVA (analysis of variance) was used to identify the variation of variables in relation to the duration of use of DMPA. Pearson's correlation-test was used to evaluate the association between the variables. All values were quoted as the means ± SD. A *P*-values of ≤ .05 were considered to be statistically significant.

4. RESULTS

4.1. Demographic and Anthropometric study of participants

This institutional based cross sectional study was conducted to investigate the effect of DMPA on lipid profile, weight, BMI and MAP of users. A total of 100 study participants (50 DMPA users, and 50 controls) were included in the study. Majority of DMPA users, 36 (72%) and 25 (50%) of the controls were married. Twenty six (52%) of DMPA users and 32 (64%) of control groups had monthly income between 650 and 3,500 ETB.

All study participants in both groups were healthy, and no woman reported history of regular exercise, consumption of alcohol and cigarette smoking.

The mean age (years) of the two groups were 27.54±5.63 (DMPA users) and 27.44±5.02 (control) and was not significantly different (P =.93). The age range in DMPA users was 19–43 years and 19–42 years in controls. Majority of study participants, 39 (78%) of DMPA users and 40 (80%) of the controls were aged 20-30 years, 10 (20%) and 9 (18%) were women aged greater than 30 years in DMPA users and controls, respectively.

The mean height (m) of study participants was 1.58±0.65 and1.60±0.60 in DMPA users and controls, respectively. The height range in DMPA users was 1.45-1.7m and 1.4-1.72 m in controls. The mean weight (Kg) in DMPA users was 55.14±6.62 and 55.52±8.30 in control group, the range in DMPA users and controls were 43–72Kg and 42–79 Kg, respectively. The mean BMI (Kg/m^2) was 22.05±3.10 and 21.55±3.05, the range being 16.2-30.7 and 15-29 in DMPA users and controls, respectively.

The MAP (mmHg) of DMPA users was 83.22±8.68, whereas 82.91±6.98 in control group with a range of 66.6-106.6 in DMPA users and 70-93.3 in controls, this difference is not statistically significant (P =.85). DMPA user received the contraceptive regularly for a mean duration of 28.86 months, range 6-96 months.

Table 4.1 Participant characteristics, including marital status; income/month; age (years); duration of DMPA use (months); weight (Kg), height (m); BMI (kg/m2) and MAP (mmHg) in Tekele Hymanot and Lomeda Health Centers, 2015.

Variables		DMPA User (n=50)	%	Control Group (n=50)	%	*P*-value
Marital status	Single	13	26	20	40	
	Married	36	72	25	50	
	Divorced	1	2	5	10	
	Total	50	100	50	100	
Income/month (ETB)	<1,400	26	52	32	64	
	1,401-3,500	22	44	15	30	
	3,501-5,000	2	4	2	4	
	>5,000	0	0	1	2	
	Total	50	100	50	100	
Age (Years)	<20	1	2	1	2	.93
	20-30	39	78	40	80	
	>30	10	20	9	18	
	Total	50	100	50	100	
Duration of use of DMPA (month)	6-24	23	46	-	-	
	27-48	21	42	-	-	
	51-96	6	12	-	-	
	Total	50	100	-	-	
Weight (Kg)	-	55.14±6.62	-	55.52±8.30	-	.80
Height (m)	-	1.58±0.65	-	1.60±0.60	-	.09
BMI (Kg/m^2)	-	22.05±3.10	-	21.56±3.05	-	.42
MAP (mmHg)	-	83.22±8.68	-	82.91±6.98	-	.85

Where: ETB= Ethiopian birr, variables statistically significant at $P \leq .05$ and P-values were obtained by student's independent t-test.

4.2. Lipid Profile

Serum TC level in DMPA users was increased by 13.2 % from controls. Mean serum TC level in DMPA users was 183.12±40.56 mg/dL and 161.76± 29.45 mg/dL in controls. The difference was statistically significant (*P* =.003, Table 4.2), the range being 124-289 mg/dL and 101-225 mg/dL in DMPA users and controls, respectively. There were 16 (32%) DMPA users with TC value ≥ 200mg/dL and 7 (14%) in control group.

Even though, there was a 9.8% increment of serum TG level in DMPA users (103±42.82 mg/dL) compared to control group (93.80± 35.16 mg/dL), the difference was not statistically significant (*P*=.24). The number of women in DMPA users with TG value of 150-200 mg/dL were 3(6%) and 2 (4%) in control group. The maximum and minimum serum TG level in DMPA users and control group was 262 mg/dL, 48 mg/dL and 178 mg/dL, 47 mg/dL, respectively.

Table 4.2 Mean serum level and mean percentile differences of TC, TG, HDL-c and LDL-c in DMPA users and controls, in Tekele Hymanot and Lomeda Health Centers, 2015.

Lipid profile	DMPA User (n=50)	Control Group (n=50)	Mean Difference	%	*P*- value
TC	183.12±40.56	161.76± 29.45	21.36	+13.2	**.003***
TG	103±42.82	93.80± 35.16	9.20	+9.8	.24
HDL-c	51±7.68	57.84±9.15	6.84	-11.8	**.001***
LDL-c	111.54±36.04	83.26±30.96	28.28	+34	**.001***

*Where: % = percentage of change, (-) = Decreased from controls, (+) = Increased from controls, values are represented as M ± SD, *****=statistically significant, P-values were obtained by student's independent t-test.*

Results in Table 4.2 reveals serum HDL-c levels in DMPA users (51±7.68 mg/dL) was significantly lower (*P*=.001) by 11.8 % from the control group (57.84±9.15 mg/dL). Our data also showed that 35 mg/dL was the minimum serum HDL-c level in both DMPA users and control group, 67 mg/dL was the maximum serum HDL-c in DMPA users and 77 mg/dL in control group. There were 5 (10%) DMPA users with HDL-c value < 40 mg/dL, and 2 (4%) in the controls. Seven (14%) of the DMPA users and 1(2%) in the control had HDL-c value between 40 and 45 mg/dL.

Table 4.3 Mean serums TC, TG, HDL-c and LDL-c levels in DMPA users related to the duration of use, in Tekele Hymanot and Lomeda Health Centers, 2015.

Lipid profile	Duration (month)	n	mg/Dl	*P*-value
TC	6-24	23	184.65±43.15	.84
	27-48	21	184.09±36.86	
	51-96	6	173.83±48.69	
	Total	50	183.12±40.56	
TG	6-24	23	101.30±41.94	.15
	27-48	21	112.90±44.94	
	51-96	6	74.83±27.83	
	Total	50	103±42.82	
HDL-c	6-24	23	51.86±7.07	.28
	27-48	21	51.38±7.65	
	51-96	6	46.33±9.68	
	Total	50	51.00±7.68	
LDL-c	6-24	23	112.56±38.72	.97
	27-48	21	110.13±33.15	
	51-96	6	112.53±41.53	
	Total	50	111.54±36.04	

Values are represented as M ± SD, P-values were obtained by one-way ANOVA.

DMPA users experienced significant greater increase (*P*=.001) in the serum LDL-c level (111.54±36.04) compared with the controls (83.26±30.96), a 34% difference. The maximum and minimum serum concentrations of LDL-c in DMPA users were 212 mg/dL, 61 mg/dL and 159 mg/dL, 17 mg/dL in control group, respectively. Seven (14%) of DMPA users had LDL-c value >150 mg/dL compared with 1 (2%) in the controls. Forty four (88%) of controls had serum LDL-c value less than 120 mg/ dL compared with 33 (66%) of DMPA users (Table 4.2).

Even though, there was significant difference in lipid profiles between DMPA users and controls (except serum TG level), the one-way ANOVA analysis (Table 4.3) didn't show statistically significant difference (*P*>.05) in lipid profile (TC, TG, HDL-c and LDL-c levels) between groups of DMPA in relation to duration of use (6-24, 27-48 and 51-96 months).

4.3. Body Weight and Body Mass Index (BMI)

Result shown in Table 4.4 demonstrated that a statistical significant (*P* =.02) mean weight gain of 1.6 Kg (+2.99%) in DMPA users in which the mean weight (Kg) was 53.54±6.42 before they starting to use DMPA and 55.14±6.62 Kg after they had been using DMPA. Weight gain varies from 1 to 14 Kg. Excessive weight gain (≥10%) was observed in 9 (18%) of DMPA users.

Table 4.4 Mean Weight, BMI and MAP of DMPA users; before they starting to use DMPA and after they had been using DMPA, in Tekele Hymanot and Lomeda Health Centers, 2015.

