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Original Article

Impact of Intermediate-term Oral Contraceptive Use on Oxidative Stress, Lipid Profile, and Liver Function in Iraqi Women: A Comprehensive Biochemical Assessment

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Abstract

Background and objectives: Oral contraceptive pills (OCPs) are commonly used for contraception, but their long-term effects on oxidative stress, lipid profiles, and liver function remain unclear. This study aimed to evaluate the impact of intermediate-term OCP use (Yasmin) on oxidative stress, lipid profile, and liver function, with particular emphasis on antioxidant markers, lipid metabolism, and hepatic enzyme activity, to better understand the potential metabolic and hepatic effects.

Methods: A case-control study was conducted in Maysan Governorate, Iraq, involving 150 women (100 OCP users and 50 non-users). Blood samples were collected from Al-Sadr Teaching Hospital and a specialized clinic between February and April 2023. Serum levels of antioxidants, lipids, and liver enzymes were measured using biochemical assays.

Results: OCP users had significantly lower levels of glutathione peroxidase vitamin E and uric acid ($p < 0.001$) compared to non-users. Lipid profiles showed that OCP users had higher levels of triglyceride and low-density lipoprotein ($p < 0.05$), whereas total cholesterol was significantly higher in non-users ($p < 0.05$). Liver enzyme activity, including alanine aminotransferase, aspartate aminotransferase, alkaline phosphatase, and total serum bilirubin, did not show statistically significant differences ($p > 0.05$). Longer duration of OCP use was significantly negatively correlated with vitamin E levels (r = −0.67), glutathione peroxidase activity (r = −0.56), uric acid levels (r = −0.45) and high-density lipoprotein (r = −0.54). Positive correlations were found between the duration of OCP use and total cholesterol (r = 0.62), triglyceride (r = 0.58), low-density lipoprotein (r = 0.60), and liver enzymes alanine aminotransferase (r = 0.66) and aspartate aminotransferase (r = 0.64).

Conclusions: Intermediate-term OCP use was associated with changes in oxidative stress and lipid metabolism, potentially increasing cardiovascular and metabolic risks. Regular monitoring of these parameters is recommended for OCP users.

Introduction

Oral contraceptive pills (OCPs) have been a cornerstone of hormonal contraception since their introduction in 1960, particularly among women aged 15 to 45 years.[1] They account for 27.3% of all reversible contraception methods in the United States.[2] Beyond preventing unplanned pregnancies, OCPs offer additional benefits, such as alleviating menstrual discomfort, managing acne, and reducing the risk of ovarian and endometrial cancers.[3–5] However, concerns persist regarding their intermediate- and long-term metabolic effects, particularly on oxidative stress, lipid metabolism, and liver function.[6] OCPs are typically composed of ethinyl estradiol (EE), a synthetic estrogen, and a progestin like drospirenone (DRSP). These components are commonly found in fourth-generation OCPs, which are widely used for their metabolic safety and contraceptive efficacy. Together, they ensure effective contraception and also influence oxidative stress, lipid profiles, and liver

Keywords: Oral contraceptive; Oxidative stress; Lipid profile; Liver function; Glutathione peroxidase; Vitamin E; Uric acid; Zinc.

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function. EE prevents ovulation by suppressing follicular development and gonadotropins, while DRSP provides anti-androgenic and anti-mineralocorticoid benefits. However, these hormonal changes may disturb oxidative balance and modulate lipid metabolism and hepatic functions, necessitating further research.[7] While these mechanisms are well understood, the biochemical effects of OCPs, particularly on oxidative stress markers, lipid profiles, and liver function, remain inadequately explored.[8,9] Hormonal changes induced by OCPs may alter antioxidant activity, potentially disturbing the body's oxidative balance.[10,11] EE, a synthetic estrogen and a key component of most combined oral contraceptives, has been shown to significantly influence oxidative stress mechanisms. Studies on cell and animal models indicate that EE can increase the production of reactive oxygen species (ROS) while reducing levels of antioxidant enzymes, such as glutathione peroxidase (GPX) and superoxide dismutase (SOD). Additionally, EE may exhibit antioxidant properties via estrogen receptor-mediated pathways under specific conditions; however, these effects are largely dose-dependent and context-specific.[12,13] These findings highlight the importance of investigating EE's impact on oxidative stress in human populations to better understand the risks associated with OCP use. Key antioxidants play crucial roles in neutralizing ROS and preventing oxidative damage, which is central to mitigating chronic conditions like cardiovascular disease.[14,15] OCP use has been linked to reduced antioxidant levels, underscoring the importance of evaluating these biochemical changes.[12,13]

Zinc and uric acid are vital components of the body's oxidative defense mechanisms. Zinc functions as a crucial trace element and a cofactor for enzymes like SOD, which neutralizes ROS and stabilizes cellular membranes.[16] Hormonal fluctuations induced by OCPs may disrupt zinc homeostasis, potentially compromising the body's antioxidant capacity. Recent findings suggest that chronic OCP use may decrease plasma zinc levels, even in populations with adequate dietary intake, emphasizing the need for its monitoring. Similarly, uric acid, traditionally considered a byproduct of purine metabolism, acts as a potent antioxidant by scavenging free radicals and contributing to total blood antioxidant capacity.[17] However, its dual role as both an antioxidant and a pro-oxidant is influenced by OCP-mediated hormonal changes. Elevated uric acid levels can exert pro-oxidant effects, increasing risks for hypertension, gout, and cardiovascular disease, making its evaluation in OCP users critical.[18–20]

OCPs significantly influence lipid profiles, with studies indicating elevated triglycerides (TG) and low-density lipoprotein (LDL) levels among long-term users.[21] EE and DRSP contribute to these changes by enhancing hepatic lipogenesis and modulating lipid transport mechanisms. Recent research also highlights the impact of OCPs on lipid subfractions, offering a more detailed understanding of their potential cardiovascular risks. These alterations underscore the importance of regular monitoring of lipid parameters to mitigate associated health complications.

