Original Article

Changes after using Contraceptives

Assessment of Circulating Biochemical, Heamatological and Oxidative Stress Markers in Females Using Contraceptives from Punjab, Pakistan

1. Waheed Jamil Ch. 2. Muhammad Binyameen Chishti 3. Madiha Ashraf 4. Arif Malik 5. Mahmood Husain Qazi

1. Asstt. Prof. of Physiology, AIMC, Lahore 2. Assoc. Prof. of Physiology, AIMC, Lahore 3. Asstt. Prof. Physiology, AIMC, Lahore 4. Assoc. Prof. of Biochemistry, The University of Lahore 5. Director, Center for Research and Molecular Medicine, The University of Lahore

ABSTRACT

Objective: To Assess the circulating Biochemical, Heamatological and Oxidative Stress Markers in Females using Contraceptives from Punjab, Pakistan.

Study Design: Case Control Study

Place and Duration of Study: This study was carried out at the Gynae Units of Jinnah Hospital Lahore from January, 2011 to December 2011.

Materials and Methods: Two injectable contraceptives (Depo-Medroxyprogesterone & NorethisteroneEnantate) and Oral contraceptive pills (COCs) were administered in females of reproductive age, the oxidative and nitrosative stress biomarkers (Malondialdehyde, Superoxide Dismutase, Catalase, Nitic oxide and Glutathione) were analyzed in these subjects.

Results: DMPA, NET-EN & COCs treatment could induce oxidative stress with a significant change in lipid profile and other biochemical markers thus affecting the normal biological system. These effects after prolonged use can generate pathological events leading to disease pattern.

Conclusion: These biomarkers can become diagnostic tools to evaluate the health of a woman using these methods as preventive measure in future

Key Words: Contraceptives, Estrogen, Progestin, Stress Biomarkers, Catalase, Glutathione, MDA, SOD

INTRODUCTION

Hormonal contraceptive methods for prevention of unplanned pregnancy have been very popular among females since the beginning of modern era. In developing countries like Pakistan, governments and organizations are running campaigns to increase the use of these methods in order to space pregnancies [1](Emokpaeet al., 2010). These hormonal preparations can be taken orally (taken by mouth), injected beneath the skin, implanted in the body tissues, absorbed from a patch on the skin, or placed inside the vagina.Oral contraceptive pills are mainly of two types, the combined pills and the progestin only pills. Combined pill is a combination of synthetic estrogens and progestin. Mestranol, a synthetic estrogen is the inactive form which is converted to ethinyl estradiol (E2) to cause the action in the body.Effect of injectable contraceptive lasts for two or three months after a single dose.Even most effective and well tolerated contraception is not 100% effective; chances of pregnancy are still 3%.DMPA acts by inhibition of pituitary gonadotropin secretions which results in loss of ovulation, amenorrhea and decline in estrogen production leading to prevention of pregnancy [2](Mia et al, 2005).Level of steroids measured in the blood reflects the difference in formulations of these injectable preparations. Blood levels of NET-EN increase rapidly attaining peak levels within 5 days. In contrast to this, DMPA gains peak levels in ten days [3](Draper et al., 2007).

Ovary is the major synthesizer of estrogen using cholesterol as raw material under the influence of pituitary hormonal secretion. They are also produced by the aromatization of androgens in fat cells, skin, bone, and other tissues. There is production of “good estrogens” and their function is to act as antioxidants exerting their effect as eliminators of damaged or cancerous cells all over the body. “Bad estrogen” is the result of inefficient estrogen metabolism.They act negatively to cause oxidation, damage to DNA, and play a role in promotion of cancer. Body produces many antioxidants which are helpful in detoxifying cancer-causing estrogens, example is glutathione. For excretion of many toxins glutathione levels are important [4](Dalessandri et al., 2004).Endogenous estrogens and exogenous other hormones can act as potential modulators in oxidative stress in otherwise healthy female, it is necessary to clarify their roles in oxidative stress.

The main action of progesterone is to strengthen as well as check the actions of estrogen. Estrogen gives the

message while progesterone controls and modifies that message. Progestins are synthetic progesterone. Chemically similar to progesterone but their action is different.Their contraceptive action is brought about by decreasing gonadotropin releasing hormone [5](Lobo and Stanczyk, 1994)causing thick cervical mucus and renders the endometrium unresponsive to implantation [6](Loose-Mitchell and Stancel, 2001). Progestin only contraceptives are the method of choice in women during lactation and the females in which estrogen is contraindicated [7](Affandi, 1998).