Variables (n=50)		Mean ± (SD)	Mean Differences	%	*P*-value
Weight (Kg)	Wta	55.14±6.63	1.6 Kg	+2.99%	**.02***
	Wtb	53.54±6.42			
BMI (Kg/m^2)	BMIa	22.05±3.10	0.67 Kg/m^2	+3.13%	**.02***
	BMIb	21.38±2.70			
MAP (mmHg)	MAPa	83.21±8.68	0.51 mmHg	+0.62%	.67
	MAPb	82.70±7.56			

*Where: Wta=weight after they had been using DMPA, Wtb=weight before they starting to use DMPA, BMIa=body mass index after they had been using DMPA, BMIb=body mass index before they starting to use DMPA, MAPa=mean arterial pressure after they had been using DMPA and MAPb=mean arterial pressure before they starting to use DMPA. % = percentage of change, (+) = Increased from baseline. *=statistically significant, P-values were obtained by student's paired t-test.*

DMPA users showed significant increase (*P*=.02) in BMI compared to their respective pretreatment value (Table 4.4). Mean BMI (Kg/m^2) at baseline was 21.38±2.70 and 22.05±3.10 after they had been using DMPA. The mean change in BMI (Kg/m^2) among DMPA users was 0.67 (+3.13%). The maximum increment of BMI among DMPA users was 5.9 Kg/m^2.

Table 4.5 Changes in mean Weight and BMI of DMPA users related to the duration of use, in Tekele Hymanot and Lomeda Health Centers, 2015.

Parameters	Duration (month)	n	Mean± SD	%	*P*-value
Change in mean weight (Kg)	6-24	23	1.78±5.06	+3.32	.96
	27-48	21	1.38±4.35	+2.58	
	51-96	6	1.66±5.24	+3.10	
	Total	50	1.60±4.70	+2.99	
Change in mean BMI (Kg/m^2)	6-24	23	.76±2.12	+3.55	.95
	27-48	21	.57±1.78	+2.67	
	51-96	6	.65±2.15	+3.04	
	Total	50	.67±1.94	+3.13	

Where: % = percentage of change, (+) = Increased from baseline. P-values were obtained by one-way ANOVA.

However, student's paired t-test showed that changes in mean weight and BMI among DMPA users were statistically significant (P =.02 and P =.02, respectively, Table 4.4), the one-way ANOVA (Table 4.5) didn't show significant (P >.05) changes in mean weight and BMI between groups of DMPA users in relation to duration of use (6-24, 27-48 and 51-96 months).

4.4. Mean Arterial pressure (MAP)

Table 4.1 showed that the MAP of DMPA users (I83.22±8.68 mmHg) and control group (82.91±6.98 mmHg) were not significantly different (P=.85). Furthermore, the change in MAP of DMPA users was not statistically significant (P=.67) where, the mean MAP of 82.70±7.56 mmHg before they staring to use DMPA and 83.21±8.68 mmHg after they had been using DMPA (Table 4.4). The mean SBP and DBP were 107 ±8.86; 70.6 ±8.18 before they starting to use DMPA and 108.4 ±12.47; 70.7±7.82 after they had been using DMPA. This difference was not significant (P =.41 and P=.94, respectively).

There were no significant difference (*P*=.93 and *P*=.18, respectively) in SBP and DBP between DMPA user (108.4 ±12.47; 70.7±7.82) and controls (108.2±11.00; 72.8±7.83), respectively.

The present study found no association between any of the variables comprising of change in weight (BMI) and lipid profiles (TC, TG, HDL-C, LDL-c, TC/HDL-c and LDL-c/HDL-c) of DMPA users.

5. DISCUSSION

Progestin-only contraceptives (POCs) are appropriate for many women who cannot or should not take estrogen. DMPA is one of the most common POC which is cost effective and a long acting form of the synthetic MPA, administered by deep intramuscular injection every 3 months for slowly releasing the progestin from the muscle (Al-Youzbaki, 2011). MPA is detected in the serum within 30 minutes of injection of 150 mg. Serum concentrations vary between individual women but generally plateau at about 1.0 ng/mL for about three months, after which there is a gradual decline. The circulating MPA initially inhibits the mid-cycle LH peak, and LH and FSH levels remain in the range of those for the luteal phase of a pretreatment control cycle (Torgrimson *et al*, 2011). Since ovulation is inhibited, serum progesterone levels remain low (< 0.4 ng/mL) for several months following an injection of DMPA. When MPA levels fall below 0.1 ng/mL , ovulation resumes. In some women, MPA can be detected in the serum for as long as 9 months after a single injection of 150 mg. Thus, return to fertility may be delayed for several months if a woman wishes to conceive after receiving one or more injections of DMPA (Mishell, 1996).

Following an injection of DMPA, serum estradiol levels initially are in the early to mid-follicular phase range (mean approximately 50 pg/nL) (Torgrimson *et al*, 2011). Serum estradiol levels begin to rise about four months after a single injection when MPA levels fall below 0.5 ng/mL. For women who have used DMPA for several years, serum estradiol levels is depleted within range of between 10 and 92 pg/mL, to be mean levels of about 40 pg/mL (Mishell, 1996). Estrogen is known to improve lipid profile, increase vasodilatation and inhibits the response of blood vessels to injury and development of atherosclerosis (Torgrimson *et al*, 2011).

DMPA is cost effective and has a prolonged duration of action. Concern about alteration in lipid profile and weight gain with result and increase risk of CVD can deter initiation of DMPA and cause early discontinuation among users.

Serum Total Cholesterol (TC): Serum TC level of DMPA users was significantly increased by 13.2 % in comparison with controls (Table 4.2). This result of the present study is in agreement with those of Yadav *et al*, (2011) study, where they have shown the TC level in long-term DMPA users was significantly higher (*P* =.001) than controls. In other study conducted by Asare *et al*, (2014) significant elevation in serum TC level was observed among DMPA users compared to controls (*P* =.018), similar to the present study. Similar observation was reported in an experimental study made on rats by Bakry and Abdullah (2009); Bakry *et al*, (2010), to investigate the effect of DMPA on estrous cycle, serum protein, body weight and serum lipid profile in rats treated with DMPA. Unlike finding of the present study, cross sectional study performed by Faddah *et al*, (2005) and cohort study by Berenson *et al*, (2009); Al-Youzbaki, (2011) reported changes in mean serum TC level of DMPA users were not significant. Contrary to the present study, a study performed by Lizarelli *et al*, (2009) aimed to determine whether the use of COC or DMPA interferes with endothelial function. They suggested a beneficial effect of DMPA, where DMPA group had lower values of TC than the control group (not used any hormonal contraception) and COC users (TC: DMPA=139.9±21.5 mg/dL vs. controls=167.1±29.2 mg/dL vs.COC=168.2±37.5, *P* =.001). This finding of Lizarelli *et al,* was supported by a cross sectional study performed on 54 young Pakistan females of age ranging from 26-32 years aimed to compare the extent of cardiovascular atherosclerotic risk associated with the lipid metabolism in women using hormonal contraceptives including COC, DMPA, NET-EN, implant and non hormonal IUCD. DMPA group poses the lowest TC value compared to other method of contraceptive next to implant (Jamil and Siddiq, 2012). This discrepancy could be due to the difference in protocol between prior and the present study. Berenson *et al*, used a cohort study (703 women participants) as opposed to cross sectional study in the present investigation relatively used small sample size (100 women). The discrepancy may also be due to age difference between the study participants. Participants were aged between 25–30 years in the study of Faddah *et al*, between 16-33 in Berenson *et al* and 20-35 years in Al-Youzbaki investigation. But, participants of the present study were aged between18-45 years. Since, the women in our study also include subjects approaching the menopause, expected to exhibit age related elevated serum TC levels. Furthermore the difference in race/ethnicity, in the food habits and life styles may contribute for this discrepancy.

Serum Triglyceride (TG): According to present study, serum TG level of DMPA users was found to be high compared to controls; however, this difference was not statistically significant (Table 4.2). This finding of the present study is consistent with study of Yadav *et al,* (2011), who demonstrated that elevation of serum TG level in DMPA users compared to controls. But, the difference was not statistically significant (P=.44). Another study suggested that serum TG level was not affected by DMPA use (Faddah *et al*, 2005). Similar study showed that the patterns of change in serum TG level was not significantly different between DMPA users and non-users (Xiang *et al*, 2007). However, others reported a significant increase in serum TG level after 6 months of DMPA use (Al-Youzbaki , 2011). Studies performed on rats by (Bakry and Abdullah, 2009; Bakry *et al*, 2010) also revealed a significant increase (P<.01) in concentration of TG in DMPA treated rats compared to controls. This may be due to the difference in study design; animals were injected daily with DMPA which may attribute its effect to be significant more than using DMPA every 3 month. On the other hand, Jamil and Siddiq (2012), reported DMPA users have lower TG value than any other contraceptive method (next to implant).

