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RESEARCH PAPER

Association of Hormonal imbalance in menopausal women with cardiovascular disease in Erbil city

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A B S T R A C T:

Menopausal women have a higher risk of cardiovascular disease (CVD), which is thought to be due to an imbalance in serum estradiol and total testosterone level. The aim of this study to find out the effect of menopause on sex hormone levels such as estrogen, testosterone, and progesterone in serum menopausal women and to elevate the relationship between these hormones and CVD. This case-control study included (224) participants (menopausal women with CVD and non-CVD) in the age range (of 40-69 years) who visited (The surgical specialty hospital cardiac center and Hawler teaching hospital). A self-administered questionnaire was used for data collection. Women with CVD represented 60.7%, who had previously been diagnosed by specialists in the cardiac center, and healthy menopausal women represented 39.3%. Parameters of serum (E2, TT, P and SHBG) and serum (Total Cholesterol, Triglycerides, HDL-C, LDL-C, and VLDL-C) were assessed for both groups. The mean value of E2 was significantly lower in menopausal women with CVD (20.066±1.773 Pg/mL vs 29.454±3.806 Pg/ml) along with serum progesterone level (0.233±0.010 ng/ml vs 0.451±0.136 ng/ml) and SHBG level (31.705±1.085 nmol/L vs 37.191±1.804 nmol/L) compared to healthy menopausal women (for all, $p<0.05$). On the other hand, a significantly elevated level of total testosterone (17.970±1.280 ng/dL vs 10.552±0.989 ng/dL) was observed in menopausal women with CVD compared with healthy women ($p<0.001$). Pearson correlation analysis showed the E2 negative correlation with LDL-C ($p<0.01$), and TT negative correlation with HDL-C ($P<0.01$). Low serum levels in terms of sex hormones, (E2, P, and SHBG) with high-level serum TT might be contributed to cardiovascular disease.

KEY WORDS: Sex hormone, Estradiol, Sex hormone-binding globulin, Total testosterone, Menopausal women, and, CVD.
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1.INTRODUCTION :

Cardiovascular disease refers to a group of illnesses affecting the heart and blood arteries (Olvera Lopez et al., 2022). Various sorts impact people of all ages. One may be born with a heart abnormality known as congenital heart disease, or one may have difficulties with heart rate known as arrhythmia, which includes coronary heart disease (CHD) and stroke, which are the top causes of death in women (Mack and Gopal, 2016).A significant association exists between menopause and an increased risk of CVD (Anagnostis et al., 2022).

The main cause of death worldwide in women is coronary heart disease (CHD). Despite a large amount of observational data, only a few studies have identified a link between endogenous testosterone and CVD risk in menopausal women, with mixed results (Lozano et al., 2012), (Sidney et al., 2016), (Chrysohoou et al., 2020) and (Islam et al., 2022). There are modifiable and non-modifiable risk factors that play an important role in the unwanted development of atherosclerosis of the coronary arteries. According to a 2019 study, age, sex, and race accounted for 63 to 80 % of prognostic performance, whereas modifiable risk variables had a little role. On the other hand, controlling modifiable risk factors resulted in significant decreases in CAD occurrences (Brown et al., 2021).Some people experience dizziness,

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exhaustion, chest pain, nausea, and shortness of breath, while others have no symptoms. High blood pressure, high cholesterol, diabetes, physical inactivity, heredity, and obesity are among the factors that contribute to CVD. This suggests that sex hormones play a role in CVD progression. Sex steroid shortage is thought to be a key role in the menopause-related alterations in CVD risk factor profile, as well as the subsequent rise in CVD risk (Lambrinoudaki et al., 2006). Menopausal women have a higher risk of CVD than premenopausal and males of the same age because after menopause, the advantage for women steadily disappears. Female estrogen's part in this process is largely responsible for this tendency (Mudali et al., 2005).