Oxidative stress is referred to imbalance between antioxidants and reactive oxygen species. Reactive oxygen species are highly reactive and unstable due to impaired electrons in their outer shell. In physiological concentrations they are beneficial while their excess can lead to damage to structures of cell. Free radicals are neutralized by antioxidants [8](Palmieri and Sblendorio, 2007). These antioxidants are of two types: enzymatic and non-enzymatic. Enzymatic antioxidants are produced in the body and are proteins i.e. enzymes. Non-enzymatic antioxidants are supplied to the body by dietary intake as vitamins and minerals [9](Agarwal et al., 2005). Free radicals are continuously produced in low concentrations and perform certain important tasks such as regulation of apoptosis, modulation of gene expression related to immune response as well as activating transcriptional factors [10](Pincemail et al., 2007).Estrogens have antioxidant property and this property is exhibited even at low plasma concentrations in vascular systems and lipid metabolism (Palmieri and Sblendorio, 2007).Evidence suggests that as oxidative stress increases, it leads to endothelial dysfunction and ultimately atherosclerosis [11](Cai and Harrison, 2000). During oral contraceptive use, Ethinyl Estradiol and progestin levels in plasma increase leading to oxidative stress. Several studies conducted to demonstrate the effect of oral contraceptives on erythrocytes for evaluation of antioxidant markers such as glutathione peroxidase (GSH-PX), catalase and superoxide dismutase activities [12](Massafra et al., 1993;[13]Subakir et al., 2000) which showed increase in activity of two of these markers (Massafra et al., 1993; Pincemail et al., 2007). The objective of this study was to assess the circulating biochemical markers, antioxidative capacity and lipid peroxidation in premenopausal women due to hormonal contraceptive therapy.

MATERIALS AND METHODS

Study Design: It was a case control study.

Target Population: Healthy women aged 25-40 years in the periphery of city of district Kasur.

Study settings: This study was carried out on women who were attending Jinnah Hospital Lahore and Basic Health Units (BHU) in Kasur.

Sample size and Specifications: 51 women were chosen for this study. 32 of them were using hormonal contraceptives preparations. 19 healthy women of the same age group were taken as control that had not used any method for the last 9 months. Among these 32 subjects, 22 of them were using injectable. Two different preparations of hormonal injectable were used. 17 of them were usinginjection Depo-medroxy-progesterone Acetate (DMPA) while remaining 5 were on injection Norethisterone Enantate (NET-EN).10 subjects had been using Combined Oral Contraceptives pills for the last 9 cycles.

Sampling: 5ml of blood was collected as a sample for study from each woman. Out of that 3ml was separated in a tube without anticoagulant. This portion was preceded by centrifugation at 3000 rpm for 20 minutes to obtain serum samples. These serum samples were stored at 2-5°C for hematological parameter analysis. The remaining 2ml was added to Ethyline Diamine Tatric Acid coated tube for performing Complete Blood Count.

Inclusion Criteria: Subjects selected were of the average age of 30 years. The range of their age was 21-41 years. They had been using these hormonal contraceptive methods for at least 15 months. As for control group they were not using any of these methods for the last 10-12 months. The mean weight and height were 55kg (range) and1.66m (1.6-1.75). Blood pressure range was less than 140/100 and more than 90/60 mm Hg.

Exclusion Criteria: All those women were excluded who had had hypertension, thyroid disease, breast feeding, obesity, diabetes, vascular disease, epilepsy, any pelvic pathology, myomas and polyps. Subjects were fully explained about study and their written consent was taken.

Following parameters were estimated: I-Stress biomarkers estimatedincluding malondialdehyde (MDA) [14](Ohkawa et al., 1979), superoxide dismutase (SOD)[15](Kakkar et al., 1984), catalase [16](Aebi, 1974), reduced glutathione (GSH) [17](Moron et al., 1979) and Nitric oxide (NO) [18](Moshage et al., 1995).

II- Haematological parameters were analyzed by Hemolytic Seismic Analyzer.

RESULTS

The level of bio-markers in oxidative stress is different when compared to each other in different formulations of hormonal contraceptives during 1st year use.

Malondialdehyde (MDA) levels are important markers of lipid per-oxidation product. Our study has shown MDA levels to be increased in all subjects of hormonal contraceptive users significantly. Highest increase was noted in the group using Injection 1, the mean was 6.840nmol/mLwhen compared with control group which was 1.345 nmol/mL. Group using Pills showed mean 4.604µmol/ml, while those using Injection 2 mean 3.692 nmol/mL. Injectable 1 group has shown 508% increase, Pills group 342% and least increase was

noted as 274% in injectable 2 group. Therefore, the result coincides with the statement that lipid peroxidation product MDA shows significant increase during first year of administration of hormonal contraceptives [19](Faddah et al., 2005).