Serum High Density Lipoprotein Cholesterol (HDL-c): Serum HDL-c level in DMPA users was significantly lower compared to controls. This supports results reported by (Enk *et al*, 1992; Kongsayreepong *et al*, 1993; Faddah *et al*, 2005; Berenson *et al*, 2009; Lizarelli *et al*, 2009), where, the DMPA users had lower serum levels of HDL-c than control group. Findings of the present study is also in agreement with findings on rats reported by Bakry and Abdullah, (2009); Bakry *et al*, (2010) that showed a significant decrease (P<.01) in serum HDL-c level in DMPA treated rats compared to controls. Whereas, other studies reported that DMPA did not cause any significant changes in mean serum HDL-c level (Yadav *et al*, 2011; Al-Youzbaki, 2011). Contrary to the present study, Jamil and Siddiq (2012) reported DMPA users have larger value of serum HDL-c compare to IUCD, implant and NET-EN group. This is possibly due to the difference in patterns of dyslipidemia that may be caused by DMPA in different ethnic group. It can also be due to the difference in food habits; Ethiopian's usually utilize imported edible palm oil containing large proportion of saturated fatty acid (Andargie *et al*, 2012, in press), which can decrease the HDL-c level in DMPA users of this study. Furthermore, DMPA users in both health centers of the present study and previous study may have been advised differently. Women in this study were very poorly advised by the staff of the health centers, with reference to their life styles.

Serum Low Density Lipoprotein Cholesterol (LDL-c): DMPA users experienced significant increase in serum LDL-c level compared to controls. Similar observations were reported by Kongsayreepong *et al*, (1993); Yadav *et al*, (2011); Asare *et al*, (2014) where, significant elevation were observed in serum LDL-c level in DMPA group compared to controls. The study done by Faddah *et al*, (2005) also revealed that the use of DMPA as contraceptive caused a significant increase ($P < .01$) in LDL-c but, after 3 and 4 years use. The result of present study is also consistent with findings on rats, that showed a significant increase ($P < 0.01$) in concentration of LDL-c in DMPA treated rats compared to controls (Bakry and Abdullah, 2009; Bakry *et al*, 2010). Whereas some studies reported that DMPA did not cause significant change in mean serum LDL-c level (Xiang *et al*, 2007; Berenson *et al*, 2009; Al-Youzbaki , 2011). Contrary to the present study, DMPA group were reported with lower values of LDL-c than control group (Lizarelli *et al*, 2009; Jamil and Siddiq, 2012).

TC/HDL-c (Castelli risk index I) and LDL-c/HDL-c (Castelli risk index II): The ratio of TC to HDL-c and LDL-c to HDL-c were increased in DMPA users compared to controls. The mean value of the ratio of TC to HDL-c and LDL-c to HDL-c were 3.59± 0.65 and 2.19±0.61 in DMPA users and 2.79±0.69 and 1.44±0.68 in control group, respectively, which were significantly different (P=.001 and P=.001, respectively). These ratios were increased by 28.67% (TC to HDL-c) and 52.08% (LDL-c to HDL-c) in DMPA users compared to controls. The present study extends the previous findings on rats that reported the ratio of TC/HDL-c and LDL-c/HDL-c were increased in response to DMPA treatment (Bakry and Abdullah, 2009). Unlike findings of the present study, purposive random sampling technique was used focused on the changes in some lipoprotein biomarkers over time, where they found that the Castelli risk indices I (TC to HDL-c) and II (LDL-c to HDL-c) were significantly increased (P =.03 and P =.01, respectively) in the COC group compared to the control group. These values were lower in DMPA users compared to COC group, but, higher than the control group, however, differences were not statistically significant (P>.05) (Asare *et al*, 2014). Another study found that the LDL-c to HDL-c ratio was increased gradually in DMPA users in comparison to control group, but differences were not statistically significant (Faddah *et al*, 2005). A study by Jamil and Siddiq, (2012) on the other hand, showed that DMPA group poses the lowest ratio of TC to HDL-c and LDL-c to HDL-c than other types of hormonal and non-hormonal contraceptive group, which were not agreed with the present findings.

Serum lipid profile of DMPA users related to duration of use: According to the results obtained from the present study, significant increase in TC, LDL-c, TC to HDL-c as well as LDL-c to HDL-c ratio level and significant decrease in HDL-c was observed among DMPA users compared to controls. However, the one-way ANOVA analysis (Table 4.3) didn't showed significant change (P >.05) in the lipid profile levels between groups of DMPA users in relation to duration of use. This finding is in agreement with the study of Al-Youzbaki, (2011) where, one-way ANOVA analysis among DMPA users didn't showed significant relationship between the change in serum TC, HDL-c and LDL-c among DMPA users in relation to the duration of use. Whereas, LDL-c to HDL-c ratio from Berenson *et al*, (2009) study showed that an initial increase at 6 months (2.4 to 2.6 mg/dL) followed by a drop back to baseline over the next 18-24 months. By the 36th month visit, the ratio had dropped further to 2.3 mg/dL. The mean HDL-c levels dropped from 45 mg/ dL at baseline to 41 mg/ dL at the 6 month visit but then steadily raised over the remainder of the follow up period and by 36th months, the level had increased to its baseline value. This alteration in lipid profile is associated with depletion of serum estrogen levels, which is known to improve lipid profiles. Even though, estrogen concentration is markedly decreased by the use of DMPA; its concentration remains constant in its minimal value with long term use of DMPA.

For women who had been using DMPA for several years, serum estrogen levels range between 10 and 92 pg/mL, with mean levels of 40 pg/mL (Mishell, 1996). Therefore, the finding of the present study may suggest that, long term use of DMPA had relatively similar effect with short term use of DMPA.

On the other hand, some studies reported duration dependent variation in lipid profiles of DMPA users. For example, TG level were initially decreased, being significant at 36 months (P <.001), but, returned to normal at 60 months of use. On the other hand, serum TC level were significantly increased at 24 and 36 months (P < .05) and become highly significant after 60 months of use (P < .001) (Liew *et al*, 1985). Studies performed by Faddah *et al*, (2005) revealed duration dependant significant decrease in mean serum HDL-c levels in DMPA users. Furthermore, a study conducted on DMPA users reported a significant increase in serum TG level after 6 months of DMPA uses with respect to the duration of use (Al-Youzbaki, 2011).

However, there were no significant association between changes in lipid profile and duration of DMPA use (Table 4.3), DMPA users showed an initial increase in serum TC level at 6-24 months (184.65mg/dL) followed by a slight drop to184.09 mg/dL over the next 27-48 months. By the 51-96 month of use, the TC level had dropped further to 173.83mg/dL. Serum TG level were 101.30 mg/dL at 6-24 months of use of DMPA followed by an increased to 112.90 mg/dL at 27-48 months, and then dropped in to 74.83 mg/dL by the 51-96 months of visit. This may probably be due to women under this group (women who had been using DMPA for 51-96 month) may had lower levels of TC and TG at the base line (before they starting to use DMPA) compared to women who had been using DMPA for 6-24 and 27-48 months. The HDL-c level of DMPA users was steadily decreased from 51.86 mg/dL at 6-24 months to 51.38 mg/dL and 46.33 mg/dL at 27-48 and 51-96 month, respectively. The LDL-c level at 27-48 months (110.13) was slightly lower than the level recorded at 6-24 months (112.56) and 51-96 months (112.53). The ratio of TC/HDL-c and LDL-c /HDL-c showed sequential increment through 6-24, 27-48 and 51-96 months (TC to HDL-c=3.54, 3.63, 3.73 and LDL-c to HDL-c=2.14, 2.19, 2.43, respectively).

Use of DMPA and risk of Coronary Heart Disease (CHD): Serum TC level is a major indicator of risk of CHD; for every 1% increase in serum TC level, a 2% increase in incidence of CHD is found. A high level of LDL-c is an independent risk factor for CHD in both men and women, but high TG level is an independent risk factor only in women (Castelli, 1998). From epidemiological studies, it is inferred that low HDL-c levels and high LDL-c levels are independent risk factors for the development of atherosclerosis and CHD (Enk *et al*, 1992). Conversely, the higher the level of HDL-c, the lower the risk of CHD. Serum LDL-c and HDL-c levels are strong predictors of CHD (Topcuoglu *et al*, 2005). High levels of HDL-c may reduce vascular endothelial uptake of LDL-c through competitive inhibition of LDL-c receptor binding. In addition, LDL-c aggregates are taken up by macrophages, since HDL-c prevents LDL-c aggregation; it reduces the influx of cholesterol and foam cell formation (Faddah *et al*, 2005).