During the transitional period of menopause, women suffer from blood vessel aging, decreased diastolic ability, insulin sensitivity, and increased blood pressure due to decreased ovarian function and changes in hormone secretion, which increase the risk of CVD development (Maturana et al., 2011). The association between estrogen and HDL cholesterol is a positive correlation and the association with LDL cholesterol is a negative correlation has been established in several research (Haring et al., 2011). Higher levels of androgens and sex hormone-binding globulin (SHBG) were linked to a lower risk of atherosclerosis in certain studies (Reinecke et al., 2002); however, others discovered a link between testosterone and CVD risk (Mudali et al., 2005). Although a higher level of estradiol was linked to a lower risk of CVD, this consideration recommends that menopausal women with higher total testosterone and lower estradiol may be at risk for CVD, CHD, and myocardial infarction (MI). Studies looking at the affiliations between sex hormone levels and CVD occasions in menopausal women, be that as it may, have yielded clashing comes about. Both high and low androgen levels have been associated with an increased risk of CVD (Sievers et al., 2010). However, the impacts of estrogen substitution treatment on CVD risk as it were account for around 50% of the diminishment seen in cardiovascular infection, recommending that there must be extra mechanisms whereby estrogen applies its cardioprotective effects (Lam et al., 2011). This study, was aimed to ensure the relationship between levels of sex hormones in the serum of menopausal women with CVD who attended a cardiac center in Erbil city.

2. MATERIALS AND METHODS

2.1. Subjects and Sample collection

This case-control study included 224 subjects (menopause women). The subjects of this study were grouped into two categories: (Group 1): One hundred thirty-six (136) menopausal women with cardiovascular disease, and (Group II): Eighty-eight (88) selected subjects served as (control) healthy women. This was carried out from September 2021 until March 2022, by a collaboration of surgical specialty hospital cardiac center and Hawler teaching hospital. A questionnaire form was filled out for every patient with CVD and healthy group. In the presence of natural menopause, menopause was defined as the absence of menses for more than 12 months. To rule out CVD in the control group, ECG, physical examination, and chest X-ray were utilized. Participants had to be in menopause between the ages of (40–69 years old) were considered. The study excluded participants who had a history of endocrine disease, hepatic disease, renal failure, diabetes mellitus type 1, hormone replacement therapies, alcoholism, cancer, and chronic kidney disease. The Ethics Committee of Erbil's Directorate of Health collected and granted informed permission from all participants. The BMI was computed by dividing the weight (kg) by the square of height (metre2). Fasting blood sugar (FBS) samples were withdrawn from participants. And other fasting blood samples were collected via vein-puncture into vacutainer tubes with a coagulant gel tube kept at 20℃ then the collected samples underwent a 10-minute centrifugation process at 3500 rpm. The separated samples were either used immediately for the study of lipid profile serum (T.cholestrol, TGs, HDL-C, LDL-C, VLDL-C), by using (COBAS C-311 Roche kit Germany) measured by using (Roche/Hitachi COBAS C-311, Germany), the hormone tests (E2), (TT), (P) and (SHBG) by using (COBAS e-411 Roche kit Germany) measured by using (Roche/Hitachi COBAS e-411, Germany), an assay is a chemiluminescent immunoassay (CLIA) the serum samples for hormone and lipid profile tests were stored at -70°C until analyzed.

2.2. Inclusion and exclusion criteria

Menopausal women with cardiovascular disease and diabetic menopausal women with hypertension and CVD medication were included, Exclusion criteria were patients taking hormones replacement therapy, taking antioxidant supplements, any history of endocrine diseases, rheumatism diseases, cancer (being under treatment and/or diagnosed with malignancies), chronic kidney disease—stage 3 or higher), liver dysfunction (including viral hepatitis, cholestasis jaundice), ischemic or hemorrhagic stroke during 12 months before admission.

2.3. Estimation of serum estradiol (E2) levels

Elecsys Estradiol II examine (Roche/Hitachi COBAS e-411, Germany) utilized a competitive test concept with a polyclonal neutralizer only guided against 17β E2 to evaluate serum estradiol. The recuperation should be inside 10% of the first worth. Patients taking high biotin measurements (> 5 mg/day) shouldn't have tests acquired until somewhere around 8 hours have passed since the last biotin infusion. In menopausal women, the estimation range E2 in menopause women is (<54.7 Pg/mL),(Johnson et al., 1993).

2.4. Estimation of serum total testosterone levels

The total testosterone measure (Roche/Hitachi COBAS e-411, Germany) utilized a monoclonal immunizer explicitly coordinated against testosterone and was likewise based on a competitive test premise. Recuperation of 0.2 ng/mL of serum esteem 1 ng/mL, incline 0.9 1.1 + catch 0.05 ng/mL, and coefficient of connection > 0.95 are the models. TT levels in menopausal women range from 0.029 to 0.408 ng/mL (2.9-40.8 ng/dL), (Thienpont et al., 1994).

