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CHANGES IN SERUM CERULOPLASMIN LEVELS WITH COMMONLY USED METHODS OF CONTRACEPTION

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ABSTRACT

It is a well established fact that long term use of oral contraceptives is hazardous to health. The most common methods of contraception used by women in reproductive age group include use of oral contraceptives and copper ' T '. One of the causative factors for the side effects of the ' pill' is presumed to be increase in serum cereloplasmin levels which has pro - oxidant activity. The present study involves the study of serum ceruloplasmin levels in two groups of subjects i.e. 30 women using oral contraceptive and 30 women using copper ' T '. 30 healthy females in reproductive age group were chosen as controls. It was observed that oral contraceptives increase the serum ceruloplasmin levels ($p < 0.001$) and the difference is highly significant as compared to no change ($p < 0.1$) in the groups using copper ' T ' as contraceptives.

KEY WORDS

Oral Contraceptives, Ceruloplasmin, Pro-Oxidant, Hazards

INTRODUCTION

Since the advent of oral contraceptives as a method of contraception, and in recent years the dilemma of HRT in post menopausal women, the hazards of long term hormonal treatment pose a problem for the use of the same, specially as oral contraceptives (1). The present study was initiated in response to references regarding the role of serum ceruloplasmin as one of the causative factors of side effects of oral contraceptives (1-3). Ceruloplasmin is a copper-containing enzyme. It oxidizes a variety of amines including epinephrine, melatonin, serotonin, paraphenylene diamine (4). It also converts ferrous ion to ferric ions and hence is required for utilization of iron, hence also known as ferroxidase I (5). The other substrate which is vulnerable to the prooxidant activity of ceruloplasmin is vitamin C or ascorbic acid. Osaki and Mc dermott proved the distinct ascorbate oxidase activity of ceruloplasmin (6). On the contrary, high levels of vit.C inhibit copper absorption by preventing its binding with metallothionine (4).This aspect has a bearing on the hazards of using oral contraceptives (1,5,7).

The present study involved estimation of serum ceruloplasmin status in commonly used methods of contraception such as oral contraceptives and copper ' T ' in females of reproductive age groups. The test group included females attending family planning OPD at D.Y.Patil Hospital and Sassoon General Hospital, Pune, Maharashtra.

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MATERIALS AND METHODS

The subjects for the study were women attending family planning O.P.D. in D.Y.Patil Hospital and Research center and Sassoon General Hospital. In all, 90 cases in reproductive age group i.e. between 25 – 35 years were studied. All of them were using contraception at lest for 1½ years.

The case distribution was as follows: -

1. Normal controls : 30
2. Women taking oral contraceptives : 30
3. Women using copper ' T ' : 30

The contentss of oral contraceptive tablets administered included

Norethisterone acetate - 1mg

Ethinylestrodiol - 30 µgm

Particular age group was chosen as most of the women in this age group use contraception, otherwise there is no age related variation after two years of age (5). The day of sample collection is important as the serum ceruloplasmin levels change during different phases of menstruation by 10 – 20 mg% (8). Hence the sample collection was done between 9^{th} – 11^{th} day of menstrual cycle. As serum ceruloplasmin levels show diurnal variation (5), the time of sample collection was between 8.30am to 10.30am. This is related to changes in ACTH levels.

Analytical methods

Venous blood samples were drawn from the subjects using disposable syringes and needles under aseptic conditions after overnight fast in the supine position. Serum was separated and the samples stored at 4 °C to 10 °C till being processed.

Serum ceruloplasmin was estimated by a colorimetric enzymatic assay based on oxidation of a dye, paraphenylene diamine first introduced by H .A. Ravin (9). Each sample was analyzed in triplicate and the mean was determined.

Principle:

The ceruloplasmin in the serum oxidizes the dye paraphenylene diamine and resulting violet colour (Bandrowski base) is measured at 540 nm on photoelectric colourimeter.

0.1ml serum was taken in three test tubes, in one tube 1ml of 0.5% sodium azide was added as a control tube. Then 8ml buffer was added to all the tubes. Then 1ml of 0.5% solution of substrate was added as rapidly as possible. The tubes are incubated at 35° – 37°C for one hour, then sodium azide is added to the test tubes. Incubate at 4° to 10°C for 30 minutes, and optical density measured at 530nm. Mg% of ceruloplasmin = (Reading of test – reading of control) X 0.002 X 87.5

Constant 87.5 was derived as in the absence of crystalline ceruloplasmin as a primary standard a highly purified ceruloplasmin was calibrated by inactivation with sodium cyanide, and the loss of PPD oxidase activity compared with the corresponding decrease in optical density of the ceruloplasmin at 605 nm in a Beckman DV spectro-photometer.

