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ASSESSMENT OF PROTEIN CARBONYLS AS BIOMARKERS OF OXIDATIVE STRESS IN WOMEN USING ORAL CONTRACEPTIVE IN D.I. KHAN DISTRICT

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Abstract

This research study was primarily conducted for the purpose to assess "increased Carbonyl formation as biomarker of oxidative stress in female using oral contraceptives in D.I. Khan District". Oxidative stress is the disturbance in balance between reactive oxygen species and antioxidant. This happens in different condition in different diseases. During this study the level of carbonyl groups have been observed. The usage of protein Carbonyl groups as biomarkers of oxidative stress has some advantages in comparison with the measurement of other oxidation products because of the relative early formation and the relative stability of carbonylated Proteins. Most of the assays for detection of protein Carbonyl groups involve derivatisation of the carbonyl group with 2, 4-dinitrophenylhydrazine (DNPH), which leads to formation of a stable dinitrophenyl (DNP) hydrazone product. This then can be detected by various means, such as spectro-photometric assay, enzyme-linked immune sorbent assay (ELISA), and one dimensional or two dimensional electrophoresis followed by Western blot immunoassay. At present, the measurement of protein Carbonyl groups after their derivatisation with DNPH is the most widely utilized measure of protein oxidation. All those female using contraceptive pills were included as participants of the study. For study fifty nine female using oral contraceptives and twenty four female not using any type of oral contraceptives were selected. To measure the effect of oral contraceptive pills on lipid profile laboratory was used. Protein carbonyl formation was taken as biomarker of oxidative stress. The results obtained from different techniques were analyzed through statistical tools. After applying statistical techniques it was found that carbonylated proteins act as indicator of

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oxidative stress. Use of contraceptive pills for long time cause many complications such as hepatopathy, cardiovascular abnormalities etc. It can be established that oral contraceptive pills increases protein Carbonyl, triglyceride, high lipoprotein, low lipoprotein and cholesterol level in female. On the other hand blood Urea and Serum Creatinine level remain the same during the use of oral contraceptive pills in female. In future, I will be examine the effect of oral contraceptive pills on other part of body like liver, heart etc.

Introduction

Functional groups containing a carbonyl group is a carbon atom double bonded to an oxygen atom. They are found in many biological compounds such as carbohydrates, nucleic acids, peptides, and proteins in the form of aldehyde, ketone, ester and amide. These compounds are involved in many biochemical reactions that are crucial for normal cell activity (Rindelaub et al., 2015).

Biomarkers are objective biological measures that can be used to determine normal physiology, disease states, exposure to toxic agents, and therapeutic reactions. Symptoms are reported by the patient, while biomarkers can be measured as evidence of biochemical or physiological changes occurring in the body. For these reasons, they have been extensively exploited in clinical medicine, disease diagnosis, drug monitoring and evaluation of environmental or metabolic regulation (Strimbu et al., 2010; Rochfort et al., 2005).

Oxidative stress is the result when the production of reactive oxygen species is greater than body antioxidants. Reactive oxygen species are produced by the normal metabolism and can be elevated by hormones, the environment, or disease. The overproduction of these reactive molecules can cause damage to lipids, nucleic acids, and proteins. Protein carbonylation, which is the irreversible addition of carbonyl groups to protein molecules, is one of the major effects of protein oxidation.

Protein carbonyl groups are predominantly formed by the oxidation of amino acid residues, principally lysine, arginine, proline and threonine (Requena et al., 2003). Carbonylated proteins can change their normal structure and biological function and are more likely to break down. But when the modified proteins build up in the cell, they can disrupt the cellular functions and lead to inflammation, damage to the tissues, ageing and chronic diseases (Nyström, 2005). Protein carbonyls are believed to be good markers of oxidative stress because they are stable and can be easily measured. They are usually quantifiable by derivatization with 2, 4-dinitrophenylhydrazine to give detectable hydrazone products.

The combined oral contraceptive, especially the combination of ethinyl estradiol plus levonorgestrel, is the most popular method used by women for contraception to avoid unwanted pregnancy (Josse et al., 2012). These drugs are effective and safe in general but long-term use can have a significant effect on oxidative balance, inflammatory responses, lipid metabolism, and renal function. On the other hand, the use of oral contraceptives has been suggested to result in higher plasma protein carbonyls, a protein oxidative modification marker, in previous studies (Zipprich et al., 2009).