The present study suggested DMPA users had the most risky lipid profile, because they had higher TC, TG (even though, statistically not significant) as well as LDL-c levels, and lower HDL-c level compared to controls. Serum TC, TG and LDL-c levels of DMPA users of the present study were increased by +13.2 %, +9.8 and +33.96, respectively, compared to controls. Furthermore, serum HDL-c level of DMPA users was decreased by 11.8% from the controls.

As a result, in DMPA users of this study, TC to HDL-c and LDL-c to HDL-c ratio were increased by 28.67 % and 52%, respectively, compared to controls. These findings have been reported to have more prognostic value than either value alone. Elevated TC to HDL-c and LDL-c to HDL-c ratio were found accurately predict CHD risk among those with elevated TG levels (Berenson *et al*, 2009). Thus, the changes we observed in lipid profiles of DMPA users can contribute to increase risk of atherosclerosis. The present study therefore, suggested that, use of DMPA induced changes in lipid metabolism similar to those associated with an increased risk of CHD. Furthermore, it appears that DMPA use indirectly increase cardiovascular risk through mechanisms involving weight gain and obesity.

DMPA has possible ill-effects which may be attributed to hormonal imbalance or toxic effect and this is indicated by a long-term atherogenic role of DMPA (Bakry *et al*, 2010). The adverse effect of DMPA on lipid metabolism is related to its weak androgenic effect which can counteract the effects of estrogen (Godsland *et al*, 1990; Topcuoglu *et al*, 2005; Berenson *et al*, 2009; Yadav *et al*, 2011; Anonymous, Pfizer New Zealand Ltd, Data Sheet, 2013).

Hence, women using DMPA have elevated synthetic progestin and low circulating estrogen to those seen in the early follicular phase of a menstrual cycle or post-menopause (Torgrimson *et al*, 2011). Menopause women are characterized by an estrogen deficient state similar to that induced by DMPA (Lange *et al*, 2015).

The role of estrogen to improve the lipid profile and its anti-atheroscleorotic effect is well documented (Tikkanen *et al,* 1978; Campos *et al*, 1988; Granfone *et al*, 1992; Stevenson *et al*, 1993; Guetta *et al*, 1996; Karjalainen *et al*, 2000; Bakry and Abdullah, 2009), thus, the suppression of estrogen by DMPA, can modify lipid profiles and endothelial function (Torgrimson *et al*, 2012). Estrogen is suggested to increase serum HDL-c levels and decrease serum TC and LDL-c levels, whereas DMPA, weak synthetic progestin, opposes the effect of estrogen (Yadav *et al*, 2011).

The mechanism of reduction in serum LDL-c level by estrogen is probably a result of accelerated conversion of hepatic cholesterol to bile acids and increased expression of LDL-c receptors on cell surfaces resulting in augmented clearance of LDL-c from the plasma (Kushwaha and Born, 1991).

Another mechanism is suggested by a study done to investigate the effect of estrogen on vLDL and LDL subclass metabolism in postmenopausal women, where, oral estrogen therapy on apolipoprotein B-100 (apoB) (in vLDL, IDL, and LDL subclasses) metabolism was to accelerate the fractional catabolic rates in all particles. Thus, estrogen therapy increases the clearance of both light and dense LDL (Campos *et al*, 1997).

The increase in HDL-c levels by estrogen is due to suppression of synthesis of key enzymes of lipoprotein metabolism, hepatic and lipoprotein lipase, and increased synthesis of the principal apoprotein of HDL, apoAI, both causes an increase in the levels of HDL_2, the HDL sub-particle considered the most active in reverse cholesterol transport (Tikkanen *et al*, 1982; Walsh *et al*, 1991; Berenson *et al*, 2009). These findings of prior study suggested that the role played by estrogen for the reduction of risk of CHD. Therefore, women with weak activity of estrogen in the plasma which can be caused by the use of DMPA are at higher risk for CHD, because of increased serum levels of TC and LDL-c and decreased HDL-c level.

Body weight and BMI: Findings from the present study demonstrated that DMPA users had significant weight gain and increased BMI after they had been using DMPA as compared to their respective pretreatment value (Table 4.4). The mean weight gain in DMPA users was 1.6 Kg (+2.99%). Excessive weight gain (BMI≥10%) was observed in 9 (18%) of DMPA users. The mean change of BMI in DMPA users was 0.67 Kg/m^2 (+3.13%). This is in agreement with a prospective cohort study performed on 97 Brazilian women, aimed to compare body weight and body composition in DMPA and copper IUD users at baseline and after 1 year of use. They found that statistically significant increase in body weight (1.9±3.5 kg) in DMPA users after 1 year of use (P =.02), resulting from an increase in fat mass of 1.6 kg (P =.03) while weight remained same (1.1±3.2 kg, P =.15) in IUCD users (Dal'Ava *et al*, 2014).

Other study, done to assess the association between progestin-only contraceptive use and changes in body weight, revealed that weight gain was greater in DMPA group than the group using a non-hormonal IUCD (Lopez *et al*, 2013), which was in agreement with findings of the present study.

Similar study by Vickery *et al*, (2014) showed that use of DMPA was associated with weight gain (mean change in weight; +2.2 kg) compared to the copper IUCD. They also suggested that only black race was associated with significant weight gain compared to other racial groups. The present study also support finding of other studies where the BMI of the DMPA users (25.24±0.63 kg/m^2) was increased significantly than the control group (21.73±0.75 kg/m^2; *P* =.008) and were in the overweight range (Asare *et al*, 2014). Excessive weight gain (BMI gain ≥10%) was observed in 11 (27.5%) of DMPA users (Bonny *et al*, 2014). Moreover, a recent study by Lange *et al*, (2015), conducted to assess dietary intake and weight gain among adolescents on DMPA, demonstrated that mean BMI increased significantly (*P* =.001) from 23.7 ±5.3 at the baseline to 25.3 ±5.7 after 12 months of use of DMPA with increased mean percentage body fat significantly (*P* <.001). The same finding was observed on rats where DMPA induces significant increase (*P* <.01) in the body weight in all treated rats (Bakry and Abdullah, 2009). While other study indicated DMPA users poses increased body weight in comparison to their controls, however, changes were not statistically significant (Tneepanichskul et al, 1999; Al-Youzbaki, 2011). This could be due to the difference in race/ethnicity which is associated with weight gain, where black women had a greater mean weight gain compared to white or other women with continued DMPA use (Enk *et al*, 1992; Vickery *et al*, 2014). It can also be due to practicing physical exercise following good counseling of study participants in the previous study.

Weight and BMI of DMPA users related to duration of use: The present study indicated that significant weight gain and increased BMI in DMPA users after they had been using the drug (student's paired t-test; *P* =.02 and *P* =.019, respectively) compared to their respective pretreatment value (Table 4.4). However, results from one-way ANOVA analysis of the mean changes in weight between groups of DMPA users [6-24 month=1.78 (+3.32%), 27-48 Month= 1.38 (+2.58%) and 51-96 Month=1.66 (+3.10%)] didn't show significant effect of duration of use (*P* =.96).

Moreover, one way-ANOVA analysis of the mean changes in BMI [6-24 month=0.76±2.12 (+3.55%), 27- 48 Month= 0 .57±1.78 (+2.67%) and 51-96 month=0.65±2.15 (+3.04%)] also didn't show significant effects of the duration of usage (P = .95).

Similar study conducted by Al-Youzbaki (2011) found that one-way ANOVA analysis of the DMPA users group revealed an increase in the mean BMI among users in relation to the duration of usage, however, the difference was not statistically significant (P =.21). Unlike the present study, a study performed by Le *et al*, (2009), aimed to examine if early weight gain in DMPA users predicts continued excessive weight gain and to identify risk factors of early weight gain in DMPA users. They reported mean weight gain was duration dependant; 0.63 kg and 8.04 kg, 1.48 kg and 10.86 kg, and 2.49 kg and 11.08 kg after 12, 24 and 36 months, at or below 5% group and above 5 % group (P<.001 at all observations), respectively. Similarly, the same finding was reported where weight gain was greater in long term use of DMPA in years 1 through 3 [MD: (2.28 to 2.77), (2.71 to 3.30), and (3.17 to 3.83), respectively] (Lopez *et al*, 2013).

The result from present study is also not agreed with findings on rats from which dose and duration dependant significant increase (P <.01) in body weight in DMPA injected mice compared with the corresponding control.

However, there were no significant differences on the mean changes in weight and BMI between groups of DMPA users, the mean weight gain (+2.58%) and BMI (+2.67%) of women who had been taking DMPA for 27-48 months were relatively low (Table 4.5), compared to those who had been taking the drug for 6-24 months (+3.32% and +3.55%) and 51-96 month (+3.10% and +3.04%). This is possibly because majority 11(52 %) of women under this group (women who had been taking DMPA for 27-48 months) belonged to low income (their monthly income was ≤1,400ETB), which is directly related to the nutritional status and subsequently, body weight of the women. Moreover, the mean weight gain and BMI of women who had been taking DMPA for 6-24 months were relatively high compared to 27-48 and 51-96 months of use, which indicates weight gain occurs mainly in the first two year of use.