2.5. Estimation of serum progesterone levels

The progesterone examination (Roche/Hitachi COBAS e-411, Germany) utilized a Biotinylated monoclonal enemy of progesterone neutralizer that was especially coordinated against progesterone and was moreover founded on a serious test premise. The accompanying standards were utilized: incline 0.9 1.1 + block 0.1 ng/mL + Pearson coefficient of connection 0.95. In menopausal women, the estimation range for progesterone is (0.1-0.8 ng/mL), (Thienpont et al., 1991).

2.6. Estimation of serum sex hormone-binding globulin (SHBG) levels

An immunoassay is used to measure the amount of sex hormone binding globulin in human serum and plasma in vitro. The Elecsys and COBAS E 411 immunoassay analyzers are intended for use with the electrochemiluminescence immunoassay, or "ECLIA." First incubation: 10 µL of a sample, a biotinylated monoclonal SHBG specific antibody, and a monoclonal SHBG specific antibody labeled with a ruthenium complex a) form a sandwich complex. Second incubation: The complex is bound to the solid phase by the interaction of biotin and streptavidin after the addition of streptavidin-coated microparticles. Recuperation inside 90% of serum worth or slant 0.9 1.1 + capture 0.95 is the rule. SHBG levels in menopausal women range from (27.1 to 128 nmol/L), (Petra, 1991).

2.7. Assay of biochemical markers

The fasting serum lipid tests were performed by using colorimetric-enzymatic methods, using a fully automated biochemistry analyzer (Roche/Hitachi COBAS C-311, Germany) for the determination of total cholesterol, triglycerides, HDL-cholesterol. LDL-cholesterol. The assay was performed according to the manufacturer's specifications. Serums were separated by centrifugation at 3500rpm for 10 minutes. Cholesterol levels are considered to be below 200 mg/dL in adults (Greiling and Gressner, 1995). Triglyceride levels are considered below 150 mg/dL in adults (Greiling and Gressner, 1995). HDL-C levels are considered (45-65 mg/dL) in Female (Nauck et al., 1997). LDL-C levels are considered to be below 100 mg/dL in adults (Rifai et al., 1992). (VLDL-C) was determined indirectly using the formula (VLDL-C = triglycerides/5). And (VLDL-C) levels in adults are considered to be between (2-30) mg/dL. The serum AIP was determined utilizing the equation (AIP=log 10 TG/HDL-c).

2.8. Statically analysis

Data were analyzed using the Statistical Package for Social Sciences (SPSS, version 26). Results were expressed as mean± SD and mean ± SE. The difference among mean values of groups was determined by using the chi-square test, and a one-way (ANOVA) test was used to examine the difference between mean values of groups. Pearson correlation coefficient (r) was calculated to assess the strength of correlation between two numerical variables. A (p-value of ≤ 0.05) was considered statistically significant.

3. RESULTS

A total of 224 menopausal women were enrolled in this study. Participants were divided into two groups, group1 included 88 healthy menopausal women who represented 39.3% of the study population, and group 2 included 136 menopausal women with cardiovascular disease represented (60.7%) of the study population. A significant difference (p<0.001) was observed in the mean value and standard deviation of age among two groups; healthy women and menopausal women with CVD CVD (56.33±5.922: 58.87±5.954), respectively (Table 1). Similarly, the duration of menopause and BMI (body mass index) illustrated significant differences (p<0.005) between healthy women and menopausal women with CVD disease. According to the Chi-square test for n (%), significant differences were found in healthy menopausal women and menopausal women with CVD for the number and % of blood pressure, family history, diabetes, and smoking (p<0.01).

Table 1. Socio-demographic characteristics of the study population

Variables	**Cases (n=136) Mean ± SD n (%)**	**Controls (n=88) Mean ± SD n (%)**	**P-value**
Age (years)	58.87±5.954	56.33±5.922	0.002
Duration of menopause (years)	10.32±5.559	6.95±4.896	<0.001
BMI (Kg/m2)	29.419±4.168	27.761±3.651	0.003
Blood pressure (mmHg)	110(80.9%)	9(10.2%)	<0.001
MAP (mmHg)	10.260 ± 1.392	9.288 ± 0.939	<0.001
Family history by (years)	67(49.3%)	28(31.8%)	0.007
Type2 Diabetic	54(39.7%)	1(1.1%)	<0.001
Current smoking	7(5.1%)	7(8.0%)	0.021

SBP: systolic blood pressure, DBP: diastolic blood pressure, FBS: fasting blood sugar, MAP: Mean Arterial Pressure

However, significantly higher serum levels of (TC, TG, LDL-C, VLDL-C, and AIP) were estimated in menopausal women with CVD when compared with healthy menopausal women (P< 0.005), and there was a significantly lower serum level of HDL-c in menopausal women with CVD when compared with healthy menopausal women (P< 0.001) (table 2).