Superoxide Dismutase (SOD) level of control group, non-users of hormonal contraceptives, was 0.473ng/mLand in injectable 1 group 0.118ng/mL. While the comparative mean levels of Pills group was 0.198µ mol/ml and injectable 2 group was 0.276µ mol/ml. The noted levels indicate that there is an overall decrease in SOD in HC user groups in comparison with control group. The result is in accordance with the statement that long term use of DMPA causes oxidative stress [20](Bakry et al., 2011).

Table No.1: Oxidative profile of women receiving contraceptives

		MDA	SOD	GSH	CAT	NO
Groups	**n**	nmol/mL	ng/mL	µg/dL	µmol/mol of protein	µmol/L
Control	19	1.34 ± 0.31	0.47 ± 0.17	9.82 ± 1.16	4.15 ± 0.82	11.20 ± 1.35
Injection-1	17	6.84 ± 1.49	0.11 ± 0.09	2.52 ± 0.81	1.39 ± 0.55	24.10 ± 2.54
Pills	10	4.60 ± 2.15	0.19 ± 0.11	1.81 ± 0.71	0.75 ± 0.58	17.19 ± 3.86
Injection-2	5	3.69 ± 0.60	0.27 ± 0.07	2.00 ± 0.49	0.93 ± 0.20	18.11 ± 1.81
P-Value		0.005	0.005	0.005	0.005	0.005

MDA: malondialdehyde; SOD: superoxide dismutase; GSH: reduced glutathione; CAT: catalase; NO: nitric oxide; Injection-1:DMPA; Injection-2: NET-EN P Value < 0.005 Significant

Table-2: Haematological profile of women receiving contraceptives

Groups	N	RBC	WBC	MCH	MCHC	PLT	BMI	Hb
Control	19	4.44 ± 0.37	8.17 ± 1.68	25.06 ± 2.22	31.97 ± 1.25	2.79 ± 67.4	21.68 ± 1.9	14.1 + 1.00
Injection-1	17	4.58 ± 0.44	8.42 ± 1.51	24.68 ± 1.74	30.12 ± 3.55	2.93 ± 44.1	21.8 ± 3.11	9.2 ± 0.81
Pills	10	4.52 ± 0.31	7.86 ± 1.70	25.13 ± 1.53	31.20 ± 2.40	2.96 ± 49.6	21.3 ± 3.36	10.8 ± 1.33
Injection-2	5	4.59 + 0.63	7.62 ± 1.50	24.74 ± 0.88	32.44 ± 1.12	2.88 ± 37.4	20.74 ± 1.08	12.4 ± 0.92
P-Value		0.759	0.719	0.905	0.113	0.850	0.571	0.000

RBC: red blood cells; WBC: white blood cells; MCH: mean corpuscle haemoglobin; MCHC: mean corpuscle haemoglobin concentration; PLT: platelets; BMI: body mass index; Hb: haemoglobin

The mean levels of reduced glutathione (GSH) of control group were 9.825µg/dL. Comparative study of HC users showed significant decreased levels during first year of use. Mean level of injectable 1 group is 2.52µg/dLwhich is 25% decrease from control group levels. Second group-Pill shows mean 1.81µ mol/ml which is 18% decrease when compared to control group. Third group-Injectable 2 has revealed mean 2.00µg/dLwhich is 20% less in comparison with non HC user control group. This decrease was statistically significant when compared to control group. Reduced glutathione, an important antioxidant shows a decrease during 1st year use is in relevance to the work of Faddah et al., 2005.

Catalase antioxidant enzyme activity has also shown significant decrease in comparison with control group. The control group revealed mean 4.152µmol/mol of proteinwhile Injectable 1 group has mean 1.397 µmol/mol of protein. Pills group has shown .07500µmol/mol of proteinand Injectable 2 group was mean 0.932µmol/mol of protein. This overall decrease is 33%, 18% and 22% respectively when compared to control non HC user group. These observations were also agreeable with the statement of Faddah et al.,2005.

Nitric Oxide (NO) levels have revealed significant increase in HC users as compared to HC non-users control group. Control group has mean 11.22 µmol/Land injectable 1 group has mean 24.14µmol/Lwhich is 215% more than control group. Pills group has mean 17.19µmol/Lwhile Injectable 2 group has mean 18.11µmol/L. These results show significant overall increase during first year use which is in contrast to the previous data stating there is no change in NO level (Faddah et al., 2005).

