

Table 2. Statistically correlations between serum cytokine (IL-6) and enzymes (ALT, ALP, GGT, LDH) in OCs user and non-OCs users' group.

	IL-6	ALT	ALP	GGT	LDH
Age					
Pearson correlation	0.71247	0.73973	-0.011	0.80246	0.71099
N	50	50	50	50	50
IL-6					
Pearson correlation	1	0.74369	0.80085	0.89463	0.7163
N	50	50	50	50	50
ALT					
Pearson correlation	0.74369	1	0.80189	0.92913	-0.0167
N	50	50	50	50	50
ALP					
Pearson correlation	0.80085	0.80189	1	0.80549	0.80191
N	50	50	50	50	50
GGT					
Pearson correlation	0.89463	0.92913	0.80549	1	0.80501
N	50	50	50	50	50
LDH					
Pearson correlation	0.7163	-0.0167	0.80191	0.80501	1
N	50	50	50	50	50

23.23±1.51 of serum GGT in control group with p=0.103 (**Table 4, Figures 1 and 2**). Statistical analysis confirmed significant difference in the average behaviors of serum ALT and ALP in OCs user and control group. Pearson correlation analysis showed positive correlation 0.802 between OCs women age and GGT, while highly negative correlation -0.016 was observed between serum ALT and LDH levels of OCs user group. A moderate nature of relationship showed by the correlation co-efficient showed. GGT and ALT had a direct relationship inverse relationship; while weak relationship between IL-6 and ALT was observed with 0.743. (**Table 2**).

OCs group women also showed significant difference in mean values 166.26±25.21 of serum LDH levels in comparison to control group LDH mean values 150.08±28.19 with p<0.0001, however, serum LDH values of both, OCs users and non-users were in reference ranges (100 to 190 u/l) (**Table 4, Figures 1 and 2**) Furthermore, statistical analysis also indicated that LDH had positive correlation with serum ALP and GGT levels with 0.801 and 0.805 respectively (**Table 2**).

DISCUSSION

Globally, the most commonly use form of hormonal contraception is the oral contraceptive pill Previous studies highlighted that estrogen progesterone combined hormonal contraception are effective in providing protection against pregnancy and are using by most of the women. The excessive hormonal contraception use is associated with various medical conditions especially cardiovascular complications. Previously developed OC pills contained increase estrogens

Table 3. Serum CRP levels of OCs user group (n=50) as compared with non-OCs users control group (n = 40). CRP was measured through latex agglutination method undiluted positive samples result showed CRP 6 mg/L.

	CRP-Negative	CRP Positive		
		Undiluted (6 mg/L)	1: 2 (12 mg/L)	1: 4 (24 mg/L)
OCs user (n=50)	29	21	-	-
Non-OCs user (n=40)	40	-	-	-

Table 4. Descriptive statistics of all study samples of OCs pills users and non-OC pills user, tested for Cytokine (IL-6) and serum enzymes (ALT, ALP, GGT, LDH).

Biochemical Parameter	OCs user (n=50)	Non-OCs user (n=40)	Df	p Value
IL-6 (pg/mL)	6.32±0.93	5.62±0.91	75	<0.0005
ALT (u/L)	30.76±2.89	24.11±1.43	76	<0.0001
ALP(u/L)	153.64±7.73	107.48±3.79	75	<0.0001
GGT (u/L)	23.59±1.66	23.23±1.51	73	0.103
LDH (u/L)	166.26±25.21	150.08±28.19	71	<0.0001

and progestins concentrations that was causing intolerable adverse effect like headache, nausea, vomiting, irregular bleeding, obesity and venous thromboembolism. Enovid 10, was the first marketed OC pill which was contained progestin norethynodrel (9.85mg) and estrogen mestranol (150µg). Today, reduce estrogens and progestins containing pills are using to control the posology. The OCs pills which are using now a days contain low hormone doses, modern progestins (0.1 to 3.0mg) and estrogens (20 to 50µg) [17]. Currently, various considerable modifications in OCs regimes have been made in order to reduce dosage frequency, menstruations duration and other related adverse effects for instance, dysmenorrhea and intermenstrual migraine. In clinical setting, the extended-release OCs pills are more appreciating because they are more effective in preventing pregnancies as compare to traditional OCs regimes and also provide improved outcomes in terms of menstrual symptoms [18]. Literature review reported many pathological conditions are associated with the use of OCs pills. Inflammatory processes are involved in OCs induced pathological conditions and many plasma inflammatory markers have been used as potential tools for early prediction of the OCs induced diseases or vascular events. Among these markers, systemic inflammatory biomarkers produced by liver such as CRP, cytokines (Tumor necrosis factor-alpha, interleukin-6) and monocyte chemotactic protein-1 [19].