Weight increase is a common phenomenon for women initiating use of hormonal contraceptives, especially DMPA (Bakry and Abdullah, 2009; Bonny *et al*, 2014; Vickery *et al*, 2014). However, the existing literature does not provide a clear-cut picture of the mechanism of DMPA-related weight gain. The previous authors were tried to report the reasons why the use DMPA can lead to weight increase. Initially, it was suggested that DMPA increases serum lipids and consequently increase the weight (Fraser and Weisberg, 1989).

Since, DMPA is a steroid contraceptive, weight increase in users of DMPA occurred principally as a result of an increase in fat mass (Dal'Ava *et al*, 2014). Others reported DMPA promotes anabolic pathway as a consequence of its androgenic action (Godsland *et al*, 1990).

The other suggested mechanisms for DMPA-associated weight gain is caused by anti-glucocorticoid activity of MPA (Guthrie *et al*, 1980) or DMPA associated significant increase in fasting blood glucose, pyruvate and insulin level (Fahmy *et al*, 1991). However, a study performed by Amatayakulte *et al,* (1980) does not support this theory. It was suggested that weight increase depends on higher appetite, dietary ingestion and fat deposition. Self-reported increase of appetite after 6 months of DMPA use was investigated by Le *et al*, (2009). This supports Leiman (1972) who reported weight gain among DMPA users was related to their higher appetite and subsequently higher dietary ingestion as a result of modifications of the hypothalamic appetite control center by DMPA. Use of DMPA causes increase in the level of gonadotropin inhibiting hormone (GnIH) releasing cells, projected to appetite regulating cells within the lateral hypothalamic area (Y Qi *et al*, 2009). Intracerebroventricular injection of GnIH, and its related peptides (GnIH-RP-1 and –RP-2) significantly stimulated food intake in chicks (Tachibana *et al*, 2005) and similar data has been obtained in rats (Johnson *et al*, 2008). This suggests a functional role for GnIH in regulation of food intake in which its activity can be increased in women using DMPA. Contrary to the prior studies, findings of Lange *et al*, (2015), aimed to examine the relationship between dietary intake and weight gain among adolescent females initiating DMPA reported that no association between total caloric intake and weight gain on DMPA. They suggested that DMPA associated weight gain cannot be explained by a simple, direct relationship to increased food consumption. Therefore, the role of appetite and dietary intake for DMPA-associated weight gain remains to be clarified.

Use of DMPA and MAP: The result in Table 4.1 illustrated that MAP between DMPA users and control group was not significantly different (*P* =.85). Moreover, changes in MAP between before they starting to use DMPA and after they had been using DMPA was not statistically significant (*P* =.67) (Table 4.4). There were no significant differences in SBP and DBP between DMPA users and controls and between before they starting to use DMPA and after they had been using DMPA.

The result obtained from the present study indicates DMPA use does not have unfavorable effect on blood pressure. These results of present study is in line with a study reported by Al-Youzbaki (2011) who found that DMPA users had increased SBP and DBP compared with the non-users after 6 and 12 months, however, it was not significant. But mean DBP increases significantly (*P*=.04) in DMPA users according to the duration of use of DMPA. In 1999, Taneepanichskul *et al,* conducted a study to assess body weight and blood pressure changes on 50 healthy Thailand women using DMPA for 120 months and 50 IUCD acceptors using an IUCD for 120 months. They found that the blood pressure changes between DMPA and IUCD users were not different. So, they suggested that long-term DMPA use didn't have negative effects on blood pressure. These observations were supportive of the findings of the present study.

Contrary to the present study, a study conducted by Asare *et al*, (2014) reported statistically significant increase (*P* =.02) in DBP among DMPA users (78.57±1.97 mmHg) compared to control group (68.75±1.81 mmHg). However, SBP didn't show statistically significant difference between DMPA users and control group.

6. CONCLUSIONS

The objective of this study was to identify possible changes that occur in lipid metabolism, body weight and blood pressure of women from the use of DMPA in selected health centers found in Addis Ababa, Ethiopia. Thus, after this study, we offer the following conclusions:

The use of DMPA induced changes in lipid metabolism similar to those associated with an increased risk of CHD manifested by high serum TC, LDL-c, TC/HDL-c and LDL-c/HDL-c ratio levels. A marked significant decrease was found in HDL-c level in the DMPA-users. Furthermore, serum TG level of DMPA users was higher compared to controls, however, this difference was not statistically significant. These changes in lipid profiles were independent of the duration of use of DMPA.

DMPA users had significant weight gain and significant increase in BMI compared with their respective pretreatment value, although, these effects appeared to be independent of the duration of use of DMPA.

DMPA users showed altered lipid profile and had significant weight gain starting from 6 months of usage, but it doesn't mean women who had been using DMPA for long term are at increased risk of continued altered lipid metabolism and excessive weight gain.

Women taking DMPA didn't show significant change in MAP compared to controls or to their respective pretreatment value, which indicates DMPA use does not have unfavorable effects on blood pressure.

Furthermore, no significant association was found between any of the variables [change in weight (BMI) and lipid profiles (TC, TG, HDL-C, LDL-c, TC/HDL-c and LDL-c/HDL-c) of DMPA users].

7. RECOMMENDATIONS

Based on the results of the present study, we recommend the following for concerned bodies:

- Routine evaluation of lipid profiles and BMI is advisable before initiating DMPA. Women who had overweight and cardiovascular problem (myocardial infarction, congestive heart failure, increased blood pressure, pulmonary embolism, tachycardia and thromboembolic disorders) should not be recommended for using DMPA as a contraceptive method.

- Personal monitoring is very advisable for each DMPA user, including regular record of body weights, heart rate and other important vital signs.

- Appropriate counseling of life style including weight bearing exercise and diet control as necessary guidelines to prevent cardiovascular risk and excessive weight gain.

- It is therefore recommended that use of DMPA should be discouraged, and if used it should be under close clinical monitoring.

- The present study is cross sectional in design, further longitudinal study (cohort in design) with larger sample size is need to be conducted to evaluate whether the changes in lipid profile, weight gain or increase in BMI indeed are associated with use of DMPA.

8. STRENGTHS AND LIMITATIONS

To start with the strength of the study, it adopted rigid criteria to select the subjects. The other possible strength is the use of anthropometric measurement instead of self-reported weight and height, as well as blood pressure.

The limitation of study includes; the lipid profile of DMPA users were not compared with their respective pretreatment values because it was not found on the client card. Furthermore, the height of DMPA users at the baseline were not documented, so, their current height was considered as height of the base line, however, this was not as such important factor, because girls usually reach their adult height and stop growing by age 14 or 15. Since, participants included in the present study were 18-24 years in which no further growing in height expected.

The other limitation lies in the fact that potential confounders including total daily caloric intake, physical activity and intake of cholesterol rich diets were not considered.

This study is cross-sectional rather than cohort in design with relatively small sample sizes.

This study was conducted only in two districts, which makes generalization to the wider population difficult. This happened due to a shortage of budget to cover more districts.

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10. ANNEXES

Annex-I: Information Sheet for study participants

Research Title: Effects of Depo-medroxyprogesterone Acetate (DMPA) on Lipid profile, Weight and Blood Pressure among women in Tekele Hymanot and Lomeda Health Center, Addis Ababa, Ethiopia

Name of PI: Muluken Fekadie

Advisor: Dr. Daniel Seifu

Dr. Menakath Menon

Dr. Solomon Kumbi

Sponsor: Addis Ababa University and University of Gondar

1. Background

Depo-medroxyprogesterone acetate (DMPA) is a highly effective contraceptive with a very low failure rate. However, the use of DMPA has been implicated in many diseases such as thromboembolism, Myocardiac infarction, Circulatory disorder and Carcinogenicity. Moreover, it's negative effect on liver, heart, diabetes, obesity, hypertension and high serum cholesterol level were well documented and available data on this topic are mostly from developed countries. Currently, there is scarcity of information on the effects of DMPA among Ethiopian women. Indeed, to the best of my knowledge, this is about the first time in our country that such a comprehensive assessment of the biochemical parameters of DMPA users is being carried out.

2. Research Objectives

The overall objective is to investigate the effects of use of DMPA on lipid profile (TC, TG, LDL-c, and HDL-c), body weight and blood pressure of women attending family planning unit in Tekele Hymanot and Lomeda Health Centers.