Although women in Dera Ismail Khan District widely use oral contraceptives information is scarce about their biochemical and oxidative effects. The purpose of the present study was to evaluate serum protein carbonyls as a biomarker of oxidative stress in women who are taking oral contraceptive medication. It also assessed serum urea, creatinine and lipid-profile parameters to explore the potential impact on renal function and lipid metabolism.

Materials and Methods

Study Design and Setting

The study was carried out as a comparative study in the Pharmaceutical Chemistry Laboratory, Faculty of Pharmacy, Gomal University Dera Ismail Khan, Pakistan. The study group consisted of women taking OCs, and age comparable women who were not taking OCs served as controls.

Participant Selection

For the study, women were required to have been taking continuous oral contraceptive use for at least a year. Informed consent was obtained in writing from all participants, after providing information on the purpose, methodology and

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importance of the study. Women who were not taking hormonal contraceptives were recruited as controls for comparison of serum protein carbonyl level and other biochemical parameters. Tuberculous, hepatitis, migraine or any chronic disease women were excluded. Additionally, people taking medication, anti-oxidants drugs, and nutritional anti-oxidants were excluded to avoid these from affecting the oxidative stress and biochemical parameters.

Blood Sampling

The venous blood was tapped from each subject in sterile, disposable syringe of about 5 mL in quantity. The blood samples were centrifuged and the serum was transferred to labelled tubes and was kept under frozen conditions till biochemical analysis.

Chemicals and Reagents Chemicals used in the protein carbonyl assay were acquired from BDH Chemicals Ltd., England, hydrochloric acid and trichloroacetic acid from Merck, Germany, ethanol and ethyl acetate from Merck, Germany, and guanidine hydrochloride from Sigma, Germany. The chemicals used were Analytical grade. A sample of 18 mL of concentrated hydrochloric acid solution was diluted with distilled water to make 100 mL of a 2 N hydrochloric acid solution. The DNPH reagent was prepared by dissolving 198 mg of DNPH in 100 mL of 2 N hydrochloric acid. 20% and 10% trichloroacetic acids were prepared by dissolving 20g and 10g of trichloroacetic acid in distilled water to make 100mL. Guanidine hydrochloride was dissolved in 2 N hydrochloric acid to make a 6 M guanidine hydrochloride solution with 15 mL of solution. A 1:1 mixture of ethanol and ethyl acetate was used.

The Estimation of the Protein

Carbonyl Content. The content of serum proteins was measured as the amount of carbonyl in serum, adopting the spectrophotometric procedure established by Levine et al. (1990). The following data is taken from the experimental results for each serum sample; two test tubes were set up for each serum sample, one filled with the DNPH reagent and one filled with 2N hydrochloric acid (the blank). 200 µL of serum was placed into each tube, 4 mL of DNPH reagent was added to the test tube and 4 mL of 2 N hydrochloric acid was added to the blank tube. These tubes were then placed in the dark at room temperature for 1 hour with vortexing every 15 minutes. After incubation 5 mL of 20% trichloroacetic acid was added to each tube to precipitate proteins. The tubes were placed on ice for 10 minutes and then centrifuged at 3,000 rpm for 5 minutes. The supernatant was removed and the protein pellets were washed with 4mL of 10% trichloroacetic acid. The centrifugation procedure was then repeated. The protein pellets were mechanically disrupted, and washed three times with an ethanol/ethyl acetate solution (1:1) to remove any remaining DNPH and lipid contaminants. The final protein precipitates were re-dissolved in 2 mL of 6 M guanidine hydrochloride. The absorbance was determined at 370 nm by UV–visible spectrophotometer. The corresponding test absorbance was subtracted from the blank absorbance to calculate the protein carbonyl content.

Biochemical Analysis

To measure renal function, serum urea and creatinine levels were measured. Creatinine was measured by alkaline picrate method which is based on the reaction of creatinine with picrate in alkaline medium to yield coloured product. The colour intensity of the resulting color was directly proportional to the concentration of creatinine. The enzymatic urease method was used for the determination of serum urea. Urease breaks down urea to form ammonia which then reacts with α-ketoglutarate and nicotinamide adenine dinucleotide in an enzyme reaction. The absorbance change was used to measure the concentration of serum urea. A lipid profile was performed to measure total cholesterol, triglycerides, high-density lipoprotein cholesterol and low-density lipoprotein cholesterol. These parameters were analyzed with women taking oral contraceptives and women not taking oral contraceptives to assess any change in lipid metabolism related to the use of oral contraceptive.