Modern OCP formulations generally have minimal effects on liver enzymes, such as alanine aminotransferase (ALT) and aspartate aminotransferase (AST).[22] Liver enzymes are critical markers for hepatic stress or dysfunction, particularly in long-term OCP users.[23] However, prolonged use may still induce subclinical hepatic stress, especially in individuals with pre-existing metabolic disorders or other risk factors. Advanced imaging studies have further revealed subtle structural changes in hepatic tissues linked to chronic OCP use, emphasizing the need for ongoing research into their long-term hepatic effects. These findings highlight the necessity of regular liver function assessments in women using OCPs, particularly those with additional health risks.

This study evaluates the intermediate-term effects of OCP use on key biochemical markers in Iraqi women, focusing on antioxidants (GPX, vitamin E, zinc, and uric acid), lipid profiles, and liver enzyme parameters. The findings aim to bridge gaps in understanding the metabolic and hepatic impacts of OCPs, offering actionable insights for clinical monitoring and risk mitigation.

Materials and methods

Study design

This case-control study was conducted in the Maysan Governorate, Iraq, involving 150 women: 100 women in the OCP user group (case group) and 50 women in the non-user group (control group). The aim was to evaluate the biochemical effects of intermediate-term OCP use on oxidative stress, lipid profiles, and liver function.

Participants

Participants were recruited from multiple healthcare facilities, including Al-Sadr Teaching Hospital, primary health centers (notably the Mother Care Unit), and specialized women's clinics. Recruitment took place between the end of February 2023 and the end of April 2023. Participants were selected based on the same inclusion and exclusion criteria as the OCP users to ensure comparability.

Women in the case group had been using fourth-generation combined OCPs daily for at least three years. This duration was selected to represent intermediate-term use, as the effect of prolonged OCP use on biochemical markers was central to the study. The OCPs used in this study were combined hormonal pills containing EE and DRSP. A commonly available trade name for this combination in Iraq is Yasmin, which contains 30 µg of EE and 3 mg of DRSP. This formulation is widely prescribed for contraception and menstrual regulation, offering additional benefits such as anti-androgenic effects.

The control group included 50 women who were not using any contraceptive methods during the study period. The control participants were matched demographically and socioeconomically to the case group to ensure comparability. All women in the control group had not used hormonal contraceptives for at least one year prior to the study, eliminating any potential confounding effects of recent hormonal use.

Inclusion and exclusion criteria

The inclusion criteria for the case group required participants to have been using OCPs daily for a minimum of three years and to have not used hormonal contraceptives for at least one year prior to the study. This ensured that any observed differences in biochemical parameters were not influenced by recent contraceptive use. It is important to note that, due to logistical constraints, no specific control for the menstrual cycle phase was implemented in either group. As a result, the timing of blood sample collection was not standardized based on menstrual cycle phase or OCP use phase.

Participants' eligibility was confirmed through a combination of medical records, interviews, and verification of OCP prescriptions. Women in the case group had a uniform usage history with no major breaks in OCP administration, ensuring consistency in exposure.

For the control group, we selected 50 women who were not using any contraceptive methods. To ensure comparability between the groups, the control participants were recruited from the same

healthcare facilities, with efforts to match them demographically and socioeconomically to the case group. Women in the control group underwent the same screening process to confirm their non-use of contraceptives and the absence of other confounding factors.

Both groups were subject to strict inclusion and exclusion criteria. Inclusion criteria for the case group required participants to have been taking OCPs daily for at least three consecutive years. For both groups, exclusion criteria included: current use of medical supplements; diagnoses of diabetes mellitus, coronary heart disease, hypertension, or chronic renal failure; presence of chronic or autoimmune diseases; or any other condition or treatment known to impact oxidative stress, lipid metabolism, or liver function. Comprehensive interviews and reviews of medical histories were conducted to ensure participants met these criteria.

Recruitment and data collection

To minimize bias, the recruitment sites were selected to include women from diverse backgrounds, encompassing both urban and rural areas. This approach aimed to capture a representative sample of the population while maintaining internal validity.

Ethical and data security

Ethical considerations were rigorously followed. The study adhered to the principles of the Declaration of Helsinki, and ethical approval was granted by the Ethics Committee of Tehran University of Medical Sciences (Approval ID: IR.TUMS.SPH.REC.1401.283). All participants provided written informed consent after receiving a full explanation of the study objectives, methods, and their rights as participants.

Confidentiality and anonymity of data were ensured throughout the study. This detailed selection and recruitment process ensured that the observed differences in biochemical parameters between the two groups could be attributed to OCP use, minimizing the influence of potential confounders.

Sample collection and processing

Venous blood samples (10 mL) were obtained from the antecubital vein of each participant. Blood samples were collected and centrifuged immediately to separate serum. The serum samples were aliquoted and then stored at −80°C in airtight tubes until batch analysis was performed. All samples were analyzed in batches within two months of collection to ensure consistency and prevent degradation of biochemical markers.

Biochemical assays

To focus the scope of this study, we selected key antioxidant markers, including vitamin E, GPX, and uric acid, as these are well-established indicators of oxidative stress influenced by hormonal changes due to OCP use. While the inclusion of pro-oxidant markers such as malondialdehyde (MDA) or ROS would provide a more comprehensive view, this was not feasible due to resource constraints and the initial scope of the investigation. Future studies are planned to address these additional markers to evaluate the oxidative balance more thoroughly.

Vitamin E and GPX Assay

Levels were measured using enzyme-linked immunosorbent assay (hereinafter referred to as ELISA) kits (Elabscience, USA) according to the manufacturer's instructions. The GPX activity was measured using a double biotin antibody sandwich method, while vitamin E levels were determined based on a biotin double antibody sandwich ELISA.

Zinc assay

Zinc levels were measured spectrophotometrically using a kit (SPINREACT, Spain), which involved specimen deproteinization and colorimetric measurement with 5-Br-PAPS as the complexant.

Uric acid assay

Uric acid levels were measured using a semi-auto chemistry analyzer (Cobas c111, Roche Diagnostics, Germany). The enzymatic method involved peroxide oxidase and subsequent formation of a red quinonimine complex.