Study of hematological parameters like RBC, WBC, MCH, MCHC, PLT and BMI have shown no change in all HC users.WBC and BMI results are not in agreement with Pantoja et al., 2010, who stated a significant increase in both WBC and BMI levels with DMPA use during 1st year of administration.

Haemoglobin levels have shown significant decrease in all the HC users. Control group showed the normal value to be 14 gm/dl in comparison with injectable 1 group which showed maximum decreased level of 9.2 gm/dl among the 3 HC-user groups. Other 2 groups

had the values of 10 gm/dl in Pills group and 12 gm/dl in Injectable 2 group.

DISCUSSION

Hormonal Contraceptives whether progesterone only injections or in combination as Pills has gained popularity all over the world. Whether these are long term reversible DMPA and NET-EN injections or combined oral contraceptive pills, their efficacy has been under observation year after year. The purpose of our study was to explore the effects of different hormonal contraceptives on some biochemical markers related to oxidative stress among users.

Our study confirmed certain oxidative stress markers to be raised and others to be decreased as compared to normal control group. Some haematological parameters were also checked and most of them were found unchanged. Previous studies data has been considered and is the basis of this study.

Oxygen metabolism generates molecules which are highly reactive, unstable as well as short lived. These are known as free radicals. Free Radicals, if not balanced with anti-oxidants, lead to cell injury and ultimately cell death. To prevent this damage, nature has provided balance between production and elimination of these free radicals endogenous defence system which, if failed, promotes oxidative stress. Although when existing in low concentrations, these free radical reactions are beneficial to the body.

Measurement of ROS as well as antioxidants in cells/tissues or body fluids can lead us to information about the health of the subject. Stable metabolites are formed as a result of these molecular reactions which are directly or indirectly measured. The quantitative measurements of oxidative damaging end products are a proof of ROS existence in the cell.

Natural and synthetic oestrogens both act as antioxidants attributed to their phenolic group. Thus oestrogens along with their metabolites show pro-oxidants or antioxidants properties which are dependent upon how many metal ions are available or what is the dose or formulation in which they are used. Decrease in antioxidant defences have been reported by oral contraceptive use (Palmieri and Sblendorio, 2007). Increased plasma lipid peroxidation levels are one of the reasons of oxidative stress. As reactive oxygen species are too reactive, and short lived, direct measurements are difficult in body fluids. For this reason their metabolic or end products are usually measured. In cell damage MDA levels are usually the most important clue to confirm the free radical involvement.

Elevation of lipid per-oxidation products (MDA level) during first year of administration of hormonal contraceptive, are in agreement with [21]Kose et al.,1993. It stated that use of DMPA shifted the plasma redox towards the oxidative side, with elevated lipid peroxidation and decrease in antioxidant levels.

Oxidative stress was indicated by the results which showed depletion of GSH, CAT, SOD levels. This is in accordance with Massafra et al., 1993.All the enzymes which catalyse the breakdown as well as destruction of free radical are decreased leading to imbalance and resultant oxidative stress.To clear intermediate toxic products of the cell GSH dependent enzymes like glutathione peroxides and glutathione transferase are important. As GSH is an important factor in the metabolism of free radicals as well as numerous intermediate metabolites. The depletion of GSH is an indicator of oxidative stress.

Along with elevated MDA, NO levels have also been increased. Vascular endothelial cells synthesize NO from L-arginine. It is a chemical messenger and act as a potent vasodilator. It also act as potent free radical scavenger [22](Choudhariet al., 2013).When the levels of NO increase pathologically it plays a different role. It becomes a free radical which is highly reactive and causes inflammation leading to injury of vascular cells (Choudhariet al., 2013). So our results show increase in NO levels which is indicative of oxidative stress.

There was no effect on blood parameters, except haemoglobin levels which were significantly decreased. This result was in contrast to the observations of Anonymous 1998, which showed no change in haemoglobin levels in OC users. This can be correlated with the fact that different concentrations of OC have different effects on blood parameters. It also depends on the regularity and occasional use.