Statistical Analysis

Data collected were summarised using appropriate descriptive statistics. The independent-samples Student's t-test was used to compare the differences among the oral contraceptive users and controls. Where more than two categories or subgroups were

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compared, analysis of variance was used. The significance of the results was calculated based on predetermined probability level.

Results and Discussion

Seven biochemical parameters were evaluated and compared between 59 OC users (subjects) and 24 non-users (controls).

Raw demographic and biochemical data for all 83 participants are provided in Supplementary Table S1.

Table 1: *Descriptive statistics of patient and control group*

Tests	N	Min	Max	Mean	Std. deviation
Serum creatinine mg/dl	83	0.50	1.6	0.9	0.2
Blood urea mg/dl	83	15.00	46.0	29.7	7.7
Triglycerides mg/dl	83	100	214	185.4	36.3
High density lipoprotein mg/dl	83	36	70	56.9	9.8
Low density lipoprotein mg/dl	83	88	171	149.8	22.78
Cholesterol mg/dl	83	88	215	187.96	35.2
Protein carbonyl nmol/ml	83	21.8	110.9	53.7	20.9
Total number	83	-----	-----	-----	-----

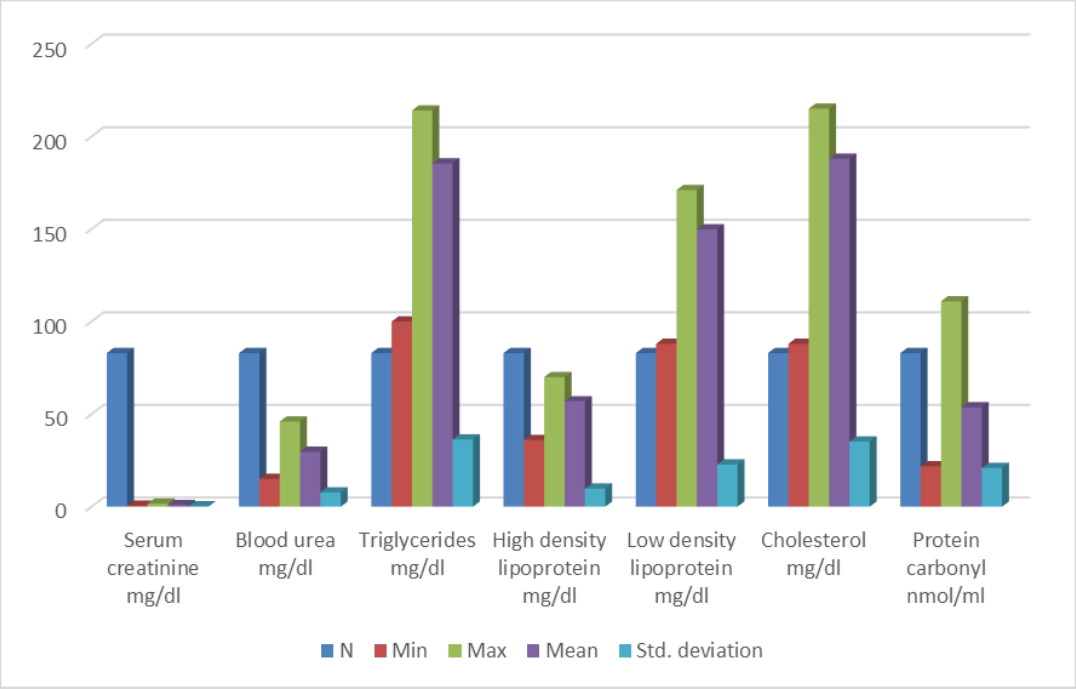


Figure 1: Showing the mean of different biochemical parameters

Table 2: *Showing the mean of different biochemical parameters*

Test	N	Min	Max	Mean	Std. Deviation
Serum creatinine mg/dl	59	0.50	1.6	0.9	0.2
Blood urea mg/dl	59	15.00	46.00	29.6	7.8
Triglycerides mg/dl	59	201	214	206.6	3.3
High density lipoprotein mg/dl	59	55	70	62.5	3.8
Low density lipoprotein mg/dl	59	151	171	162.5	4.8
Cholesterol mg/dl	59	200	215	206.4	4.1
Protein carbonyl nmol/ml	59	43.1	110.9	63.7	16.05
Total number	59	-----	-----	-----	-----

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Fifty-nine women with mean age 25±1 were selected. The levels of serum parameters were determined. Table 2 and Fig. 1 demonstrate that the values of all parameters were in normal range except protein carbonyl.