Lipid profile

Total cholesterol, TG, high-density lipoprotein (HDL), and LDL were measured using a semi-auto chemistry analyzer (Cobas c111, Roche Diagnostics, Switzerland).

Liver enzymes

ALT, AST, alkaline phosphatase (ALP), and total serum bilirubin levels were also measured using the Cobas c111 device.

Statistical analysis

All statistical analyses were performed using SPSS (version 26, IBM Corporation). Descriptive statistics, including mean, standard deviation, median, quartiles, and range, were calculated for all variables. The normality assumption was tested by the Shapiro-Wilks test. Independent sample t-tests were employed to compare the mean values between the OCP users and the control group. To further evaluate the magnitude of the differences observed between groups, effect sizes were calculated using Cohen's d for continuous variables. Effect sizes provide a standardized measure to determine the practical relevance of statistically significant findings and were interpreted as small (0.2), medium (0.5), or large (0.8). Parameters without statistically significant differences were excluded from effect size analysis. Multiple logistic regression analysis was conducted to assess the predictive accuracy of biochemical parameters and other variables in discriminating between OCP users and non-users. The Receiver operating characteristic (ROC) analysis was used to compute the area under the curve (AUC) and the Youden index to find the optimum cut-off value and its associated sensitivity and specificity values. Pearson correlation coefficients were calculated to evaluate the association between the duration of contraceptive use and the biochemical parameters. A p-value of less than 0.05 was considered statistically significant.

Results

Demographic and anthropometric characteristics

Table 1 presents the comparison of demographic and basic anthropometric characteristics between OCP users and non-users. OCP users had a significantly higher number of children ($p = 0.008$), weight ($p = 0.049$), and BMI ($p = 0.004$) compared to non-users. However, no significant differences were observed in age or height between the two groups.

Biochemical parameters, lipid profile, and liver enzyme levels

As detailed in Table 2 and Figure 1, OCP users exhibited significantly lower levels of GPX, vitamin E, and uric acid compared to the control group ($p < 0.001$ for all). Zinc levels did not differ significantly between the groups ($p = 0.136$). As depicted in Figure 2,

Table 1. Basic anthropometric characteristics between two study groups

Variables	OCP users (n = 100)[a]	Non-users (n = 50)[a]	*p*-value
Age (years)	33.4 ± 8.7	33.4 ± 8.5	0.99
Weight (kg)	69.9 ± 9.4	66.7 ± 9.2	0.04
Height (cm)	160.9 ± 6.1	162.1 ± 5.9	0.24
BMI (kg/m2)	27.1 ± 3.8	25.3 ± 3.1	0.004
Number of children	4 ± 2	3 ± 2	0.008
Duration of OCP use (years)	5.7 ± 3.4	–	–

[a]Means ± SD. BMI, body mass index; cm, centimeters; kg, kilograms; OCP, oral contraceptive pill; SD, standard deviation.

lipid profiles showed that OCP users had higher levels of TG and LDL ($p < 0.05$ for both), whereas total cholesterol was significantly higher in non-users ($p < 0.05$). No significant differences were observed in HDL levels. Liver enzyme activity (Fig. 3), including ALT, AST, ALP, and total serum bilirubin, was comparable between the two groups. To account for potential confounders, multivariable regression analysis was performed with OCP use as the dependent variable. The predictors included vitamin E, LDL, BMI, age, and uric acid. The regression analysis revealed significant associations with vitamin E ($p < 0.001$), LDL ($p < 0.001$), BMI ($p = 0.004$), and uric acid ($p = 0.021$). These results highlight the independent impact of OCP use on oxidative stress and lipid metabolism parameters. The detailed results are provided in Table 3.

In this study, effect size calculations were performed for parameters that demonstrated statistically significant differences between OCP users and non-users, as these differences are both statistically relevant and potentially clinically meaningful. Cohen's d was specifically calculated for vitamin E ($p < 0.001$), glutathione peroxidase ($p < 0.05$), uric acid ($p = 0.003$), and lipid profile components such as triglycerides ($p = 0.036$), LDL ($p = 0.046$), and total cholesterol ($p = 0.03$), as these parameters exhibited notable changes between the two groups. Parameters like liver enzyme levels (ALT, AST, ALP, and total bilirubin), which did not show statistically significant differences ($p > 0.05$), were excluded from effect size calculations, as the lack of statistical significance suggests limited clinical or practical relevance. The results of these calculations, along with corresponding means, standard deviations, and *p*-values, are summarized in Table 4.

ROC analysis and cut-off values

Table 5 presents the results of the ROC analysis, which was conducted to determine the optimal cut-off values for the biochemical parameters that showed significant differences between OCP users and non-users. The AUC values indicated moderate discriminatory power for vitamin E (AUC = 0.699), glutathione peroxidase (AUC = 0.564), uric acid (AUC = 0.628), and BMI (AUC = 0.645). The corresponding cut-off values with their associated sensitivity and specificity were identified for each parameter, as detailed in Table 4.

Table 2. Comparison of biochemical parameter levels between two study groups

Variables	OCP users (n = 100)[a]	Non-users (n = 50)[a]	Cohen's d	*p*-value
Antioxidants				
Vitamin E (nmol/mL)	20.5 ± 6.3	24.6 ± 6.1	−0.59	<0.001
Glutathione peroxidase (ng/mL)	16.6 ± 5	17.3 ± 4.2	−0.15	<0.001
Zinc (mg/dL)	62.9 ± 17.8	67.8 ± 21.3	−0.27	0.36
Uric acid (mg/dL)	3.6 ± 0.7	4.0 ± 0.8	−0.55	0.003
Lipid profile				
Total cholesterol (mg/dL)	181.5 ± 38.8	195.6 ± 38.2	−0.37	0.03
Triglycerides (mg/dL)	125.3 ± 51.7	112.8 ± 45.9	0.25	0.03
VLDL (mg/dL)	25.1 ± 10.3	22.6 ± 9.2	0.24	0.35
LDL (mg/dL)	105.8 ± 33	94.1 ± 36	0.34	0.04
HDL (mg/dL)	74.7 ± 27.7	77.1 ± 26.1	0.09	0.60
Liver function test				
AST (U/L)	15.2 ± 6.4	14.7 ± 7.2	0.08	0.67
ALT (U/L)	10.0 ± 3.6	10.8 ± 5.8	−0.15	0.52
ALP (U/L)	61.8 ± 23.5	61.2 ± 14.1	0.02	0.52
Total serum bilirubin (mg/dL)	0.4 ± 0.4	0.5 ± 0.3	−0.25	0.42