Using Holmberg - Laurell factor for conversion of optical density at 605 nm to ceruloplasmin concentration the net optical density attained in the PPD oxidase assay may be converted directly to mg% ceruloplasmin whole serum by multiplying O.D. by 87.5

Calibration of colorimeteric PPD oxidase activity as ceruloplasmin :

Sample	O.D. 605mu	PPD oxidase activity
Active enzyme	0.059	0.870
CN-inactivated enzyme	0.009	0.050

* O.D. 605 X 4.585 X $\frac{100}{32}$ = mg% ceruloplasmin

\# $\frac{\text{Mg \% Ceruloplasmin}}{\text{O.D. 530mn}}$ = Factor for conversion of O.D. in PPD oxidase test for mg% ceruloplasmin in serum in std. test.

(* derived from data of Holmberg – Laurell)

The constant 4.585 is the ratio between concentration of ceruloplasmin copper, expressed in micrograms per 100ml of a solution of pure enzyme, and the optical density at 605mu of the active enzyme in 1 cm path cuvette. The constant 0.32 is the concentration of copper in human ceruloplasmin expressed in percent.

(# The mean value of this conversion factor is based on 6 determinations with 3 different preparations of purified ceruloplasmin in 87.5%. Thus, net O.D. of PPD oxidase activity of 0.1ml of serum in the standard test, multiplied by 87.5 expresses the results directly in terms of mg ceruloplasmin in 100ml of serum.

The normal values of serum ceruloplasmin quoted in literature very between 20 – 50 mg/100ml of serum. (2,9,10).

Statistical analysis:

The data was analyzed using student's ' t ' test.

RESULTS

i. Simple colorimetric assay for estimation of serum ceruloplasmin is fairly sensitive method (sensitivity of 98%) for estimating serum ceruloplasmin controls.

ii. The difference between the ceruloplasmin controls and women taking oral contraceptives is highly significant ($p < 0.001$). Oral contraceptives increase serum ceruloplasmin levels.

iii. The difference between the serum ceruloplasmin in controls and women using copper 'T' is statistically non significant ($p < 0.1$).

The values of serum ceruloplasmin in control and different methods of contraception are illustrated in Table I.

DISCUSSION

The rise in serum ceruloplasmin after administration of oral contraceptive is in accordance with other workers (2,9,10). Certain side effects of oral contraceptives have been attributed to chronic increase in serum ceruloplasmin. The oestrogen component is mainly responsible for the increased level of serum ceruloplasmin while progesterone causes a less drastic rise (5). Considering the operon concept of Monod and Jacob oestrogen act as an inducer for synthesis of ceruloplasmin RNA templates causing subsequent increase in synthesis of the protein (10).

Recent evidence suggests that ceruloplasmin exhibits potent prooxidant activity and causes oxidative modification of important bimolecules like low density lipoproteins (LDL). This newly described prooxidant activity of ceruloplasmin may help to explain epidemiological studies indicating that ceruloplasmin

Table I: Value of Serum Ceruloplasmin in different methods of Contraception.

Sr. No.	Category	No. of Cases	Mean & SD Ceruloplasmin in mg%	Probability
1.	Control	30	44.8 ±10.4	
2.	Group using Oral Contraceptive	30	76.3 ± 14.0	P < 0.001
3.	Group using Copper ' T '	30	38.2 ± 6.7	P < 0.1

is an independent risk factor for cardiovascular disease (3,13,14).

Hence women on oral contraceptives may be considered a high risk group.

Secondly, ceruloplasmin also oxidizes compounds like ascorbic acid, epinephrine, melatonin, serotonin and other amines. Under physiological conditions this oxidation is minimized by common metabolite citrate (6). Any condition leading to rise in serum ceruloplasmin can lead to increased oxidation of the above mentioned substrates. Reduced levels of vitamin C have been detected in the plasma, leukocytes and platelets and urine of women taking oral contraceptives with a mean reduction of 30 to 40% (1,5,7).

Ascorbic acid is involved in post translational modification of collagen. It is also important for reduction of folate to tetrahydrofolate required for purine synthesis.

According to Stich et al the cupric ion catalysed oxidation of ascorbate can induce mutation and DNA damage (5). This could possibly be the mechanism by which long term use of oral contraceptives can give rise to carcinogenic effects and cervical dysplasia.

Several studies indicate that women receiving oral contraceptives are in induced hypovitaminotic C condition due to raised serum ceruloplasmin and require supplementary vitamin C (1,5,7).

SUMMARY

1. Colorimetric assay is a simple and fairly sensitive method for estimating serum ceruloplasmin.

2. Oral contraceptives cause a highly significant rise in serum ceruloplasmin level.

3. Use of copper ' T ' as a method of contraception does not alters serum ceruloplasmin level.

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