With this background information, we intended to investigate serum pro-inflammatory cytokine (IL-6) and liver enzymes as biomarkers related to bodily inflammation process and hepato cellular damage. These biomarkers were evaluated in serum samples of OCs user (n=50) and non-OCs user (n=40) group through ELISA and photometry-based assay. The study results revealed significant elevation in serum IL-6 levels in OCs user group as compare to non-OCs user group with $p < 0.0005$. These findings support previously studies performed on OCs user to determine the hormonal contraceptive effects on plasma inflammatory cytokines levels and found both, oral and vaginal contraceptive users showed significantly high ($p < 0.0001$) IL-6 and CRP levels as compare to non-users. Furthermore, positive correlation was observed between IL-6 and CRP [20]. In contrast, Rooijen [20] observed significant high CRP level in OCs user with unaffected serum TNF- α and IL-6 levels, they attributed that elevation of CRP is not linked with direct metabolic hepatic activation but by generation of CRP by non-hepatocytes [21].

In present study, we found that 42% OCs users showed serum CRP levels (6 mg/L), and 58% OCs user women and control group showed negative CRP test. (**Table 1**). The elevation in serum CRP level in OCs group was also in agreement with the findings observed by Piltonen [21] they studied combined hormonal contraceptives effects in women [22]. Karim [22] conducted a prospective study on postmenopausal women and found significantly increase in four inflammatory markers (CRP, IL-6, serum amyloid A & sICAM-1) in 122 women with history of cardiovascular events in comparison to control

group comprise of 224 women with no history of cardiovascular events and concluded these biomarkers as potentially predictors of future cardiovascular events [23]. Rooijen [20] explained that plasma CRP is hepatic response to inflammation and there consider as inflammatory marker. They also showed significant high level of CRP among OCs users as compare to control [21]. Divani [13] evaluated hormonal contraceptive effects among female subjects who were using combined OCs and combined vaginal contraceptive and observed elevated CRP level in both oral and vaginal contraceptive users and CRP levels were positively correlated with IL-6 and sTNF-RI [14]. High plasma CRP levels have been confirmed in previously performed studies on OCs user women as compare to control and they concluded that elevated plasma CRP is related with moderate to high risk of future cardiovascular events [8]. In another study, metabolic and cardiovascular parameters were evaluated in OCs user women and those women who were using levonorgestrel-releasing intrauterine device and study results revealed significantly high systolic and diastolic blood pressure and elevated inflammatory indices (C-reactive protein). They concluded that OCs usage linked with adverse findings in cardiovascular, metabolic, and inflammatory parameters that can serve as predictor of cardiovascular events and metabolic disorders [24].

Earlier studies have documented the use of OCs pills linked with the development of benign hepatic adenoma. The mechanism of development of tumor by progestins is still not clear, however, it is proposed that procarcinogenes are converted into carcinogens by the progestins induced homooxygenases [25]. In this study, serum enzymatic (ALT, ALP, GGT, LDH) profile was assessed among OCs users and non-users' group. In the present work, liver health status of OCs users was assessed through the estimation of serum ALT levels, and found significant elevation in comparison to non-OCs user group with $p < 0.0001$, though, serum ALT values were in normal ranges. While in OCs user group, serum ALP was not only significantly raised as compare to control group with $p < 0.0001$, but was also high from reference range. But no statistically significant difference was observed in serum GGT level between OC user and non-user group with $p = 0.103$. Serum LDH levels of OC user group were in normal ranges but in comparison to control group, LDH level was significantly high with $p < 0.0001$ (**Table 1, Figure 1**). In OC user group, average behaviors of serum ALT, ALP and LDH was significantly change, a positive correlation was observed between LDH and ALP and highly negative correlation between serum ALT and LDH levels of OCs user group. (**Table 2**) Similarly, increase in serum LDH was found among OCs users by Naz [25] they reported increased use of OCs pills can alter normal lipids and serum enzymes profile among OCs users [26]. Oral contraceptives pills induced hepatotoxicity was also studied in female rabbits by Ekhato [26] they found total bilirubin, AST and ALT levels were significantly increased in OCs given female

rabbits and suggested that regardless of OCs pills dosages, hepatotoxicity is associated with them [27]. Also, Walch [27] observed significantly increase ($p < 0.05$) in liver enzymes (ALT, AST and ALP) activities in patients that were receiving DMPA. This increase in enzyme activity was attributed to altered liver enzymes metabolism and/or integrity of liver membrane may be compromised.

CONCLUSION

In conclusion, we observed even though estrogens produce in women body, but its daily intake in the form of OCs pills may increase estrogen concentration in the body. The prolong OCs pills use elevates systemic inflammatory marker and liver enzymes in blood that in clinical terms can be associated with acute/chronic inflammation or low degree of severity disease. This increase in biochemical parameters may be due to altered hepatic metabolism or due to inflammatory response. These biochemical parameters must be examining continuously as estrogens has mitogenic activity.

RECOMMENDATIONS

We recommend that OCs user women must undergo periodic liver tests for early detection and diagnosis of OCs induce hepatotoxicity. Furthermore, intake of liver tonic can maintain liver membrane integrity.

ETHICAL APPROVAL

All procedures of present work involving human participants were performed according to the ethical standards of the institutional research committee. The study protocol was approved by the institutional ethical review board and to hide the identity of subjects, all samples were labeled with specific codes.

DISCLAIMER

None.

CONFLICT OF INTEREST

None.

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