[a]Means ± SD. ALP, alkaline phosphatase; ALT, alanine aminotransferase; AST, aspartate aminotransferase; dL, deciliters; HDL, high-density lipoprotein; LDL, low-density lipoprotein; mg, milligrams; OCP, oral contraceptive pill; SD, standard deviation; U/L, units per liter; VLDL, very low-density lipoprotein.

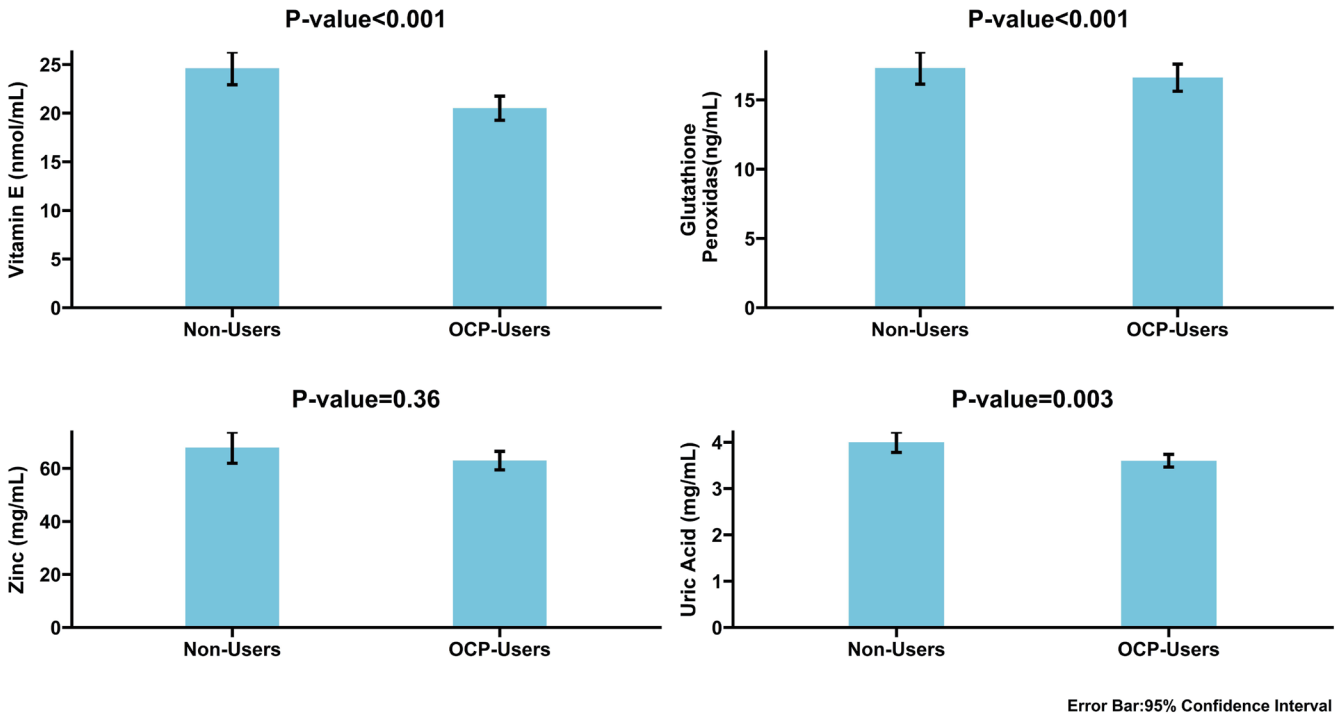

Fig. 1. Comparative analysis of antioxidants between oral contraceptive pill (OCP) users and non-users. The bar plots represent the mean levels of antioxidants, including Vitamin E, Glutathione peroxidase, Zinc and Uric acid, in OCP users and non-users. Error bars indicate the 95% confidence intervals. Statistical significance is noted with respective *p*-values for each parameter.

