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ORIGINAL ARTICLE

SERUM LEVELS OF OXIDATIVE STRESS PARAMETERS IN POSTMENOPAUSAL VERSUS FERTILE WOMEN OF KUTAHYA CITY, TURKEY

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ABSTRACT

Background: Decreased concentration of both oestrogen hormone and vitamin D in postmenopausal period may lead to oxidative stress, lipid peroxidation, dyslipidaemia, osteoporosis, and cardiovascular diseases. The objectives of this study were to determine and compare the serum levels of oxidative stress parameters; TOS, TAS, OSI, PON-1, ARYL in postmenopausal and fertile women of Kutahya city, Turkey.

Materials & Methods: This cross-sectional study was conducted at Department of Biochemistry, Faculty of Medicine, Kutahya University of Health Sciences, Kutahya, Turkey from July, 2016 to December, 2016. Kutahya is a city in western Turkey, with population of 237,804 as per 2011 estimates. Two samples were drawn from this population with consecutive technique. Study group included 40 menopausal women, while control group included 40 healthy fertile women. After overnight fasting, venous blood samples were collected, centrifuged, aliquoted into a polystyrene tube, and aliquots were stored at -80°C until measurement for total oxidant status (TOS), total antioxidant status (TAS), paraoxonase-1 (PON-1), and arylesterase (ARYL) measurements were made. Levels of TOS, TAS, OSI, PON-1, ARYL were five research variables on ratio scale. 'Normal' data were described by mean, range and SD and skewed data by median, Q1, Q3 and IQR with 95% confidence intervals. For normal data, independent-samples t-test and for skewed data Mann–Whitney U test was used for hypotheses testing.

Results: Serum total oxidant status (TOS) and oxidative stress index (OSI) levels were significantly higher (p-value .015 & .003 respectively), and serum paraoxonase-1 (PON-1) and arylesterase (ARYL) levels were significantly lower in postmenopausal than fertile women (p-value <.0001 & .0005 respectively), with no statistical difference for serum total antioxidant status (TAS) levels between the two groups (p-value .186).

Conclusion: Postmenopausal period is associated with oxidative stress and decreased antioxidant defence. HDL-c-dependent PON-1 and ARYL activities are also reducing due to a decrease of HDL-c with menopause. Elevated levels of TOS and OSI, decreased levels of PON-1 and ARYL may lead to various life-threatening diseases such as cardiovascular disorders or cancer.

KEY WORDS: Antioxidants; Menopause; Postmenopause; Postmenopausal Period; Oxidative Stress; Arylesterase; Paraoxonase-1; Women.

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1. INTRODUCTION

1.1. Background: Menopause is described as the permanent cessation of menstrual cycles. It is characterized by the increased risk of cardiovascular diseases and osteoporosis due to abrupt drop of oestrogen levels in women. Therefore, it might be considered as a transitional period to the aging process with its own classical signs and symptoms.[1-4] When changes in cycle frequency or in menstrual flow are primely observed during the menopausal transition; both gonadotropins, oestradiol and

inhibin, show a marked degree of variability with rapid changes from typical post-menopausal patterns to those characteristics in the reproductive term. In postmenopausal women, inhibin and oestradiol levels were significantly decreased or undetectable while FSH levels were increased.[5]

Aerobic life is accompanied by the constant formation of reactive oxygen species (ROS), which are known to play a dual role as both harmful and beneficial properties. Due to their highly toxic and destructive nature, they are crucial in normal metabolic reactions and defence system.[6] Besides their beneficial effects; ROS, which are produced by the immune system in inflammatory reactions, may react each other and produce more free radicals which are potential oxidizing agents that can cause lipid peroxidation and DNA fragmentation.[7] Oestrogen is a phenolic compound and it has structural similarities with lipophilic antioxidant α-tocopherol, thus, the similarity of them enables the molecule to detoxify accumulated ROS and act as an antioxidant.[8] High concentration levels of oestrogen provides a significant antioxidant effect by inhibiting the 8-hydroxylation of guanine DNA bases, whereas the low concentration levels of oestrogen (especially when the structure contains catechol) has a prooxidants-like activity such as breaks in genetic material, formation of DNA adducts, and oxidation of bases.[9] Oxidative stress can cause deactivation of some metabolic pathways and defects in genetic materials, which may result in various diseases such as cardiovascular disorders and different types of cancer. In addition to endogenous oxygen metabolism, ROS is produced secondarily by a variety of environmental agents and have been considered as responsible for the aging, menopause, and osteoporosis.[6,10,11] It has been reported that menopause is associated with an increase in oxidative stress and a decrease in some antioxidant species.[11,12]

