Genotoxic damage in cultured human peripheral blood lymphocytes of oral contraceptive users

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Abstract Synthetic progestins and estrogens have been reported to be toxic in various experimental models. Their prolonged use has been reported to induce cancer in humans. In the present study the effects of oral contraceptives were studied among users using chromosomal aberrations, sister chromatid exchanges and DNA damage as a parameter, in cultured human peripheral blood lymphocytes. The study was performed on 25 women (users) and 25 age match controls. No significant difference was observed in chromosomal aberrations and DNA damage. A significant increase was observed in sister chromatid exchanges (SCEs) among users. The results obtained and the risk of oral contraceptives (OCs) genotoxicity have been discussed.

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1. Introduction

Oral contraceptives are the combination of estrogen and progesterone. They are used to treat various hormonal disturbances, premenstrual syndrome and ovarian cysts [1]. The composition of pills varies from country to country due to the responsiveness variation from individual to individual or one population to another [2]. Earlier reports suggest the genotoxic effects of synthetic progestins in cultured human peripheral blood lymphocytes [3–7]. In these studies the genotoxic effects were observed at very high doses and, the reactive oxygen species (ROS) was suggested as a causative agent for the genotoxic damage [8,9]. The conversion of estrogen into catechol estrogens and quinines, via redox reactions causes oxidative damage to DNA [5,10]. There are both positive as well as negative reports regarding the genotoxic effects of estrogens and synthetic progestins [11–13]. In the present study an attempt has been made to investigate the possible genotoxic effects among oral contraceptives (OCs) users (females) by using chromosomal aberrations, sister chromatid exchanges and DNA damage as a parameter.

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2. Subjects and methods

2.1. Chemicals

RPMI 1640, fetal calf serum, phytohaemagglutinin-M, antibiotic-antimycotic mixture (Invitrogen); 5-bromo-2-deoxyuridine, Giemsa stain (Merck, India), Tris HCl, EDTA, Triton X, Trichloro acetic acid and diphenylamine, colchicine (SRL, India).

2.2. Human lymphocyte culture

A sample of heparinized venous blood was obtained from 25 women taking an OCs preparation containing levonorgestrel and ethinylestradiol as a content for 12 months as a means of contraception following their first pregnancy. Age matched healthy 25 controls were evaluated along with the above subjects. A written informed consent was obtained from all subjects. The mean age of 25 women using OCs was 28.5 years and of the 25 controls was 27.5 years. Individuals answered a questionnaire relating to lifestyle factors. The OC users were not having any other addictions such as smoking, alcohol drinking etc. nor have any history of disease. Briefly, heparinized blood sample (0.5 ml), was obtained from each female and was placed in a sterile cultured tube containing 7 ml of RPMI 1640 medium, supplemented with fetal calf serum (1.0 ml), antibiotic–antimycotic mixture (1.0 ml) and phytohaemagglutinin-M (0.1 ml). The culture tubes were placed in the incubator at 37 °C for 48 h [14].

2.3. Chromosomal aberration analysis

After 47 h, an amount of 0.2 ml of colchicine (0.2 μg/ml) was added to the culture tubes. Cells were centrifuged at 800 g for 10 min. The supernatant was removed and 8 ml of prewarmed (37 °C) 0.075 M KCl (hypotonic solution) was added. Cells were re-suspended and incubated at 37 °C for 15 min. The supernatant was removed by centrifugation, at 800 g for 10 min, and subsequently 5 ml of chilled fixative (methanol–glacial acetic acid; 3:1) was added. The fixative was removed by centrifugation and the procedure was repeated twice. The cells were stained in 3% Giemsa solution in phosphate buffer (pH 6.8) for 15 min. About, 300 metaphases were examined for the occurrence of different types of abnormality. Criteria to classify different types of aberration were in accordance with the recommendation of Environment Health Criteria 48 for Environment Monitoring of Human Population [15].