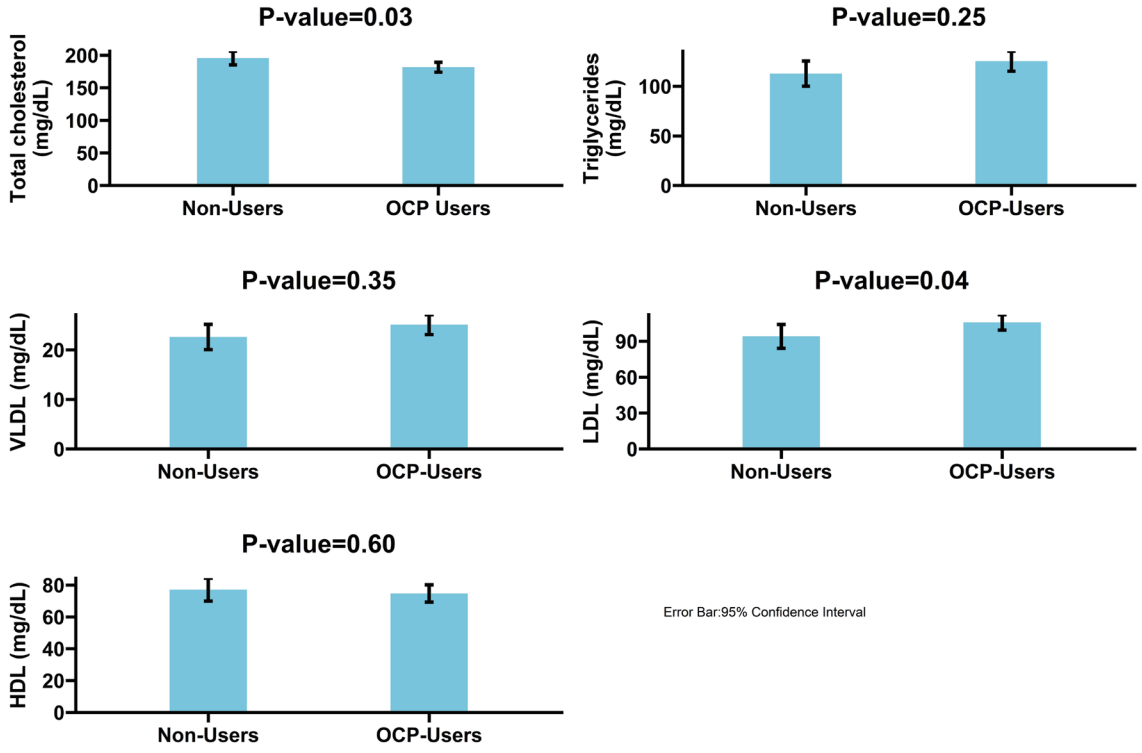

Fig. 2. Comparative analysis of lipid profile between oral contraceptive pill (OCP) users and non-users. The bar plots depict the mean levels of lipid profile components, including Total Cholesterol, Triglycerides, very low-density lipoprotein (VLDL), low-density lipoprotein (LDL), and high-density lipoprotein (HDL), in OCP users and non-users. Error bars indicate the 95% confidence intervals. Significant differences between groups are marked with their corresponding *p*-values.

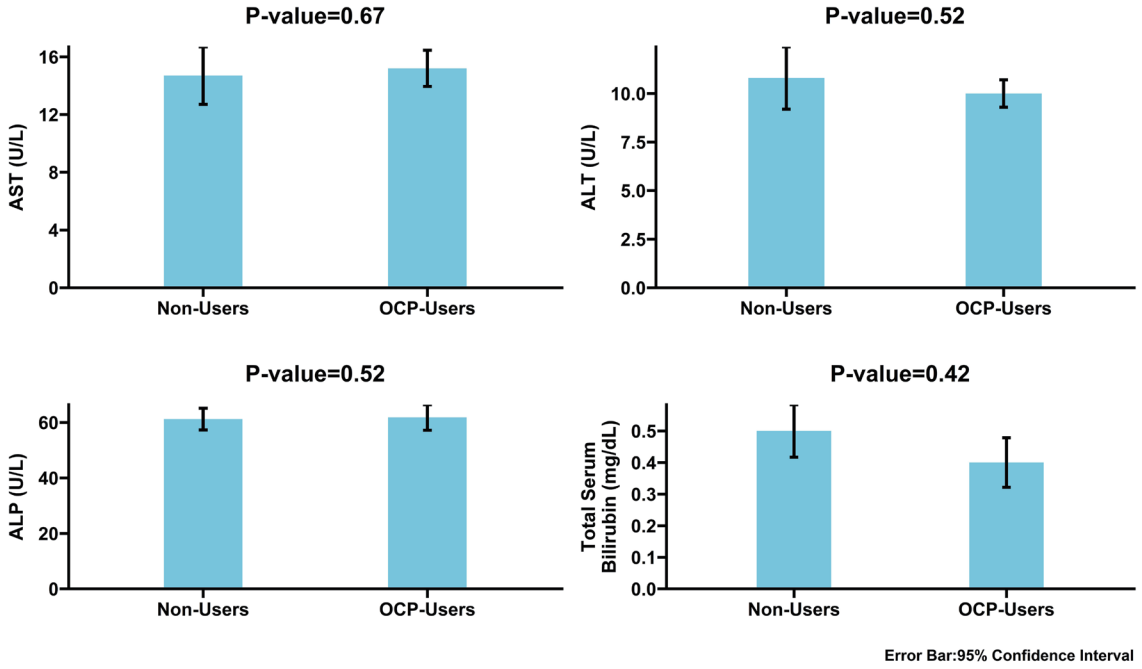

Fig. 3. Comparative analysis of liver function tests between oral contraceptive pill (OCP) users and non-users. The bar plots represent the mean levels of liver function markers, including aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), and total serum bilirubin, in OCP users and non-users. Error bars indicate the 95% confidence intervals. No statistically significant differences were observed between the groups, as indicated by the respective *p*-values.

Correlation analysis

Pearson correlation analysis was conducted to assess the relationship between the duration of contraceptive use and various biochemical indices, as detailed in Table 6. The results revealed that the duration of contraceptive use was significantly negatively correlated with vitamin E levels (r = −0.67, $p = 0.001$) and GPX activity (r = −0.56, $p = 0.004$), indicating that longer use of oral contraceptives is associated with a greater decrease in these antioxidants. A significant negative correlation was also observed between the duration of contraceptive use and uric acid levels (r = −0.45, $p = 0.021$). On the other hand, positive correlations were found between the duration of contraceptive use and total cholesterol (r = 0.62, $p = 0.003$), TG (r = 0.58, $p = 0.007$), and LDL cholesterol (r = 0.60, $p = 0.005$), while a significant negative correlation was observed with HDL cholesterol levels (r = −0.54, $p = 0.009$). Additionally, the levels of liver enzymes ALT (r = 0.66, $p = 0.001$) and AST (r = 0.64, $p = 0.002$) were positively correlated with the duration of contraceptive use, suggesting potential hepatic stress with prolonged use. These results highlight the associations between the duration of contraceptive use and alterations in biochemical indices.

Table 3. Results of the multivariable regression analysis assessing the relationship between biochemical parameters and OCP use, adjusted for confounders

Predictor	Coefficient (β)	95% CI	*p*-value
Vitamin E	−0.12	(−0.18, −0.06)	<0.001
LDL	0.15	(0.08, 0.22)	<0.001
BMI	0.10	(0.03, 0.17)	0.004
Age	0.02	(−0.01, 0.05)	0.12
Uric acid	−0.08	(−0.14, −0.02)	0.02

BMI, body mass index; CI, confidence interval; LDL, low-density lipoprotein; OCP, oral contraceptive pill.