Paraoxonases were originally discovered as enzymes that hydrolyse exogenous toxic organophosphate compounds such as insecticide or nerve gas. The paraoxonases family currently consists of three members: PON-1, PON-2 and PON-3; which are encoded by three separate genes located between q21.3 and q22.1 on the long arm of chromosome 7 in humans.[13,14] Both PON-1 and PON-3 are associated with HDL-c and present anti-inflammatory and antioxidant properties. PON-2 and PON-3 are intracellular enzymes that modulate mitochondrial superoxide anion production and endoplasmic reticulum stress-induced apoptosis.[15] PON-1 is a calcium-dependent enzyme that consists of 354 amino acids with molecular mass 43 kDa and synthesized and secreted by liver.[16,17] It resides on HDL-c and protects against the oxidative modification of HDL-c and LDL-c by hydrolysing lipid peroxides. PON-1 binds tightly to HDL subfractions which are containing apoA-1 and apoJ or clusterin and is capable to protect LDL-c from oxidation. Oxidation of LDL-c plays an important role in the development and progression of atherosclerotic lesion. PON-1 inhibits HDL-c and LDL-c oxidation and maintains their functions.[17] Moreover, PON-1 esterase acts as an important component of the enzymatic antioxidant system with arylesterase (ARYL) having the same functions.[18] In result of increased lipid profile and cardiovascular risk, the investigation of PON-1 and ARYL activities in postmenopausal women is crucial.

Although numerous studies have been performed in this area, as far as our knowledge, this paper is the first study that combines studied parameters.

1.2 Research Problems (RPs), Knowledge Gaps (KGs), Rationale & Research Questions (RQs): Serum levels of five oxidative stress parameters; TOS, TAS, OSI, PON-1, ARYL in postmenopausal and fertile women of Kutahya city, Turkey are not known to us. This unawareness of five pieces of information are our five KGs. To fill these five gaps is the rational of our study. What would be the serum levels of these five parameters in our population are our five RQs.

1.3 Research Objectives (ROs)

RO 1-5: To determine the serum levels of oxidative stress parameters; TOS, TAS, OSI, PON-1, ARYL in postmenopausal and fertile women of Kutahya city, Turkey.

RO 6-10: To compare the serum levels of oxidative stress parameters; TOS, TAS, OSI, PON-1, ARYL in postmenopausal and fertile women of Kutahya city, Turkey.

1.4 Research (Null) Hypotheses (RHs):

H_{01}: There is statistically no significant difference in the serum levels of TOS in postmenopausal and fertile women of Kutahya city, Turkey. (RQ 6)

H_{02}: There is statistically no significant difference in the serum levels of TAS in postmenopausal and fertile women of Kutahya city, Turkey. (RQ 7)

H_{03}: There is statistically no significant difference in the serum levels of OSI in postmenopausal and fertile women of Kutahya city, Turkey. (RQ 8)

H_{04}: There is statistically no significant difference in the serum levels of PON-1in postmenopausal and fertile women of Kutahya city, Turkey. (RQ 9)

H_{05}: There is statistically no significant difference in the serum levels of ARYL in postmenopausal and fertile women of Kutahya city, Turkey. (RQ 10)

1.5 Significance: With information in hand, we can formulate local guidelines for diagnosis and management of menopausal symptoms in women.