Table 3: ***Showing the mean value of different biochemical parameters of control***

Test	N	Min	Max	Mean	Std. Deviation
Serum creatinine mg/dl	24	0.6	1.3	0.8	0.2
Blood urea mg/dl	24	18.00	41.00	29.9	7.7
Triglycerides mg/dl	24	100.00	185.00	133.5	26.6
High density lipoprotein mg/dl	24	36.00	52.00	43.2	5.7
Low density lipoprotein mg/dl	24	88.00	146.00	118.6	19.0
Cholesterol mg/dl	24	88.00	190.00	142.5	36.6
Protein carbonyl nmol/ml	24	21.8	36.5	29.08	4.1
Total number	24	~~~~	~~~~	~~~~	~~~~

Table 3 show the results of seven biochemical parameter obtain through different techniques are in normal range.

Table 4: ***t test showing the variation between two groups regarding serum creatinine level***

Test	Category of the respondents	N	Mean	Std. Deviation	t	Sig.
Serum creatinine mg/dl	Subjects	59	0.9	0.2	1.7	0.0
	Control	24	0.8	0.19		

As shown in the table, serum creatinine levels did not differ significantly between the experimental and control groups, $t(83) = 1.7$, $p > 0.05$.

H0: Between two groups the difference is not significant

Table 5: ***t test showing difference in two groups regarding blood urea***

Test	Category of the respondents	N	Mean	Std. Deviation	t	Sig.
Blood urea mg/dl	Subjects	59	29.6	7.8	0.1	0.9
	Control	24	29.4	7.8		

H0: The different between two groups regarding blood urea is not significant

Table 6: ***t test showing difference in two groups regarding triglycerides***

Test	Category of the respondents	N	Mean	Std. Deviation	T	Sig.
Triglycerides mg/dl	Subjects	59	206.6	3.3	20.8~	0.000
	Control	24	133.5	26.6		~

Triglyceride levels were significantly elevated in the subject group compared to controls ($t = 20.8$, $p < 0.05$).

H0: The difference between two groups regarding triglyceride is significant

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Table 7: ***t test showing difference in two groups regarding HDL***

Test	Category of the respondents	N	Mean	Std. Deviation	t	Sig.
High density lipid mg/dl	Subjects	59	62.5	3.8	17.6	0.001
	Control	24	43.2	5.7	~	~

H0: The difference between two groups regarding HDL is significant

Table 8: ***t test showing difference in two groups regarding LDL***

Test	Category of the respondents	N	Mean	Std. Deviation	t	Sig.
Low density lipoprotein mg/dl	Subjects	59	162.5	4.8	~	~
	Control	24	118.6	19.01	16.5	0.000

H0: The difference between two groups regarding LDL is significant

Table 9: ***Difference between two groups regarding cholesterol***

Test	Category of the respondents	N	Mean	Std. Deviation	t	Sig.
Cholesterol mg/dl	Subjects	59	206.4	4.1	~	~
	Control	24	142.5	36.6	13.3	0.000

Cholesterol levels showed a statistically significant increase in the subject group compared to the control group ($t = 13.3$, $p < 0.05$).