Discussion

This study aimed to evaluate the biochemical effects of intermediate-term OCP use on oxidative stress markers, lipid profile, and liver function in Iraqi women. The results indicate significant alterations in several key biochemical parameters, suggesting that prolonged OCP use may impact oxidative stress balance and lipid metabolism, with potential implications for cardiovascular and metabolic health.

Our findings demonstrate a significant reduction in the levels of vitamin E, GPX, and uric acid among OCP users compared to non-users. The moderate to large effect size for vitamin E (Cohen's d = −0.66) underscores the substantial reduction in antioxidant capacity among OCP users, which may increase their susceptibility to oxidative stress and its associated health risks. These results align with previous studies, such as those by Palan *et al.*[24] and Kowalska and Milnerowicz,[25] which also reported decreased antioxidant levels in women using OCPs. The reduction in vitamin E and GPX is particularly concerning, as these antioxidants play critical roles in protecting cells from oxidative damage and maintaining overall cellular health. The decreased levels of uric acid further suggest a compromised antioxidant defense, as uric acid is known to scavenge free radicals, particularly in the plasma. However, contrary findings by De Groote *et al.*[26] and Oubeid *et al.*[27] suggest variability in the effects of OCPs on antioxidant levels, possibly due to differences in study populations, contraceptive formulations, or methodologies. It is important to acknowledge that focusing solely

Table 4. Comparison of biochemical parameters between OCP users and non-users with effect sizes

Variable	OCP users (Mean ± SD)	Non-users (Mean ± SD)	Cohen's d	*p*-value
Vitamin E (nmol/mL)	20.5 ± 6.3	24.6 ± 6.1	−0.66	< 0.001
Glutathione peroxidase (ng/mL)	16.6 ± 5.0	17.3 ± 4.2	−0.15	< 0.05
Uric acid (mg/dL)	3.6 ± 0.7	4.0 ± 0.8	−0.54	0.003
Triglycerides (mg/dL)	125.3 ± 51.7	112.8 ± 45.9	0.25	0.03
LDL (mg/dL)	105.8 ± 33.0	94.1 ± 36.0	0.34	0.04
Total cholesterol (mg/dL)	181.5 ± 38.8	195.6 ± 38.2	−0.36	0.03

Cohen's d: effect size; dL, deciliters; LDL, low-density lipoprotein; mg, milligrams; ng/mL, nanograms per milliliter; nmol/mL, nanomoles per milliliter; OCP, oral contraceptive pill; SD, standard deviation.

Table 5. The area under the curve and associated cut-off values

Characteristics	AUC (95%CI)	Sensitivity	Specificity	Cut off*
Vitamin E (nmol/mL)	0.699 (0.61–0.78)	0.54	0.82	20.35
Glutathione peroxidase (ng/mL)	0.564 (0.47–0.66)	0.38	0.82	15.38
Uric acid (mg/dL)	0.628 (0.53–0.72)	0.44	0.80	3.39
Number of children	0.63 (0.53–0.73)	0.38	0.82	4.5
Weight (kg)	0.587 (0.49–0.68)	0.38	0.82	72.5
BMI (kg/m2)	0.645 (0.55–0.74)	0.48	0.8	27.4

*Greater and equal classified as contraceptive non-user. AUC, area under the curve; CI, confidence interval; BMI, body mass index; kg, kilograms; mg/dL, milligrams per deciliter; nmol/mL, nanomoles per milliliter; ng/mL, nanograms per milliliter; kg/m^2, kilograms per square meter.

on antioxidant changes without examining pro-oxidants limits the depth of our analysis. While our study primarily targeted antioxidant markers, future investigations should integrate assessments of pro-oxidants and oxidative markers to elucidate the complete oxidative balance in OCP users.

Interestingly, our study did not observe a significant difference in zinc levels between OCP users and non-users. This finding contrasts with some studies that have reported alterations in zinc metabolism due to OCP use. For instance, Fallah *et al*.[18] found that OCPs could affect zinc levels, potentially compromising the body's antioxidant defenses. Zinc is a crucial cofactor for enzymes like SOD, which neutralize ROS and stabilize cellular membranes. The lack of significant changes in zinc levels in our study might suggest that the impact of OCPs on zinc metabolism is less pronounced or may vary depending on individual differences in diet, absorption, and overall zinc status. This discrepancy could be attributed to differences in the population studied, the duration of OCP use, or the specific types of OCPs used, all of which can influence the metabolism and bioavailability of zinc. While zinc did not show significant alteration in this study, it remains a critical element in the body's antioxidant defense, and its interaction with OCPs warrants further investigation, especially in populations with different dietary zinc intake or genetic predispositions affecting zinc metabolism. Comprehensive analyses that also account for changes in pro-oxidants may further clarify these complex interactions.[28,29]

Table 6. Pearson correlation coefficients between duration of contraceptive use and biochemical indices

Biochemical index	Pearson correlation coefficient (r)	*p*-value
Vitamin E	−0.67	0.001
Glutathione peroxidase	−0.56	0.004
Zinc	−0.23	0.14
Uric acid	−0.45	0.02
Total cholesterol	0.62	0.003
Triglycerides	0.58	0.007
LDL cholesterol	0.60	0.005
HDL cholesterol	−0.54	0.009
ALT	0.66	0.001
AST	0.64	0.002

ALT, alanine aminotransferase; AST, aspartate aminotransferase; HDL, high-density lipoprotein; LDL, low-density lipoprotein.