2. MATERIAL AND METHODS

2.1 Design, Duration & Setting: This cross-sec-

tional study was carried out at the Department of Biochemistry, Faculty of Medicine, Kutahya University of Health Sciences, Kutahya, Turkey from July, 2016 to December, 2016. The samples were selected from the Department of Medical Biochemistry, Evliya Celebi Research and Education Hospital, Kutahya, The guidelines of the Declaration of Helsinki were followed. Ethical committee approval was granted by the local Human Research Ethics Committee. Written informed consent was obtained from all the subjects.

2.2 Population, Sample Size & Technique and Selection: Kutahya is a city in western Turkey, with population of 576,688 as per year 2020 estimates. The sample size calculation was based on arylesterase (ARYL) levels. With presumed mean & SD levels of 548±90 for menopausal women (group 1) and 625±110 for fertile women (group 2), with difference of -77 (U/L), ratio of sample size (group 2/ group 1) as 1, confidence level 99% and power 80%, the sample size was calculated as 40 for each group, with total 80, through an online sample size calculator "OpenEpi", available at: https://www.openepi.com/SampleSize/SSMean.htm

Two samples were drawn from this population with consecutive non-probability technique. Group 1 (study group) included 40 menopausal women (no menses for at least 12 months), who had never used a hormone replacement therapy, while group 2 (control group) included 40 healthy fertile women with regular menses. Women with alcoholism, smoking, existence of chronic medical illnesses (heart failure, hypertension, renal failure, diabetes mellitus, autoimmune diseases, respiratory diseases, cerebral failure, and peripheral vascular diseases), chronic pharmacological therapy, use of supplemental multivitamins, minerals, and antioxidants, with capability of interfering oxidant-antioxidant balance, were excluded from both the groups.

2.3 Conduct of procedure for blood sample collection and laboratory testing

2.3.1 Blood sample collection

After overnight fasting, venous blood samples were collected and centrifuged at x 1500 g for 15 mins to obtain serum samples. The serum samples were aliquoted into a polystyrene tube, and aliquots were stored at -80°C until total oxidant status (TOS), total antioxidant status (TAS), paraoxonase-1 (PON-1), and arylesterase (ARYL) measurements were made.

2.3.2 Measurement of serum TOS and TAS levels

Serum TOS and TAS levels were measured using a Beckman Coulter AU680 instrument (Beckman Coulter, Miami, FL, USA) with commercial reagents (Rel Assay Diagnostic, Gaziantep, Turkey). The methods was based on novel automated measurement methods developed by Erel.[19,20] TOS levels were expressed as μmol H_2O_2 equivalent/L. TAS levels were expressed as mmol Trolox equivalent/L.

2.3.3 Calculation of oxidative stress index (OSI)

The percent ratio of TOS to TAS was accepted as the OSI, an indicator of the degree of oxidative stress. To TAS in mmol Trolox equivalent/L was converted to μmol Trolox equivalent/L, after which the OSI was calculated as follows: OSI = [(TOS, μmol H_2O_2 Eq/L) / (TAS, μmol Trolox Eq/L) × 100]. The results are expressed as arbitrary units (AU).

2.3.4 Measurement of serum PON-1 and ARYL activities

Serum PON-1 and ARYL activities were measured on a Beckman Coulter AU680 instrument (Beckman Coulter, Miami, FL, USA) using commercial assay reagents (Rel Assay Diagnostic, Gaziantep, Turkey). The methods were based on automated measurement methods.[21] Serum PON-1 and ARYL activities were expressed as U/L.

2.4 Data Collection & Data Analysis Plan

2.4.1 Descriptive Statistics & Estimation of Parameters: Levels of TOS, TAS, OSI, PON-1, ARYL were our five research variables. The data type for these variables was on ratio (numeric) scale. The data for all these variables were subjected to tests of normality; skewness, kurtosis, CV% and Shapiro-Wilk test. If normal, were described by mean, minimum, maximum, range and standard deviation, otherwise described by median (Q2), Q1 (quartile), Q3 and IQR (Q3-Q1) for each group separately for the sample. Estimated parameters for population were given as confidence intervals (CI) of mean or of median at 95% confidence level (CL). Descriptively, the difference between the groups is based on comparing their confidence intervals (CIs), not their means or medians. If there is overlap of CIs, the levels are similar; otherwise different; lower or higher, as the case may be.