Table 2: Arithmetic means of biochemical parameters in the study population

Variables	**Cases (n=136)**	**Controls (n=88)**	**P-value**

	Mean ± SD	**Mean ± SD**	
T. Cholesterol(mg/dl)	194.90±51.869	173.28±31.969	0.001
Triglycerides(mg/dl)	188.09±65.820	156.26±63.360	0.007
HDL-C(mg/dl)	40.54±9.084	48.59±11.888	< 0.001
LDL-C(mg/dl)	98.431±39.395	74.173±29.260	< 0.001
VLDL-C(mg/dl)	37.618±13.164	32.889±12.220	0.007
AIP	0.651±0.191	0.401±0.223	< 0.001
FBS(mg/dl)	169.15±59.076	123.61±27.688	< 0.001

TC: Total cholesterol, TG: triglycerides, HDL-C: high-density lipoproteins cholesterol, LDL-C: low-density lipoproteins cholesterol, AIP: atherogenic index of plasma

Sex-Hormone parameters, which were illustrated in menopausal women with CVD appeared to be associated with lower mean values of serum E2, SHBG, P, and E2/TT ratio as compared to healthy menopausal women (P < 0.01). On the other hand, higher mean values of serum TT were estimated in menopausal women with CVD compared to healthy menopausal women (P < 0.01) (table 3).

Table 3. Sex hormone parameters in healthy women and menopause women with CVD

Sex-Hormone parameters	**Mean**	**SE**	**95% CI Lower**	**95% CI Upper**	**P-value**
E2(Pg/mL)					
• Case(n=136)	20.066	1.773	16.558	23.573	0.014
• Control(n=88)	29.454	3.806	21.888	37.020	
TT(ng/dL)					
• Case(n=136)	17.970	1.280	15.437	20.503	<0.001
• Control(n=88	10.552	0.989	8.585	12.519	
SHBG(nmol/L)					
• Case(n=136)	31.705	1.085	29.558	33.851	0.006
• Control(n=88	37.191	1.804	33.604	40.777	
P(ng/mL)					
• Case(n=136)	0.233	0.010	0.213	0.253	0.050
• Control(n=88	0.451	0.136	0.180	0.723	
E2/TT					
• Case(n=136)	1.676	0.131	1.417	1.935	<0.001
• Control(n=88	5.389	0.862	3.674	7.104	

Data are mean ±SE. E2: estradiol; TT: total testosterone; SHBG: sex hormone-binding globulin, P: Progesterone

Correlation of sex-hormone with lipid profiles in menopausal women with CVD. Correlation of sex hormones with lipid profile (table 4) shows the results from Pearson correlation coefficient (r) analysis between sex hormones and lipid parameters in menopausal women with CVD. Because BMI and age might mediate the

relationship between hormones and CVD, these variables were excluded from multivariate analysis. In menopausal women with CVD, E2 had a strong negative association with Tc (r= -

1.76, p =0.041) and LDL (r= -0.219, p= 0.011), whereas no statistical significance was found with TG and HDL-c (p>0.05). Progesterone showed a significant was found positive correlation with serum HDL-c(r=0.182, p=0.034) and there was a negative significant correlation for LDL-c, VLDL-c, and AIP (p<0.005).The SHBG showed a positive association with HDL-c, also SHBG showed a strong negative association with TC (r= -0.195, p=0.023),TG(r= -0.198, p=0.021), VLDL-c(r= -0.176, p=0.041) and AIP(r= -0.236, p=0.006).Total testosterone showed significant negative associations with HDL-c (r= -0.207, p= 0.016). The association between the E2 /TT ratio and lipid profile is the most remarkable finding in this study, according to Pearson correlation (r) analysis. Lower AIP and higher TC and LDL were closely linked to the E2/TT ratio. In particular, the E2/TT ratio had a negative association with TC (r= -0.49, p=0.003) and LDL (r= -0.228, p=0.007).