CONCLUSION

These biomarkers can become diagnostic tools to evaluate the health of a woman using these methods as preventive measure in future

REFERENCES

1. Emokpae MA, Uadia PO, Osadolor HB. Effect of duration of use of hormonal contraceptive pills on total lipid and lipoproteins in Nigerian women. Int J on Pharma and Bio Sci 2010; 1(3):1-5.
2. Mia AR, Siddqui NI, Islam MN, Khan MR, Shampa SS, Rukunuzzaman M. Effects of prolonged use of injectable hormonal contraceptive on serum lipid profile. Mymensingh Med J 2005; 14(1):19-21.
3. Draper BH, Morroni C, Hoffman M, Smit J, Bekinska M, Hapgood J, et al. Depot medroxyprogesterone versus Norethisterone enanthate for long-acting progestogenic contraception. Cochrane Database Syst Rev 2006; 3:CD005214.
4. Dalessandri KM, Firestone GL, Fitch MD, Bradlow HL, Bjeldanes LF. Pilot study: effect of 3, 3'-diindolylmethane supplements on urinary hormone metabolites in postmenopausal women

with a history of early-stage breast cancer. Nutrition and Cancer 2004; 50(2):161-167.
5. Lobo RA, Stanczyk FZ. New knowledge in the physiology of hormonal contraceptives. Am J Obstet Gynecol 1994;170:1499-1507.
6. Loose-Mitchell DS, Stancel GM. Estrogens and Progestins. Goodman & Gilman's Pharmacological Basis of Therapy. 12th ed. 2001.
7. Affandi B. An integrated analysis of vaginal bleeding patterns in clinical trials of Implanon. Contraception 1998; 58:99-107.
8. Palmieri B, Sblendorio V. Oxidative stress detection: what for? Part II. European Rev for Med and Pharma 2007; 11:27-54.
9. Agarwal A, Gupta S, R Sharma. Role of Oxidative stress in female reproduction Reprod Bio and Endo 2005; 28(3):1477-7827.
10. Pincemail J, S Vanbelle , Gaspard U, Collette G, Haleng, Cheramy-Bien JP, et al. Effect of different contraceptive methods on the oxidative stress status in women aged 40-48 years from the ELAN study in the province of Lie`ge. Belgium. Human Reprod 2007; 22(8): 2335-2343.
11. Cai H, Harrison DG. Endothelial dysfunction in cardiovascular diseases the role of oxidant stress. Circ Res 2000; 87:840-844.
12. Massafra C, Buonocore G, Berni S, Gioia D, Giuliani A, Vezzosi P. Antioxidant erythrocyte enzyme activities during oral contraception. Contraception 1993; 47:591-596.
13. Subakir SB, Abdul Madjid O, Sabariah S and Affandi B. Oxidative stress, vitamin E and progestin breakthrough bleeding. Hum Reprod 2000; 15(3):18-23.
14. Ohkawa H, Onishi N, Yagi K. Assay for lipid peroxidation in animal tissue by thiobarbituric acid reaction. Anal Biochem 1979; 95: 351-358.
15. Kakkar P, Das B, Viswanathan PN.A modified spectrophotometric assay of superoxide dismutase. Ind J Biochem Biophys 1984;21: 131-132.
16. Aebi H. Methods in enzymatic analysis. New York: Academic Press; 1974.p.674-684.
17. Moron MS, Depierre J, Mannervik B. Levels of glutathione, glutathione reductase and glutathione -S- transferase activities in rat lung and liver. Biochem Biophys Acta 1979;582: 67-78.
18. Moshage H, Kok B, Huizenga JR, Jansen PL. Nitrite and nitrate determinations in plasma: a critical evaluation. Clin Chem 1995;41(6):892-896.
19. Faddah LM, Al-Rehany MA, Abdel-Hamid NM, Bakeet AA. Oxidative Stress, Lipid Profile and Liver Functions in Average Egyptian Long Term Depo Medroxy Progesterone Acetate (DMPA) Users. Molecules 2005; 10:1145-1152.
20. Bakry S, Khattab E, Mansour A, Abu-Amer W and Abu-Shaeir W. Parentral toxicity of Medroxy-progestrone Acetate. Aus J Basic & App Sci 2011; 5(3):420-429.
21. Kose K, Dogan P and Ozesmi C. Contraceptive steroids increase erythrocyte lipid peroxidation in female rats. Contraception 1993; 47:421-425.
22. Choudhari SK, Chaudhary M, Bagde S, Gadbail AR, Joshi V. Nitric oxide and cancer: a review. World J Surg Oncol 2013; 11:118.

Address for Corresponding Author:
Waheed Jamil Ch.
Asstt. Prof. of Physiology,
AIMC, Lahore
E-mail :doctorwjc@gmail.com
Phone No: +923004256730-1