Despite the stable zinc levels observed in our cohort, the significant reductions in vitamin E, GPX, and uric acid suggest that OCP use still imposes oxidative stress, potentially overwhelming the antioxidant defenses that zinc supports. This emphasizes the complex interplay between different antioxidants and highlights the need for comprehensive monitoring of oxidative stress markers in women using OCPs.[30,31] The observed reductions in antioxidant levels, such as vitamin E, GPX, and uric acid, in OCP users indirectly suggest an imbalance in oxidative stress. These findings align with previous studies that reported compromised antioxidant defenses in similar populations. While our study did not measure pro-oxidant markers like MDA or ROS, the significant depletion of antioxidants may indicate increased oxidative stress. This underscores the need for future investigations to incorporate both antioxidant and pro-oxidant markers, providing a holistic view of oxidative balance in OCP users. Such an approach could elucidate

the interplay between reduced antioxidant defense and elevated oxidative stress, offering deeper insights into the potential health risks associated with OCP use. Furthermore, the lack of significant change in zinc levels could indicate that while zinc remains stable, its protective role might be insufficient to counterbalance the oxidative stress induced by intermediate-term OCP use. Therefore, even in the presence of adequate zinc levels, the overall antioxidant capacity may be compromised, necessitating further investigation into additional protective measures or supplementation strategies for women on intermediate-term OCP therapy.

The impact of OCP use on lipid metabolism is another critical finding of this study. Our results indicate that women using OCPs had significantly higher levels of TG and LDL cholesterol compared to non-users. These findings are consistent with previous research, which has shown that OCPs can adversely affect lipid metabolism, leading to elevated TG and LDL levels. The medium effect sizes observed for uric acid (Cohen's d = −0.54) and lipid profile components, such as LDL (Cohen's d = 0.34) and triglycerides (Cohen's d = 0.25), suggest meaningful alterations in lipid metabolism that could elevate cardiovascular risk over time. It is important to consider the role of baseline body composition differences, such as BMI and weight, in the observed lipid profile alterations. BMI was significantly higher in OCP users compared to non-users (Table 1), which may partially explain the elevated levels of triglycerides and LDL cholesterol observed in this group. Increased adiposity is known to influence lipid metabolism through mechanisms such as enhanced hepatic lipogenesis and altered lipid transport pathways, which could amplify the hormonal effects of OCPs on lipid profiles. Our multivariable regression analysis confirmed BMI as an independent predictor of LDL levels (p = 0.004), suggesting that body composition plays a contributory role alongside OCP use.

However, the persistence of significant differences in TG and LDL levels after adjusting for BMI (Table 3) highlights the independent impact of OCP components, such as EE and DRSP, on lipid metabolism. These findings align with previous studies reporting that hormonal contraceptives can modulate lipid profiles through estrogen-mediated stimulation of hepatic triglyceride synthesis and progestin-related alterations in LDL receptor activity. Future studies should consider more comprehensive assessments of body composition, including fat distribution and lean mass, to better delineate the interplay between OCP use and metabolic alterations. Such alterations in lipid profiles are concerning because they are well-established risk factors for cardiovascular diseases, including atherosclerosis and coronary artery disease. The mechanism behind these changes may be related to the hormonal components of OCPs, particularly estrogen and progestins, which are known to influence lipid metabolism.[21,32] Estrogen can increase the synthesis of TG in the liver and modulate LDL receptor activity, leading to higher circulating levels of these lipids. This hormonal effect, combined with individual factors such as diet and physical activity, might explain the variability in lipid responses among different studies and populations. Interestingly, while TG and LDL levels were elevated in OCP users, total cholesterol was significantly lower in this group compared to non-users. This finding suggests a complex interaction between OCPs and lipid metabolism, where certain lipid fractions may be more affected than others. Further research is needed to elucidate these mechanisms and to determine whether specific formulations of OCPs are associated with a more favorable lipid profile.[6,32,33] The finding of higher total cholesterol levels in non-OCP users is intriguing and may point to a compensatory metabolic mechanism or differences in underlying physiological or lifestyle factors between the two groups. One potential explanation is that non-OCP users may have differences in dietary habits or lipid metabolism that contribute to elevated total cholesterol levels. It is also possible that non-OCP users, particularly those with higher baseline cholesterol, may have refrained from using hormonal contraceptives due to medical advice or contraindications. Additionally, hormonal regulation could play a role. The absence of exogenous hormonal influence in non-OCP users might lead to unopposed endogenous hormonal activity, potentially driving cholesterol synthesis through mechanisms such as increased hepatic hydroxymethylglutaryl-CoA reductase activity. This hypothesis aligns with studies suggesting that endogenous estrogen fluctuations can modulate lipid metabolism, potentially leading to higher total cholesterol levels in certain populations.[34–36] Further investigation is required to explore these possibilities, including assessments of dietary intake, physical activity levels, and detailed lipid subfraction analyses to better understand the observed differences.

Beyond systemic oxidative stress and lipid metabolism alterations, recent research highlights the potential impact of OCP use on ocular microvascular structures. Specifically, OCPs containing drospirenone and ethinyl estradiol have been associated with reduced deep capillary plexus vessel density, as observed via optical coherence tomography angiography.[37] This finding suggests that the systemic vascular effects of OCPs may extend to microcirculatory systems, including the retina, underscoring the importance of comprehensive monitoring of both systemic and ocular vascular health in OCP users. Additionally, women using combined oral contraceptive pills were found to have significantly lower choroidal vascular index (CVI) values compared to non-users, despite no differences in choroidal thickness measurements.[38] This reduction in CVI indicates that OCPs may selectively impact the vascular components of the choroid, potentially contributing to ocular microvascular changes over time. Together, these findings emphasize the need for regular assessment of ocular vascular health in women using OCPs, as parameters like reduced deep capillary plexus vessel density and CVI could serve as early indicators of vascular pathology.