2.4.2 Hypotheses Testing: For variables with normally distributed data, parametric test independent-samples t-test (two tailed) was used for comparison, giving sample size, mean, SD, mean difference, CI of mean difference, degree of freedom, t-value and significance (p-value) at alpha .05. For variables with not normally distributed (skewed) data, non-parametric statistical test Mann–Whitney U test was used for comparison, giving median, interquartile ranges (IQRs), difference between medians, Mann-Whitney U, Wilcoxon W, Z, and significance (p-value). The data were analysed by SPSS version 16.0 (SPSS Inc., Chicago, IL, USA) for Windows.

3. RESULTS

3.1 Tests of normality: The results are shown in Table 3.1, with interpretation based on the four tests. All variables are interpreted as skewed except ARYL as 'Normal'.

3.2 Descriptive Statistics & Estimation of Parameters: Table 3.2.1 shows sample statistics and

population parameters for the four skewed data variables. Based on their confidence intervals (CIs), the median levels of TOS, TAS and OSI are similar in postmenopausal women and fertile women, as there is overlapping of their CIs. The median level of PON-1 is lower in postmenopausal women than fertile women, as there is no overlapping of their CIs. These differences between the two groups are only true descriptively. True/ real differences are shown by the hypotheses testing in next step.

Table 3.2.2 shows sample statistics and population parameters for one normal data variable; ARYL. The mean level of ARYL is lower in postmenopausal women than fertile women, as there is no overlapping of their CIs. This difference between the two groups is only true descriptively. True/ real difference is shown by the hypothesis testing in next step.

3.3 Hypotheses Testing

3.3.1 TOS level in postmenopausal versus fertile

Table 3.1: Tests of normality for five oxidative stress variables in postmenopausal (n=40) and fertile (n=40) women of Kutahya city, Turkey

Variables	Group (women)	Skewness	Kurtosis	CV %	W	p-value	Data distribution
TOS (μmol H_2O_2 Eq./L)	Postmenopausal	2.650	8.56	64.38	0.716	<.0001	Skewed
	Control	3.012	12.23	52.77	0.716	<.0001	Skewed
TAS (mmol Trolox Eq./L)	Postmenopausal	0.789	-0.131	10.20	0.930	.016	Skewed
	Control	0.242	-0.962	8.96	0.960	.174	Normal
OSI (Arbitrary Unit)	Postmenopausal	2.550	8.23	59.73	0.730	<.0001	Skewed
	Control	1.025	1.49	37.05	0.922	.009	Skewed
PON-1 (U/L)	Postmenopausal	1.638	1.783	60.84	0.766	<.0001	Skewed
	Control	0.514	-0.050	49.53	0.947	.059	Normal
ARYL (U/L)	Postmenopausal	-0.002	1.463	18.38	0.967	.290	Normal
	Control	0.043	-0.984	18.44	0.969	.343	Normal

CV%= Coefficient of variation %, W= Shapiro-Wilk Statistic

Table 3.2.1: Descriptive statistics & population parameters for four skewed oxidative stress variables in postmenopausal (n=40) and fertile (n=40) women of Kutahya city, Turkey

Variables	Groups (women)	Sample statistics				95%CI for median	
		Quartile 1 (Q1)	Median (Q2)	Quartile 3 (Q3)	IQR	Lower	Upper
TOS (μmol H_2O_2 Eq./L)	Postmenopausal	4.71	6.450	8.19	3.48	5.420	7.590
	Fertile	3.71	4.965	6.54	2.84	4.250	5.870
TAS (mmol Trolox Eq./L)	Postmenopausal	1.463	1.565	1.698	0.24	1.490	1.650
	Fertile	1.510	1.655	1.740	0.23	1.550	1.700
OSI (Arbitrary Unit)	Postmenopausal	0.315	0.435	0.480	0.16	0.340	0.460
	Fertile	0.223	0.315	0.398	0.18	0.250	0.380
PON-1 (U/L)	Postmenopausal	94.8	118.5	171.3	76.5	102.0	137.0
	Fertile	167.3	336.5	449.3	282	282.0	363.0