3. Study Procedure

To achieve the goals of this study, a total of 100 study subjects will be included. Out of this, 50 healthy women who had been using DMPA for at least 6 months, and 50 healthy age-matched controls, will be selected. Women 18-45 years of age on DMPA, and non-users of hormonal contraceptives will be recruited by principal investigator and health professionals. Demographic information, other relevant clinical data and 5 ml blood samples will be collected from each study participant. Study participants will be asked for any of the following history of disease conditions: thromboembolic disorders, known or suspected pregnancy, missed abortion, cardio vascular disease, severe uncontrolled hypertension, severe liver dysfunction and any medication. Only those women who are free of listed disease conditions and drugs will be included in the study, and blood sample from the participants will be taken immediately. Sample processing and laboratory analysis including measurement of lipid profile, will be done in clinical laboratory unit of Black Lion Hospital.

4. Risks and Discomfort

Your participation in the study, will not have any adverse effect, and has minimum invasive procedures. You may have minor discomfort and pain during blood drawing and there may also be mild redness, or swelling on the site from where the blood was taken. But, this will be minimized, as the procedure will be carried out by experienced health professionals in the health center with a standard aseptic conditions.

5. Benefits

You will not receive direct benefit by participating in this research. However, you will be assisted by public health professionals to gain an improved understanding on the adverse effects of DMPA. At the same time you will get to see some biochemical parameters and clinical assessment of your health condition for free.

6. Participant's Role

If you volunteer to participate in this study, you will be asked different socio- demographic and other health related questions. In addition, you will be requested to give 5 ml of blood for lipid profile test and you will be asked to measure your blood pressure, height and weight.

7. Participant's Right

You have the right to refuse participation or quit participation at any point of the interview process if you are not comfortable. Refusing to take part in the study will not result in any penalty or loss of benefits or right to which you are otherwise entitled.

8. Confidentiality

The information you provide is confidential and will only be used for the objective mentioned above. Information about your health collected from the study, will be stored by code numbers. No personal identification will be mentioned in the results of the study which may be published for scientific purposes.

9. Communication

In case you have any questions, unclear ideas and doubt about the study please feel free to contact the following individuals through their addresses:

Name	***Email***	***Mobile***
o Muluken Fekadie	mulukenfekadie@gmail.com	+251918037027
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o Dr Menakath Menon	menakathmenon@gmail.com	+251923244176
o Dr Solomon Kumbi	solkumbi@gmail.com	+251911223838

Thank you very much!!

Annex-II: በጥናቱ ተሳታፊዎች የሚሰጥ መረጃ (በአማርኛ የተዘጋጀ)

የጥናቱ ርዕስ፡ በተከለሃይማኖት እና ሎሜዳ ጤና ጣቢያ በመርፌ የሚሰጥ የወሊድ መቆጣጠሪያ (ዲፖ) በሚጠቀሙ ሴቶች የደም ስብ ልኬት፣ የሰዉነት ክብደት እና የደም ግፊት ላይ የሚያመጣዉ ተጽእኖ ማትናት

ዋና ተመራማሪ፡ ሙሉቀን ፈቃዴ (ዲገሪ ነረስ እና ዲገሪ ኬሚስትሪ)

አማካሪ: ዶ/ር ዳንኤል ሰይፉ

ዶ/ር መናካዝ መነን

ዶ/ር ሶሎሞን ቁምቢ

የምርምሩ ወጭ የሚሸፈነዉ፡ በአዲስ አባባ ዩኒቨርስቲ እና በጎንደር ዩኒቨርስቲ

1. የጥናቱ ጽንሰ ሀሳብ

ዲፖ ቀላልና እርግዝናን የመከላከል አቅሙ ከፍተኛ የሆነ የወሊድ መቆጣጠሪያ ነው፡፡ምንም እንኳን በብዙ ሴቶች ዘንድ ተወዳጅ የወሊድ መቆጣጠሪያ ቢሆንም ትንሽ የማይባሉ የጎንዮሽ ጉዳቶች እንደሚያስከትል ይታወቃል፡፡ከእነዚህም መካከል የልብ እና ተያያዥ ችግሮች፣ የማይፈልግ የስብ በደም ቧንቧ ላይ መከማቸት ፣ የደም ግፊት እና የመሳሰሉት ይገኙበታል፡፡ ከላይ ስለተጠቀሱት ችግሮች በአደጉት ሀገሮች ላይ ሰፊ ጥናት ቢካሄድም መድሐኒቱ በታዳጊ ሀገር ሴቶች ኢትዮጵያን ጨምሮ ስለሚያስከትለዉ ችግር ብዙ ጥናት አልተካሄደም፡፡ ስለሆነም በዚህ ጥናት መድሀኒቱን በሚወስዱ ኢትዮጵያዉያን ሴቶች ላይ እያስከተለ ያለዉን የልብ እና ተያያዥ ችግሮች፣ የማይፈልግ የስብ በደም ቧንቧ ላይ መከማቸት ፣የሰዉነት ክብደት እና የደም ግፊት ሁኔታን ለመዳሰስ ይሞከራል፡፡

2. ጥናቱ ዓላማ

የጥናቱ ዋና ዓላማ በደም ዉስጥ ያለን ስብን ፣ የሰዉነት ክብደት እና የደም ግፊት መጠንን በተከለሃይማኖት እና ሎሜዳ ጤና ጣቢያ በመርፌ የሚሰጥ የወሊድ መቆጣጠሪያን (ዲፖ) በሚወስዱ እና በማይወስዱ ሴቶች መካከል ያለዉን ልዩነት ማነጻጽር ነው፡፡

3. የጥናቱ ሂደት

ይህን ጥናት ለማካሄድ ከ18 – 45 ዓመት የሆኑ 50 ዲፓ ተጠቃሚዎች እና 50 ምንም አይነት የሆርሞን የወሊድ መቆጣጠሪያ የማይጠቀሙ በድምሩ 100 ጤነኛና ምን ዓይነት መድሃኒት የማይወስዱ ሴቶች በጥናቱ ውስጥ ይካተታሉ፡፡ ከጤና ጋር ተዛማጅነት ያላቸው የግል መረጃዎች ከተሰበሰቡ በኃላ ከተሳታፊዎች 5 ሲሲ (1 የሻይ ማንኪያ) የደም ናሙና ተወስዶ ምርምራ በላቦራቶሪ ይካሄድበታል፡፡

4. ከጥናቱ ጋር የተያያዘ ጉዳት/ አለመመቸት

የደም ናሙናውን የሚወስደው ባለሙያ ብቁ የሆነና ንፁህ የሆኑ የህክምና መሳሪያችን ስለሚጠ ቀም እርስዎ በዚህ ጥናት ውስጥ በመሳተፈዎ ለከፋ ጉዳት የሚጋለጡበት ሁኔታ አይኖርም፡፡ ደም በሚወሰድበት ወቅት አነስተኛ ህመም ሊሰማወት ይችላል፡፡ እንዲሁም የመቅላት፣ እና የማበጥ ሁኔታ ደም ከተወሰደበት ቦታ ላይ ሊታይ ይችላል፡፡ ነገር ግን እነዚህ ሁኔታወች የከፋ ጉዳት የሚያስከትሉ አይደሉም፡፡

5. በጥናቱ የመሳተፍ ጥቅም

እርስዎ በዚህ ጥናት ላይ በመሳተፍዎ በደም ዉስጥ ያለዉን አላስፈላጊ ስብን ፣ የአጥንት ጥንካሬን ፣ የደም ግፊት መጠን ምርመራን በነጻ ያገኛሉ፡፡ እንዲሁም ሥለ አጠቃላይ ጤና አጠባበቅና ተያያዥ ጉዳዮች እና ስለ ዲፓ የጎንዮሽ ጉዳት እና መደረግ ስለሚገባዉ ጥንቃቄ የባለሙያ የምክር አገልግሎት ያገኛሉ፡፡

6. የጥናቱ ተሳታፊ ድርሻ

በዚህ ጥናት ለመሳተፍ ፍቃደኛ ከሆኑ ከጤናዎ ሁኔታ ጋር የተያያዙና ሌሎች የ ግ ል መረ ጃ ዎ ች ን እ ን ዲ ሰ ጡ ይ ጠ የ ቃ ሉ ፡ ፡ በ መ ቀ ጠ ል ም የ ሰ ዉ ነ ት ክ ብ ደ ተ ወ ን እ ና የ ደ ም ግ ፊ ተ ወ ን እ ን ዲ ለ ኩ ና 5 ሲ ሲ መ ጠ ን ያ ለ ው የ ደ ም ና ሙ ና ለ ተ ጠ ቀ ሰ ዉ ዓ ላ ማ እ ን ድ ን ወ ስ ድ ይ ጠ የ ቃ ሉ ፡ ፡