H0: The difference between two groups regarding cholesterol is significant

Table 10: ***Difference protein carbonyl values nmol/ml between control and subjects***

Test	Category of the respondents	N	Mean	Std. Deviation	t	Sig.
Protein carbonyl nmol/ml	Subjects	59	63.7	16.05	~	~
		24	29.08	4.1	10.4	0.000

H0: Difference regarding protein carbonyl nmol/ml between the two groups is significant

Age matched group analysis of subject and control

Table 4.11: ***Descriptive statistics of biochemical parameters stratified by age group and contraceptive use (Mean ± SD)***

Parameter | A1: Subjects (20-25y) n=15 | B1: Control (20-25y) n=15 | A2: Subjects (26-30y) n=20 | B2: Control (26-30y) n=7 | A3: Subjects (>30y) n=24 | B3: Control (>30y) n=6 |
Serum Creatinine (mg/dl) | 0.9 ± 0.2 | 0.8 ± 0.2 | 0.9 ± 0.2 | 0.8 ± 0.1 | 0.9 ± 0.2 | 0.9 ± 0.2 |
Blood Urea (mg/dl) | 29.2 ± 7.2 | 29.0 ± 8.0 | 29.7 ± 8.1 | 28.7 ± 8.9 | 29.7 ± 8.2 | 31.0 ± 7.2 |
Triglycerides (mg/dl) | 206.4 ± 4.0 | 131.6 ± 30.6 | 206.9 ± 3.2 | 128.7 ± 22.9 | 206.5 ± 3.0 | 142.5 ± 24.1 |
HDL (mg/dl) | 62.8 ± 4.1 | 44.3 ± 5.2 | 61.3 ± 3.9 | 41.8 ± 5.6 | 63.4 ± 3.5 | 43.8 ± 7.3 |
LDL (mg/dl) | 161.2 ± 4.9 | 117.5 ± 20.0 | 162.7 ± 4.9 | 125.4 ± 19.9 | 163.2 ± 4.8 | 115.1 ± 20.2 |
Cholesterol (mg/dl) | 207.2 ± 5.0 | 146.7 ± 35.2 | 206.9 ± 4.0 | 140.2 ± 39.1 | 205.5 ± 3.5 | 137.6 ± 42.2 |
Protein Carbonyl (nmol/ml) | 65.9 ± 21.0 | 29.1 ± 3.8 | 60.2 ± 10.6 | 28.8 ± 4.7 | 65.3 ± 16.2 | 29.3 ± 4.5 |

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Table 12: ***Age-matched independent t-test comparisons between Subjects and Controls***

Parameter	A1 vs B1 (t, p-value)	A2 vs B2 (t, p-value)	A3 vs B3 (t, p-value)
Serum Creatinine	t = 0.9, p = 0.3	t = 1.1, p = 0.2	t = 0.6, p = 0.5
Blood Urea	t = 0.0, p = 0.9	t = 0.2, p = 0.7	t = -0.3, p = 0.7
Triglycerides	t = 8.4, p < 0.001	t = 15.6, p < 0.001	t = 13.7, p < 0.001
HDL	t = 9.1, p < 0.001	t = 10.3, p < 0.001	t = 9.8, p < 0.001
LDL	t = 8.6, p = 0.002	t = 8.0, p < 0.001	t = 11.2, p < 0.001
Cholesterol	t = 5.9, p = 0.001	t = 8.1, p < 0.001	t = 8.5, p < 0.001
Protein Carbonyl	t = 5.1, p = 0.001	t = 8.9, p < 0.001	t = 5.5, p < 0.001

Age wise group

Table 11: ***Age matched group (A1 group) and control (B1) in serum creatinine mg/dl***

Test	Age group	Sample size	Mean	Std. Deviation	t	Sig.
Serum creatinine mg/dl	A1	15	0.9	0.2	0.9	0.3
	B1	11	0.8	0.2	~	~

Table 12: ***Age matched group (A1 group) and control (B1) in blood urea mg/dl***

Test	Age group	N	Mean	Std. Deviation	t	Sig.
Blood urea mg/dl	A1	15	29.2	7.2	0.0	0.9
	B1	11	29.0	8.0	~	~

Table 13: ***Age matched group (A1 group) and control (B1) in triglycerides***

Test	Age group	N	Mean	Std. Deviation	t	Sig.
Triglycerides mg/dl	A1	15	207	3.9	8.4	0.000
	B1	11	134.6	30.9	~	~

Table 14: ***Age matched group (A1 group) and control (B1) in HDL***

Test	Age group	N	Mean	Std. Deviation	t	Sig.
High density lipoprotein mg/dl	A1	15	62.3	3.9	9.1	0.000
	B1	11	44	5.6	~	~