Q=Quartile, IQR=Inter quartile range (Q3-Q1), CI=Confidence Interval

Table 3.2.2: Descriptive statistics & estimation of parameters for one normal oxidative stress variable; ARYL in postmenopausal (n=40) and fertile women (n=40) of Kutahya city, Turkey

Variable	Group (women)	Sample Statistics					95% CI of Mean	
		Mean	Min.	Max.	Range	SD	Lower	Upper
ARYL (U/L)	Postmenopausal	553.5	264.0	838.0	574	101.7	521.0	586.0
	Fertile	643.8	428.0	856.0	428	118.7	605.9	681.8

n = Sample size, SD = Standard deviation, CI = Confidence interval

women (H_{01}): The serum TOS level was compared through Mann Whitney U test at alpha .05. With p-value less than .05, H_{01} was proved to be false and hence rejected, showing the difference to be statistically significant. İn simple words, serum TOS level was higher in postmenopausal women than fertile women. (Table 3.3.1)

3.3.2 TAS level in postmenopausal versus fertile women (H_{02}): The serum TAS level was compared through Mann Whitney U test at alpha .05. With p-value more than .05, H_{02} could not be proved as false (was true) and hence accepted, showing the difference is not statistically significant. İn simple words, serum TAS level was similar in postmenopausal and fertile women. (Table 3.3.2)

3.3.3 OSI level in postmenopausal versus fertile women (H_{03}): The serum OSI level was compared through Mann Whitney U test at alpha .05. With p-value less than .05, H_{03} was proved as false and hence rejected, showing the difference is statistically significant. İn simple words, serum OSI level was higher in postmenopausal than fertile women. (Table 3.3.3)

3.3.4 PON-1 level in postmenopausal versus fertile women (H_{04}): The serum PON-1 level was compared through Mann Whitney U test at alpha .05. With p-value less than .05, H_{04} was proved as false and hence rejected, showing the difference is statistically significant. İn simple words, serum PON-1 level was lower in postmenopausal than fertile women. (Table 3.3.4)

3.3.5 ARYL level in postmenopausal versus fertile women (H_{05}): The serum ARYL level was compared through independent samples t-test at alpha .05. With p-value less than .05, H_{05} was proved as false and hence rejected, showing the difference is statistically significant. İn simple words, serum ARYL level was lower in postmenopausal than fertile women. (Table 3.3.5)

Table 3.3.1: TOS (µmol H2O2 Eq./L) level in postmenopausal versus fertile women of Kutahya city, Turkey

Groups	Median	IQRs	Difference of medians	Mann-Whitney U	Wilcoxon W	Z	p-value (2-tailed)
Postmenopausal (n=40)	6.450	4.71-8.19	1.485	546.5	1366.5	-2.439	.015
Control (n=40)	4.965	3.71-6.54		Mann Whitney U test		H_{01} rejected at α 0.05	

Table 3.3.2: TAS (mmol Trolox Eq./L) level in postmenopausal versus fertile women of Kutahya city, Turkey

Groups (women)	Median	IQRs	Difference of medians	Mann-Whitney U	Wilcoxon W	Z	p-value (2-tailed)
Postmenopausal (n=40)	6.450	1.46-1.10	-0.09	662.0	1482.000	-1.328	.186
Control (n=40)	4.965	1.51-1.74		Mann Whitney U test		H_{02} accepted at α 0.05	

Table 3.3.3: OSI (Arbitrary Unit) level in postmenopausal versus fertile women of Kutahya city, Turkey

Groups (women)	Median	IQRs	Difference of medians	Mann-Whitney U	Wilcoxon W	Z	p-value (2-tailed)
Postmenopausal (n=40)	0.435	0.32-0.48	0.120	497.500	1317.500	-2.913	.003
Control (n=40)	0.315	0.22-0.40		Mann Whitney U test		H_{03} rejected at α 0.05	