7. የ ጥ ና ቱ ተ ሳ ታ ፊ ዉ መ ብ ት

በ ጥ ና ቱ ላ ይ መ ሳ ተ ፍ በ እ ር ስ ዎ መ ሉ ፈ ቃ ደ ኝ ነ ት ላ ይ ብ ቻ የ ተ መ ሰ ረ ተ ነ ው ፡ ፡ ስ ለ ሆ ነ ም በ ጥ ና ቱ ለ መ ሳ ተ ፍ ባ ይ ስ ማ ሙ ም ን ም አ ይ ነ ት ቅ ጣ ት የ ማ ያ ስ ከ ት ል ሲ ሆ ን ማ ን ኛ ዉ ም እ ር ስ ዎ ሊ ያ ገ ኙ የ ሚ ገ ባ ዉ ን ህ ክ ም ና ና ተ ያ ያ ዥ መ ብ ት የ ማ ያ ሳ ጣ መ ሆ ኑ ን እ ና ረ ጋ ግ ጣ ለ ን ፡ ፡

8. የ ጥ ና ቱ መ ረ ጃ ዎ ች ም ስ ጢ ራ ዊ ነ ት

ር ስ ዉ ን በ ተ መ ለ ከ ተ የ ም ን ና ገ ኘ ው መ ረ ጃ በ ጥ ና ቱ ወ ቅ ት ም ሆ ነ ከ ዚ ያ በ ኃ ላ ባ ሉ ት ጊ ዚ ያ ት እ ን ዲ ሁ ም ከ ጥ ና ቱ የ ተ ገ ኘ ው መ ረ ጃ ሚ ስ ጥ ራ ዊ ነ ት የ ሚ ጠ በ ቅ ሲ ሆ ን መ ረ ጃ ዎ ቹ ም የ ሚ ያ ዙ ት በ ስ ም ሳ ይ ሆ ን በ ል ዩ ኮ ድ ነ ው ፡ ፡ ይ ኸ ው መ ረ ጃ በ ጥ ን ቃ ቄ የ ሚ ያ ዝ ና የ ተ ፈ ቀ ደ ለ ት ተ መ ራ ማ ሪ እ ና ለ ህ ክ ም ና ባ ለ መ ያ ዉ ብ ቻ ይ ህ ም እ ጅ ግ አ ስ ፈ ላ ጊ በ ሆ ነ ጊ ዚ ብ ቻ ካ ል ሆ ነ በ ስ ተ ቀ ር ለ ሌ ላ ለ ማ ን ም ሰ ው አ ይ ሰ ጥ ም ፡ ፡ ማ ን ኛ ዉ ም ከ ር ስ ዎ ጋ ር የ ተ ያ ያ ዘ ዉ ጤ ት በ ል ዩ ኮ ድ ብ ቻ የ ሚ ያ ዝ ሲ ሆ ን ዉ ጤ ቱ ም ለ ሳ ይ ን ሰ ዊ ዓ ላ ማ ብ ቻ ስ ም በ ማ ይ ገ ል ፅ ሁ ኔ ታ እ ን ዲ ታ ተ ም ይ ደ ረ ጋ ል ፡ ፡

9. ስለጥናቱ መረጃ ማግኘት ቢፈልጉ፡

ይህ ጥ ና ት በ አ ዲ ስ አ በ ባ ዩ ኒ ቨ ር ስ ቲ ህ ክ ም ና ጤ ና ሳ ይ ን ስ ኮ ሌ ጅ የ ስ ነ -ም ግ ባ ር ቅ ኝ ት ኮ ሚ ቴ ተ ገ ም ግ ሞ ፀ ድ ቋ ል ፡ ፡ ጥ ና ቱ ን በ ተ መ ለ ከ ተ ግ ል ጽ ያ ል ሆ ነ ማ ን ኛ ዉ ን ም ጥ የ ቄ ካ ለ ዎ ት ነ ፃ ሆ ነ ው ከ ዚ ህ በ ታ ች ባ ሉ ት አ ድ ራ ሻ ዎ ች መ ጠ የ ቅ ይ ች ላ ሉ ፡ ፡

ሰ ም	ኢ ሜ ል	ሰ ል ክ
○ መ ሉ ቀ ን ፈ ቃ ዴ ፡	mulukenfekadie@gmail.com	+251918037027
○ ዶ /ር ዳ ን ኤ ል ሰ ይ ፉ	daniel.seifu@aau.Edu.Ef	+251911232754
○ ዶ /ር መ ና ካ ዝ መ ነ ን ፡ ኢ ሜ ል	menakathmenon@gmail.com	+251923244176
○ ዶ /ር ሶ ሎ ሞ ን ቁ ም ቢ	solkumbi@gmail.com	+251911223838

በ ጣ ም እ ና መ ሰ ግ ና ለ ን !!

Annex-III: Consent form for study participants (English Version)

Study title: Effects of Depo-medroxyprogesterone Acetate (DMPA) on Lipid profile, Weight and Blood Pressure among women in Tekele Hymanot and Lomeda Health Center, Addis Ababa, Ethiopia

Principal Investigator: Muluken Fekadie (BSc in Chemistry, BSc in Nursing)

I have read the information sheet attached as well as the principal investigator's statement, regarding the project attached with this consent form. I had the opportunity to ask questions and am satisfied with the answers given by the investigators. I am also informed that my refusal or withdrawal to participate in the study, will not affect my treatment or other services from the health institute.

With full understanding of the importance of the study, I agreed voluntarily to give information about me, and allow the investigators to take the required blood sample (5 ml or one tea spoon). Therefore, I hereby give my consent to participate in this study.

_____	_____	____/____/___
Code of Participant	Signature	Day/month/year

Witness for illiterate participants:

_____	_____	____/____/___
Name of Witness	Signature of Witness	Day/month/year
_____	_____	____/____/___
Name of the researcher	Signature of researcher/physician	Day/month/year

Annex-IV: ለ ጥ ና ቱ ተ ሳ ታ ፊ ዎ ች ሴ ቶ ች የ ሚ ሰ ጥ የ ፈ ቃ ደ ኝ ነ ት ማ ረ ጋ ገ ጫ ቅ ፅ (በ አ ማ ር ኛ የ ተ ዘ ጋ ጀ)

የ ጥ ና ቱ ር ዕ ስ ፡ በ ተ ከ ለ ሃ ይ ማ ኖ ት እ ና ሎ ሜ ዳ ጤ ና ጣ ቢ ያ በ መ ር ፌ የ ሚ ሰ ጥ የ ወ ሊ ድ መ ቆ ጣ ጠ ሪ ያ (ዲ ፖ) በ ሚ ጠ ቀ ሙ ሴ ቶ ች የ ደ ም ስ ብ ል ኬ ት ፣ የ ሰ ዉ ነ ት ክ ብ ደ ት እ ና የ ደ ም ግ ፊ ት ላ ይ የ ሚ ያ መ ጣ ዉ ተ ጽ እ ኖ ማ ት ና ት

ዋ ና ተ መ ራ ማ ሪ ፡ መ ሉ ቀ ን ፈ ቃ ዴ (ዲ ገ ሪ ነ ረ ስ እ ና ዲ ገ ሪ ኬ ሚ ስ ት ሪ)