Regarding liver function, our study found no significant differences in liver enzyme levels (ALT, AST, ALP, and total serum bilirubin) between OCP users and non-users. These results suggest that intermediate-term OCP use does not markedly impact liver enzyme activity in this cohort, which is in line with some studies that have reported minimal hepatic effects of modern OCP formulations. However, it is important to note that liver enzymes are only one aspect of liver function, and normal enzyme levels do not entirely rule out hepatic stress or subclinical liver conditions. The liver plays a crucial role in metabolizing OCPs, and chronic exposure to these hormones could potentially lead to liver stress or alter hepatic metabolism. Although our study did not find evidence of significant liver dysfunction, the positive correlation between the duration of OCP use and liver enzyme levels (as indicated by the Pearson correlation analysis) suggests that prolonged OCP use might exert some degree of hepatic stress over time. This finding underscores the importance of regular liver function monitoring in intermediate-term OCP users, particularly those with other risk factors for liver disease.[39,40] Interestingly, liver enzyme levels, including ALT, AST, ALP, and total bilirubin, did not show statistically significant differences between the groups, indicating that intermediate-term OCP use may not induce overt hepatic dysfunction in this cohort. However, the positive correlations observed between OCP duration and liver enzyme levels warrant further investigation into potential subclinical hepatic effects. These results emphasize the importance of monitoring antioxidant markers and

lipid profiles in OCP users to mitigate potential health risks. Future research should explore whether dietary interventions, lifestyle modifications, or antioxidant supplementation can counterbalance these biochemical changes and reduce associated risks. Additionally, longitudinal studies are recommended to assess the long-term implications of these findings and determine whether specific OCP formulations may be associated with more favorable biochemical outcomes.

This study has several limitations that should be considered. The relatively small sample size and the specific geographic focus limit the generalizability of the findings. The cross-sectional design prevents the establishment of causality, and the lack of dietary and lifestyle data may confound the results. Additionally, the study did not differentiate between various OCP formulations, which could influence the biochemical outcomes. Future research should include larger, more diverse populations and longitudinal designs, incorporate detailed dietary and lifestyle assessments, and explore the impact of different OCP formulations. Investigating protective strategies, such as antioxidant supplementation, is also recommended to mitigate the potential risks associated with intermediate-term OCP use.

Future directions

This study provides valuable insights into the biochemical impacts of intermediate-term oral contraceptive use, particularly in relation to oxidative stress, lipid metabolism, and liver function. However, several aspects require further exploration to deepen our understanding and address the limitations of this research. Future investigations should focus on a more comprehensive analysis of oxidative stress mechanisms by simultaneously assessing pro-oxidants and oxidative markers, such as ROS, MDA, advanced oxidation protein products, and hs-CRP, alongside antioxidant markers. This dual approach will help clarify the dynamic balance between oxidative and antioxidative processes influenced by oral contraceptive use. Additionally, the hormonal composition of the oral contraceptives used by participants, including estrogen and progesterone levels, should be directly measured and analyzed. Such measurements, combined with subanalyses based on the generation, dosage, and specific formulations of the contraceptives, will enable a more nuanced understanding of their physiological effects. The correlation between antioxidant depletion and pro-oxidant induction, as well as the influence of baseline differences such as BMI and metabolic health, must be examined through covariate analyses to ensure that observed biochemical changes are directly attributable to contraceptive use and not confounded by other factors. Furthermore, longitudinal studies tracking participants before, during, and after contraceptive use are needed to establish causality and assess the long-term implications of these changes on cardiovascular and hepatic health. Advanced techniques, such as proteomics, metabolomics, and genomic analyses, should be incorporated to uncover the molecular pathways modulated by oral contraceptives, providing deeper insights into the mechanisms behind their impact on oxidative stress, lipid metabolism, and liver function. These techniques may also help identify biomarkers that predict individual susceptibility to adverse effects, paving the way for personalized contraceptive regimens. Moreover, exploring clinical strategies such as antioxidant supplementation, dietary interventions, and personalized medicine approaches tailored to hormonal profiles and genetic predispositions could offer preventive solutions and optimize the safety of oral contraceptive use. Evaluating cardiovascular and hepatic risks more comprehensively, through methods such as vascular imaging and liver fibrosis screening, will further enhance our understanding of the broader health implications. Future research in these directions will contribute significantly to the development of safer and more effective contraceptive options, ultimately improving the quality of care for women.

Conclusions

This study provides valuable insights into the biochemical effects of intermediate-term OCP use on oxidative stress markers, lipid profiles, and liver function in Iraqi women. Our findings demonstrate that prolonged OCP use is associated with significant reductions in key antioxidants, including vitamin E, GPX, and uric acid. These reductions suggest a compromised antioxidant defense, potentially increasing the risk of oxidative stress-related conditions. Although zinc levels remained stable, the overall antioxidant capacity may still be insufficient to counterbalance the oxidative stress induced by OCPs. Moreover, the study revealed significant alterations in lipid metabolism, with higher levels of TG and LDL cholesterol observed in OCP users. These changes heighten the risk of cardiovascular diseases, emphasizing the need for careful monitoring of lipid profiles in women using OCPs. On the other hand, liver function appeared largely unaffected by intermediate-term OCP use, as indicated by stable liver enzyme levels. However, the positive correlation between the duration of OCP use and liver enzyme levels suggests potential hepatic stress over time, warranting regular liver function assessments. In light of these findings, it is crucial for healthcare providers to monitor the antioxidant status, lipid profiles, and liver function of women on intermediate-term OCP therapy. Further research is needed to explore potential protective strategies, such as antioxidant supplementation, to mitigate the risks associated with prolonged OCP use.

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Conflict of interest

The authors declare no conflicts of interest.

Author contributions

Study project (FN), experimental tests and result interpretation (FN, ZM, AT, HHS), the first draft manuscript (FN, HHS), editing of the manuscript (FN, ZM), data analysis and interpretation, and validation and analysis of the obtained results of the study (FN, AT). All authors have read and approved the final edition of this manuscript.

Ethical statement

The study adhered to the principles of the Declaration of Helsinki, and ethical approval was granted by the Ethics Committee of Tehran University of Medical Sciences (Approval ID: IR.TUMS.

SPH.REC.1401.283). All participants provided written informed consent after receiving a full explanation of the study objectives, methods, and their rights as participants.

Data sharing statements

The datasets used and/or analyzed during the study are available from the corresponding author upon reasonable request.

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