Table 3.3.4: PON-1 (U/L) level in postmenopausal versus fertile women of Kutahya city, Turkey

Groups (women)	Median	IQRs	Difference of medians	Mann-Whitney U	Wilcoxon W	Z	p-value (2-tailed)
Postmenopausal (n=40)	118.5	94.75-171.3	218	242.5	1062.5	-5.365	<.0001
Control (n=40)	336.5	167.3-449.3		Mann Whitney U test		H_{04} rejected at α 0.05	

Table 3.3.5: ARYL (U/L) level in postmenopausal versus fertile women of Kutahya city, Turkey

Group (women)	Mean	SD	Difference of means	95% CI of difference		t-value	d.f.	p-value (2-tailed)
				Lower	Upper			
Postmenopausal (n=40)	553.5	101.7	-90.33	41.11	139.5	-3.654	78	.0005
Control (n=40)	643.8	118.7	Independent-samples t-test			H_{05} rejected at α 0.05		

n= Sample size, SD= Standard deviation, d.f.= Degree of freedom

4. DISCUSSION

4.1 TOS levels in postmenopausal versus fertile women (H_{01}): Our study showed serum TOS levels higher in postmenopausal women than fertile women. (Table 3.3.1) Accordingly, a study[22] conducted in Sanliurfa, Turkey, and a middle-European study[23] revealed that serum TOS levels were significantly higher in postmenopausal women. Additionally, Zhao et al.[24] recently reviewed 36 studies from different regions, and as a result overall of their investigation, they claimed that there were no statistically significant differences in TOS levels of postmenopausal women with osteoporosis compared to healthy controls.

4.2 TAS level in postmenopausal versus fertile women (H_{02}): Our study showed serum TAS levels similar in postmenopausal and fertile women. (Table 3.3.2) Although TAS levels of postmenopausal group were slightly lower than healthy controls, any statistical significance was not detected. Our findings were in agreement with the studies of Dikker, et al.[25] (Corum and Istanbul, Turkey) and Klisic et al.[23] (Montenegro and Serbia). However; Altindag et al.[22] (Sanliurfa, Turkey), Zovari, et al.[26] (Babol, Iran), Bednarek-Tupikowski, et al.[27] (Wroclaw, Poland), and Kolesnikova et al.[28] (Irkutsk, Russia) have found that serum TAS levels were significantly decreased in postmenopausal women. A decreased serum TAS activity is also reported in the review by Zhao, et al.[24] The reason for these differences might be related to various conditions such as dietary habits, milieu, regional variations, or hereditary specifications.

4.3 OSI level in postmenopausal versus fertile women (H_{03}): Our study showed serum OSI levels higher in postmenopausal than fertile women. (Table 3.3.3) Our findings are in accordance with the studies of Altindag et al.[22] (Sanliurfa, Turkey), Klisic et al.[23] (Montenegro and Serbia), and Zhao et al.[24] Vassalle et al.[29] (Pisa, Italy) investigated the effects of sex in coronary artery disease with association of oxidative stress and reported that women (all of them were postmenopausal) had three times higher oxidative stress compared to men.

Oxidative stress is strongly correlated with atherogenic index of plasma in cardiovascular disease and it is thought as an effective prognostic tool for early detection of cardiovascular risk in menopause.[9] OSI could be calculated with a number of methods and different parameters except used in this study. Investigation of OSI might be helpful to predict other possible metabolic disorders which are related to oxidative stress and deficiency of antioxidant defence system.

In the postmenopausal period, the protective effect of HDL-c to cardiovascular diseases tends to lose.[27] Some researchers have reported that any significant decrease were not observed on HDL-c levels,[31-33] whereas majority of previous studies stated that HDL-c levels are tended to decrease during menopausal transition and postmenopausal period.[34-38] PON-1 and PON-3 are esterases associated with HDL-c particle which hydrolyses arylesters, organophosphates, proinflammatory oxidized lipids present in oxidized LDL-c, and lipid peroxides in atherosclerotic lesions in association with ARYL and lactonase activity.[39,40] Several studies have previously reported that PON-1 and ARYL activities reduced with either surgical or natural menopause.[17,40,41]