ይ ህ ጥ ና ት በ አ ዲ ስ አ በ ባ ዩ ኒ ቨ ር ስ ቲ አ ስ ተ ባ ባ ሪ ነ ት ፤ በ መ ር ፌ የ ሚ ሰ ጥ የ ወ ሊ ድ መ ቆ ጣ ጠ ሪ ያ (ዲ ፖ) በ ሚ ወ ስ ዱ ሴ ቶ ች በ ደ ም ዉ ስ ጥ ባ ለ ስ ብ ፣ በ ሰ ዉ ነ ት ክ ብ ደ ት እ ና በ ደ ም ግ ፊ ት መ ጠ ን ላ ይ የ ሚ ያ መ ጣ ዉ ን ተ ጽ እ ኖ ም ን ም አ ይ ነ ት የ ሆ ር ሞ ን የ ወ ሊ ድ መ ቆ ጣ ጠ ሪ ያ ከ ማ ይ ወ ሰ ዱ ሴ ቶ ች ጋ ር ማ ወ ዳ ደ ር ሲ ሆ ን እ ኔ በ ዚ ህ ጥ ና ት ዉ ስ ጥ እ ን ድ ሳ ተ ፍ ፍ ቃ ደ ኛ ነ ቴ ን ተ ጠ ይ ቂ ያ ለ ው ፡ ፡ በ ጥ ና ቱ ወ ቅ ት ም የ ሰ ዉ ነ ት ክ ብ ደ ቴ እ ና የ ደ ም ግ ፊ ቴ እ ን ደ ሚ ለ ኩ ና 5 ሲ ሲ የ ደ ም ና መ ና እ ን ደ ም ሰ ጥ ተ ነ ግ ሮ ኛ ል ፡ ፡ የ ተ ወ ሰ ደ ው ና መ ና ለ ጥ ና ቱ አ ላ ማ ብ ቻ እ ን ደ ሚ ዉ ል ና ም ር መ ራ እ ን ደ ሚ ካ ሄ ድ በ ት ም ተ ነ ግ ሮ ኛ ል ፡ ፡ በ ጥ ና ቱ ወ ቅ ት ም ደ ም ሲ ወ ሰ ድ ከ መ ጠ ነ ኛ ህ መ ም በ ስ ተ ቀ ር ም ን ም አ ይ ነ ት የ ከ ፋ ች ግ ር እ ን ደ ሌ ለ ዉ ተ ገ ነ ዘ ቢ ያ ለ ሁ ፡ ፡ እ ኔ የ ም ሰ ጠ ው እ ን ዲ ሁ ም ከ ጥ ና ቱ የ ሚ ገ ኘ ው መ ረ ጃ ሚ ስ ጥ ራ ዊ ነ ት እ ን ደ ሚ ጠ በ ቅ ፤ የ ጥ ና ቱ ዉ ጤ ት ም ለ እ ኔ ብ ቻ እ ን ደ ሚ ነ ገ ረ ኝ ተ ነ ግ ሮ ኛ ል ፡ ፡ የ ዚ ህ ጥ ና ት ዉ ጤ ት ም ለ ሚ መ ለ ከ ተ ው አ ካ ል ብ ቻ እ ን ደ ሚ ገ ለ ፅ አ ው ቄ አ ለ ሁ ፤ በ ጥ ና ቱ ለ መ ሳ ተ ፍ የ ሚ ሰ ጥ ም ን ም አ ይ ነ ት ማ ባ በ ያ አ ይ ኖ ር ም ተ ብ ያ ለ ሁ ፡ ፡ ስ ለ ጥ ና ቱ የ ተ ገ ል ፀ ው ን መ ረ ጃ አ ን ብ ቤ አ ለ ው/ተ ነ ቦ ል ኛ ል ፡ ፡ ጥ ያ ቄ ዎ ች ን እ ን ድ ጠ ይ ቅ እ ድ ል ተ ሰ ጥ ቶ ኛ ል ፡ ፡ የ ጠ የ ቅ ሁ ት ም ጥ ያ ቄ በ በ ቂ ሁ ኔ ታ ተ መ ል ሶ ል ኛ ል ፡ ፡ በ ጥ ና ቱ አ ለ መ ሳ ተ ፍ ከ ዚ ህ ተ ቋ ም በ ማ ገ ኘ ው አ ገ ል ግ ሎ ት ላ ይ ም ን ም አ ይ ነ ት ተ ፅ እ ኖ አ ይ ኖ ረ ው ም ተ ብ ያ ለ ሁ ፡ ፡ በ መ ሆ ኑ ም የ ጥ ና ቱ ዓ ላ ማ ና ጥ ቅ ም በ ሚ ገ ባ ስ ለ ተ ገ ነ ዘ ብ ኩ ፤ ስ ለ እ ኔ ማ ን ኛ ው ን ም መ ረ ጃ ለ መ ስ ጠ ት እ ና 5 ሲ ሲ (አ ን ድ የ ሻ ይ ማ ን ኪ ያ) የ ደ ም ና መ ና እ ን ዲ ወ ሰ ድ በ መ ሉ ፍ ቃ ደ ኝ ነ ት መ ስ ማ ሙ ቴ ን በ ፊ ር ማ ዬ አ ረ ጋ ግ ጣ ለ ሁ ፡ ፡

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የ ተ ሳ ታ ፊ የ ሚ ስ ጥ ር ቁ ጥ ር የ ተ ሳ ታ ፊ ፊ ር ማ ቀ ን /ወ ር /ዓ .ም

ማ ን በ ብ ና መ ፃ ፍ ለ ማ ይ ች ሉ የ ጥ ና ት ተ ሳ ታ ፊ ዎ ች ፤ ም ስ ክ ር

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የ ም ስ ክ ር ስ ም የ ም ስ ክ ር ፊ ር ማ ቀ ን /ወ ር /ዓ .ም

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የ ተ መ ራ ማ ሪ ው የ ተ መ ራ ማ ሪ ው ፊ ር ማ ቀ ን /ወ ር /ዓ .ም

Annex V (English version)

Questionaire

To be answered by both DMPA users and control group individuals:

1. Code_____Age_____Sex_____Ethnicity_____Martial status_____
2. Education: A) Illiterate B) Primary C) Secondary and above
3. Occupation: A) Private B) Government C) Others_____
4. Income: per day _____ per month_____
5. Height (m) _____
6. Weight (kg) *(for both)* _____before use of DMPA (*only for DMPA users*)_____
7. Blood pressure *(for both)* _____ before use of DMPA (*only for DMPA users*)_____
8. Do you practice regular exercise? Yes_____ No_____
9. Suspected pregnancy?____ yes, _____ no breast-feeding____yes,_____
10. Do you have any of the following health problems?
    A) Cardiovascular disease C) Liver injury
    B) Kidney failure D) Others_____
11. If your answer is yes for question number 10, would you list the drug(s) you are taking for managing the disease?
    A) _____ C)_____
    B)_____ D)_____
12. Do you use other substances? A) Alcohol B) Cigarette C) Khat D) Not taking any of the items mentioned above
13. What type of contraceptive method you are using?
    A) DMPA
    B) Other hormonal contraceptives
    C) None users or non-hormonal contraceptive users or hormonal contraceptive at least before 12 months

Question number 14 and 15 will be answered by only DMPA users.

14. If you are a DMPA user, for how long you were using?_____months.
15. How old are you when you started DMPA?_____

Annex VI (Amharic version)

መጠይቅ

በዲፖ ተጠቃሚ አና ተጠቃሚ ባልሆኑ የሚመለስ

1. የሚስጥር ቁጥር _____ ዕድሜ _____ ብሄር _____ የጋብቻ ሁኔታ_____

2. የትምህርት ደረጃ ሀ) ያልተማረ ለ) 1ኛ ደረጃ መ) 2ኛ ደረጃና ከዚያ በላይ

3. ስራ ሀ) የግል ለ) የመንግስት መ) ሌላ_____

4. የገቢ ሁኔታ የቀን_____ ወርሃዊ _____

5. ቁመት (በሜ) _____

6. ክብደት (በኪ.ግ) *(ለሁለቱም)* _____ ዲፖ ከመጠቀምወ በፊት *(ለዲፖ ተጠቃሚወች)*_____

7. የደም ግፊት ልኬት(mmHG) *(ለሁለቱም)*_____ዲፖ ከመጠቀምወ በፊት *(ለዲፖ ተጠቃሚወች)*_____

8. መደበኛ የአካል እንቅስቃሴ ያደርጋሉ; ሀ) አወ ለ) አላደርግም

9. ነፍሰጡር ነወት? _____አወ_____ አይደለም ያጠባሉ? _____አወ_____ አይደለም

10. ከሚከተሉት በየትኛዉ ሕመም ተይዘዉ ያዉቃሉ?

ሀ) ከ ልብ ጋር ተያያዥነት ያለቸዉ ሕመሞች ለ) የጉበት ሕመም

መ) የኩላሊት ሕመም ሰ) ሌሎች_____

11. ለ10ኛዉ መጠይቅ መልሰዎ አወ ከሆነ ፣ እባክዎ የሚወስዷቸዉን መድሀኒቶች ቢዘረዝሩልን

ሀ)_____ ለ)_____
መ)_____ ሰ)_____

12. ከሚከተሉት የትኛዉን ይጠቀማሉ ?

ሀ) አልኮል ለ) የሲጋራ
መ) ጫት ሰ) ምንም አልጠቀምም

13. ምን ዓይነት የወሊድ መከላከያ ዘዴ ይጠቀማሉ?

ሀ. ዲፖ
ለ. ሌሎች (ሆረሞናል)
መ. ምን ዓይነት የወሊድ መከላከያ ዘዴ አልጠቀምም ወይም ሆረሞናል ያልሆኑ ወይም ሆረሞናል ቢያንስ ከ12 ወር በፊት

ከጥያቄ ተራ ቁጥር 14-15 በዲፖ ተጠቃወች ብቻ የመለሱ ናቸው

14. የዲፖ ተጠቃሚ ከሆኑ ለምን ያክል ጊዜ ተጠቀሙት?_____ በወራት
15. ዲፖ መጠቀም ሲጀምሩ ስንት አመተት ነበር? _____