Table 15: ***Age matched group (A1 group) and control (B1) in LDL***

Test	Age group	N	Mean	Std. Deviation	t	Sig.
Low density lipoprotein mg/dl	A1	15	161.3	5.1	8.6	0.002
	B1	11	119.1	16.7	~	~

Table 16: ***Age matched group (A1 group) and control (B1) in cholesterol mg/dl***

Test	Age group	N	Mean	Std. Deviation	t	Sig.
Cholesterol mg/dl	A1	15	207.4	5.1	5.9	0.001
	B1	11	146.2	37.06	~	~

Table 17: ***Age matched group (A1 group) and control (B1) in protein carbonyl nmol/ml***

Test	Age group	N	Mean	Std. Deviation	t	Sig.
Protein carbonyl nmol/ml	A1	15	66.6	22.7	5.09	0.001
	B1	11	29.3	3.9		

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Table 18: *Age matched group (A2 group) and control (B2) in serum creatinine mg/dl*

Test	Age group	N	Mean	Std. Deviation	t	Sig.
Serum creatinine mg/dl	A2	20	0.9	0.2	1.1	0.2
	B2	7	0.8	0.1	--	--

Table 19: *Age matched group in blood urea*

Test	Age group	N	Mean	Std. Deviation	t	Sig.
Blood urea mg/dl	A2	20	29.7	8.1	0.2	0.7
	B2	7	28.7	8.9	--	--

Table 20: *Age matched group in triglycerides mg/dl*

Test	Age group	N	Mean	Std. Deviation	t	Sig.
Triglycerides mg/dl	A2	20	206.6	3.3	15.6	0.000
	B2	7	125.3	23.2	--	--

Table 21: *Age matched group (A2 group) and control (B2) in high density lipoprotein mg/dl*

Test	Age group	N	Mean	Std. Deviation	t	Sig.
High density lipoprotein mg/dl	A2	20	61.3	4.09	10.3	0.000
	B2	7	42	5.3	--	--

Table 22: *Age matched group (A2) and control (B2) in low density lipoprotein mg/dl*

Test	Age group	N	Mean	Std. Deviation	t	Sig.
Low density lipoprotein mg/dl	A2	20	162.9	4.9	8.04	0.000
	B2	7	120.7	22.7	--	--

Table 23: *Age matched group cholesterol mg/dl*

Test	Age group	N	Mean	Std. Deviation	t	Sig.
Cholesterol mg/dl	A2	20	206.3	3.8	8.08	0.000
	B2	7	141.5	36.4	--	--

Table 24: *Age matched group in protein carbonyl nmole/ml*

Test	Age group	N	Mean	Std. Deviation	t	Sig.
Protein carbonyl nmol/ml	A2	20	58.5	9.1	8.9	0.000
	B2	7	28.5	4.4	--	--

Table 25: *Age matched group in serum creatinine mg/dl*

Test	Age group	N	Mean	Std. Deviation	t	Sig.
Serum creatinine mg/dl	A3	24	0.9	0.2	0.6	0.5
	B3	6	0.9	0.2	--	--

Table 26: *Age wise third group in blood urea mg/dl*

Test	Age group	N	Mean	Std. Deviation	t	Sig.
Blood urea mg/dl	A3	24	29.7	8.2	-.3	0.7
	B3	6	31.0	7.2	--	--

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Table 27: ***Age matched group in triglycerides mg/dl***

Test	Age group	N	Mean	Std. Deviation	t	Sig.
Triglycerides	A3	24	206.3	3.06	13.7	0.000
mg/dl	B3	6	142.5	24.1	--	--

Table 28: ***Age matched group high density lipoprotein mg/dl***

Test	Age group	N	Mean	Std. Deviation	t	Sig.
High density lipoprotein mg/dl	A3	24	63.5	3.5	9.8	0.000
	B3	6	43.8	7.3	--	--

Table 29: ***Age matched group in low density lipoprotein mg/dl***

Test	Age group	N	Mean	Std. Deviation	t	Sig.
Low density lipoprotein mg/dl	A3	24	162.8	4.8	11.2	0.000
	B3	6	115.1	20.2	--	--

Table 30: ***Age matched group (A3 group) and control (B3) in cholesterol mg/dl***

Test	Age group	N	Mean	Std. Deviation	t	Sig.
Cholesterol mg/dl	A3	24	206.04	3.8	8.5	0.000
	B3	6	137.6	42.2	--	--

Table 31: ***Age matched group (A3 group) and control (B3) in protein carbonyl nmol/ml***

Test	Age Group	N	Mean	Std. Deviation	t	Sig.
Protein carbonyl nmol/ml	A3	24	66.1	16.01	5.5	0.000
	B3	6	29.3	4.5	--	--

Comparison between different age group

One-way ANOVA showed no significant age-related differences in any of the evaluated biochemical parameters among the subjects ($P > 0.05$).