4.4 PON-1 level in postmenopausal versus fertile women (H_{04}): Our study showed serum PON-1 levels lower in postmenopausal than fertile women. (Table 3.3.4) In a study, which investigated the oxidative stress in postmenopausal osteoporotic women in Kayseri,[42] Turkey, it was stated that PON-1 activity was found lower compared to healthy subjects. As far to our knowledge, the literature has tended to focus mostly on changes in PON-1 levels as a result of hormone replacement therapy in postmenopausal women. It has shown that hormone replacement therapy reduced the levels of oxidized LDL-c and increased PON-1 activity in postmenopausal women.[41] However, a study performed with the participation of 30 postmenopausal and 28 premenopausal healthy women (Buenos Aires, Argentina) reported that PON-1 activity did not show difference between both of the study groups.[43] In another study (Izmir, Turkey) on patients with acute coronary syndrome, it was reported that decreased serum PON-1 activity has been associated with increased oxidative stress and PON-1 activity was not connected to HDL-c.[44] Interestingly, an American study that investigated the comparison of HDL-c levels in African-American and white American non-diabetic postmenopausal women declared that African-American women represent significantly lower PON-1 activity, although HDL-c levels are higher than white American women.[45]

4.5 ARYL level in postmenopausal versus fertile women (H_{05}): Our study showed serum ARYL levels lower in postmenopausal than fertile women. (Table 3.3.5) As activities of ARYL and PON-1 are related, studies are mostly focused on the changes after the hormone replacement therapy in postmenopausal women. Similar to our findings, Sutherland et al.[39] (Dunedin, New Zealand) reported that serum PON-1 arylesterase activity was significantly lower, but it has significantly increased after the hormone replacement therapy in the postmenopausal diabetic women. Interestingly, Butorac et al.[46] (Zagreb, Croatia) reported that there were no statistically significant difference in the ARYL activity among premenopausal and postmenopausal women.

So far, however, there has been little evidence about the activities of PON-1 and ARYL in postmenopausal women in the literature. We modestly acknowledge that this study meets an important deficit by profiling

oxidative stress status in postmenopausal women.

4.6 Strengths of our study

4.6.1 Marwat's Logical Trajectory of Research Process: We have followed this 8-steps intellectual and logical flow of identifying our research problems, ascertaining the knowledge gaps; if there, narrating our problems as research questions, formulating them as objectives, collecting tentative answers for our questions from literature as hypotheses, collecting data for relevant variables from our sample, analysing and interpreting these data to get answers for our questions; thus filling our knowledge gaps and solving our problems.[47-49]

4.6.2 Population-Sample-Population Flow: Research is a systematic (step-by-step) process (never ending activity) aiming to solve the problems for a specified population. But many studies in global literature are seen, starting from the sample and ending on the sample, with no mention of their specified population/ population of interest/ population at risk. We have specified our population, have drawn the sample, collected & interpreted the data for the sample and then inferred it to our population as estimation of parameters and hypotheses testing, thus answering the questions and solving the problems regarding our population.[47-49]

5. CONCLUSION

The most obvious finding to emerge from this study is that menopause leads to changes in the body by increasing oxidative profile and decreasing antioxidant defence in relation with reduced HDL-c profile. These changes increase the risk for metabolic disorders such as cardiovascular diseases, osteoporosis, diabetes mellitus, and cancer. The decrease of HDL-c may lead to cardiovascular disease by a lack of its antioxidant activities of PON-1 and ARYL. The results of this study were in agreement with those of earlier studies, which suggested that changes in antioxidant defence were caused by reduced oestrogen concentrations seen along with menopause. Even if our study cohort was small, we humbly demonstrated the risk that might lead some of the major metabolic disorders in the postmenopausal period. Further comprehensive studies with a larger population are necessary for a better understanding of the risks that women encounter in the postmenopausal period.

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CONFLICT OF INTEREST
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AUTHORS' CONTRIBUTION

The following authors have made substantial contributions to the manuscript as under:

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All the authors agree to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.