2.4. Sister chromatid exchanges

For sister chromatid exchange analysis, bromo deoxyuridine (10 μg/ml) was added at the beginning of the culture and the mitotic arrest was attempted, 1 h prior to harvesting by adding 0.2 ml of colchicine (0.2 μg/ml). Hypotonic treatment and fixation were performed as described for chromosomal aberration analysis. The sister chromatid average was taken from an analysis of metaphase during second cycle of division. A total of 25 well spread metaphases were scored per individual [16].
Quantitative assay for DNA fragmentation was performed according to the protocol of Burton [17]. After incubation of 48 h, the cell suspension containing 1–10 × 10⁶ cells in a 1 ml volume was prepared for each donor. About, 0.8 ml of cell suspension was transferred to a micro centrifuge tube and 0.7 ml of ice cold lysis buffer (5 mM Tris Cl, pH 8.0/20 mM EDTA/0.5% (v/v) Triton X-100) was added. The tubes were vortexed and allowed to lyse for 30 min at 4 °C. The tubes were centrifuged for 15 min at 15,000 g (4 °C) and the supernatant was transferred to a labeled conical glass tube. About 0.65 ml of 5% trichloroacetic acid (TCA) was added to the pellet in a micro centrifuge tube and 1.5 ml of 10% TCA to the sample in a labeled glass tube. The sample was precipitated overnight at 4 °C. The tubes were centrifuged for 10 min at 2500 g at room temperature and the supernatant was removed and about 0.65 ml of 5% TCA was added to the pellet. The tubes were boiled for 15 min at 100 °C in a water bath. After cooling down to room temperature the sample was centrifuged at 2500 g for 5 min. About 0.5 ml of each supernatant (from both glass and micro centrifuge tubes) was added to the labeled glass tube. About, 1 ml of diphenylamine reagent was added to each tube and was incubated for 4 h at 37 °C [18]. Finally the absorbances were noted at 600 nm in a spectrophotometer and the results were expressed in the percentage of DNA fragmented as follows:

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\% \text{Fragmented DNA} = \frac{\text{absorbance of supernatant}}{\text{absorbance of supernatant} + \text{pellet}}
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Student’s *t*-test was used for the analysis of data obtained for chromosomal aberrations and sister chromatid exchanges and \(\chi^2\) (Chi-square) was applied for the analysis of DNA damage.

### 2.6. Statistical analysis

The frequency of cells with aberration in OC users was almost similar to their age matched controls. Gaps and breaks of chromosome as well as chromatid were observed, but dicentric, exchanges and rearrangements were not observed. The mean values obtained were not statistically significant (Table 1). A significant difference \((P > 0.005)\) in the number of SCEs/cell was observed between OC users (3.9 ± 0.076) and their age matched control (2.62 ± 0.068) (Table 2). The mean value for the DNA damage was (0.1172 ± 0.0020) in OC users and (0.1148 ± 0.0025) in controls. The values were not statistically significant (Table 3). The results of the present study suggest that the OC users have significantly higher frequencies of SCEs/cell, but the CAs and DNA damage were not significant.

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damage/chromosomal aberrations, but are able to increase the number of SCEs/cell. The study performed by Biri et al. [25] also showed the increase in SCEs/Cell in OC users. The SCEs are the cytological manifestation of interchanges between DNA replication products at apparently homologous loci. It is more sensitive indicator of genotoxic effects than structural aberrations [26,27]. The induction of SCE is also correlated with the induction of cancer [28]. Besides various reports on the genotoxicity of steroids some of the progestogens have been studied in short and long term toxicology studies in rodents and dogs or monkeys for their carcinogenic activity, no significant or unusual toxicities were reported [29]. In some cases the oral contraceptives have also been suggested to be having a role in preventing ovarian cancer by both suppressing ovulation and altering the tumor promoting milieu [30]. The results obtained in the present study are contrary to the studies performed by other workers [13,31,32]. This may be due to the new hormonal formulations and preparations developed in an attempt to reduce the adverse effects of OCs, such as the reduction in the estrogen content. Estrogen such as ethynylestradiol, in the liver undergoes aromatic hydroxylation and the product, 4-hydroxyestrone, 3,4-dihydroxy 1,3,5 (10)-oestratien-17-one (4-OHE) is carcinogenic in male Syrian golden hamster kidney tumor model [33,34]. The genotoxic potential of steroids is determined by the metabolic conditions in the test system and the human body. The extrapolation from the experimental data to humans is not only difficult but also complex. The most of the studies conducted for genotoxic potential of steroids involve the concentration in microgram per ml range whereas the therapeutic plasma concentration ranges from nanogram or picogram per ml [7]. The present study was conducted on the women taking OCs for one year and the increase in SCEs may be due to the variable hormonal profile among them. Further, the occupation and work place, polymeric metabolizing genes and efficiency of DNA repair can also affect the response against OCs.

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References

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