Discussion

This current study aimed to assess the impact of Oral Contraceptive Usage on Oxidative Stress, Kidney Function and Lipid Metabolism in females of Dera Ismail Khan District. Oral contraceptive users had significantly higher serum protein carbonyl levels and significantly different lipid parameters but not significantly different serum creatinine or blood urea. The results in this report indicate that the use of oral contraceptives may cause oxidative and metabolic alterations without any detectable decrease of renal function in the group of women studied.

No significant difference was observed between the oral contraceptive users and the controls in regard to serum creatinine ($t = 1.7$, $p = 0.07$). Likewise, blood urea was not different between the groups ($t = 0.1$, $p = 0.9$). The results suggest that taking oral contraceptives did not harm the kidney-function markers assessed. The findings are in contrast, however, to findings of Brändle et al. (1992) and Kurzweil et al. (2005) who found changes in kidney markers in long-term oral contraceptive use. This disagreement could be linked to various factors such as hormonal formulation, duration of treatment, dosage, age, nutritional status, sample size, or health status. Furthermore, mild and early renal changes may not be detectable in serum urea and creatinine, so it must be taken with caution that there are no significant alterations.

However, women taking oral contraceptives exhibited marked changes in lipid profile. Users had significantly higher levels of triglycerides, low density lipoproteins and total cholesterol than controls; and high density lipoproteins were also significantly different in users and controls. Our results agree with those previously reported by Stocco et al. (2013) who found significant changes in the levels of lipids in women who used combined oral contraceptives. They also corroborate Santos et al. (2008) who found higher

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lipid profile levels in women who used oral contraceptives as compared to those who did not use oral contraceptives. These modifications could be produced by the action of estrogen and progestogen on lipid synthesis and production of lipoproteins, as well as cholesterol transport in the liver. High levels of triglycerides, LDL and total cholesterol can lead to cardiovascular risk, especially if they continue to rise over time in women who have obesity, hypertension, diabetes, smoking habit and/or family history of cardiovascular disease.

The most prominent result was the significantly elevated protein carbonyl level in oral contraceptive users. The mean protein carbonyl concentration was 63.7 in users and 29.08 in controls ($t = 10.4$, $p < 0.05$). These results corroborate an increase in protein oxidation and justify the use of protein carbonyls as markers of oxidative stress. It is consistent with Dalle-Donne et al. (2003), who have found that protein carbonyl levels are higher in conditions with oxidative stress, and with Oberg et al. (2004) and Fernando et al. (2016), who have shown that protein carbonyls are a good marker of oxidative cellular damage. The relationship between the increased level of protein carbonyls and the dyslipidaemia may suggest a systemic coupling between oxidation and lipid metabolism.

No significant age related difference was seen in the biochemical parameters analysed. In all, use of oral contraceptives was linked to higher oxidative stress and to changes in lipid metabolism but not to any notable changes in markers of kidney function. These findings confirm that biochemical monitoring should be done periodically among long-term users.

Conclusion and Recommendations

Conclusion

The researchers find that women taking the pill showed significantly higher levels of protein carbonyl in their blood than women who did not take the pill, suggesting higher levels of oxidative stress and protein oxidation. Protein carbonyls could thus be useful markers of oral contraceptive-related oral oxidative damage. However, oral contraceptive users also exhibited substantially changes in triglycerides, HDL, LDL and total cholesterol, indicating lipid metabolic changes that may lead to a greater cardiovascular risk in the longer term. However, there were no significant differences between the oral contraceptive users and the controls for the serum creatinine and blood urea. In the present cohort, therefore, there was no evidence of detectable impairment of renal function with oral contraceptive use. There was no significant difference in the biochemical parameters measured among the different age groups. In conclusion, the results indicate that estrogens from OCPs are more closely linked with oxidative stress and lipid alterations than with conventional renal-function markers.

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