Table 4. Pearson correlation coefficients (r) of Sex-hormones, SHBG, and lipid profile in menopause women with CVD

Sex-hormone Parameters		**T.C (mg/dl)**	**T.G (mg/dl)**	**HDL-c (mg/dl)**	**LDL-c (mg/dl)**	**AIP**
E2 (Pg/ml)	r	-1.76*	-0.014	0.109	-0.219**	-0.098
	p-Value	0.041	0.870	0.219	0.011	0.269
Progesterone (ng/ml)	r	-0.167*	-0.092	0.182*	-0.247**	-0.208
	p-Value	0.050	0.288	0.034	0.004	0.015
SHBG (nmol/L)	r	-0.195**	-0.198**	0.060	-0.207*	-0.236**
	p-Value	0.023	0.021	0.489	0.016	0.006
TT (ng/dl)	r	-0.039	0.107	-0.207*	0.082	0.149
	p-Value	0.656	0.213	0.016	0.344	0.084
E2/TT	R	-0.49**	-0.53	0.123	-0.228**	-0.123
	p-Value	0.003	0.536	0.155	0.007	0.153

Association between Sex hormones and SHBG in menopausal women with CVD the endogenous hormones were moderately or highly correlated with one another. Among the menopausal women with CVD, the serum level of E2 strongly correlated with TT (r=0.301, p< 0.001), E2/TT ratio (r = 0.358, p< 0.001) and P (r=0.409, p< 0.001) (table 5). Testosterone was negatively associated with the E2 /TT ratio (r = -0.444, p< 0.001). The E2/TT ratio was significantly associated with P (r = 0.296, p< 0.001). SHBG was positively associated with TT (r= 0.010, p=0.908) which was not significant.

Table 5. Correlation between sex hormones and SHBG in menopausal women with CVD

Sex-hormone parameters		**E2 (Pg/ml)**	**TT**	**E2/ TT**	**Progesteron e**
TT (ng/dl)	r	0.301**			
	p-Value	<0.001			
E2/TT	r	0.358**	-0.444		

	p-Value	< 0.001	< 0.001		
Progesterone	r	0.409**	-0.009	0.296*	
(ng/ml)	*p-Value*	<0.001	0.917	<0.001	
SHBG	r	-0.048	0.010	-0.018	0.049
(nmol/L)	*p-Value*	0.582	0.908	0.833	0.568

According to the Pearson correlation analysis, the duration of menopause was highly significantly correlated with E2, P, and TT in menopausal women with CVD ($p < 0.05$), while regarding E2/TT and SHBG non-significant correlation with the duration of menopause ($p>0.05$) was observed. Considering the duration of the disease, results revealed the presence of a negative correlation between the duration of disease with E2, E2/TT, and Progesterone ($p=<0.05$). Age, results revealed the presence of a negative correlation between age with E2 ($p = 0.009$) and P ($p=0.015$). Fasting blood sugar (FBS), results revealed the presence of a negative correlation with SHBG ($p=0.745$). On the other hand, a high negative correlation between BMI with E2, E2/TT, P, and SHBG ($p <0.05$). While TT positive correlation with BMI was observed in (table 6).

Table 6. Pearson correlation coefficients for the association of sex hormones and SHBG, duration of menopause, duration of disease, age, fasting blood sugar, and BMI in menopause women with CVD

Sex-hormone parameters		**Duration of Menopause (Years)**	**Duration of disease (Years)**	**Age (Years)**	**FBS (mg/dl)**	**BMI (Kg/m^2)**
E2	r	-0.204*	-0.192*	-0.225**	0.41	-0.214*
(Pg/ml)	*p-Value*	0.017	0.025	0.009	0.634	0.013
TT	R	-0.173*	0.002	-0.113	0.025	0.075
(ng/dl)	*p-Value*	0.044	0.977	0.190	0.770	0.383
E2/TT	r	-0.010	-0.234**	-0.012	0.017	-0.365**
	p-Value	0.905	0.006	0.893	0.846	<0.001
Progesterone	r	-0.168	-0.334**	-0.207*	-0.028	-0.304**
(ng/ml)	*p-Value*	0.050	< 0.001	0.015	0.748	<0.001
SHBG	r	-0.071	-0.150	-0.160	-0.028	-0.241*
(nmol/l)	*p-Value*	0.412	0.081	0.063	0.745	0.0305

4. DISCUSSION

The association between sex hormones and CVD has been extensively studied. Estrogen has been linked to inflammatory processes, oxidative stress, angiogenesis, and lipid metabolism, implying that it may be beneficial to the cardiovascular system (Masood et al., 2010). Maturana et al., (2011), indicated the relationship between estradiol, testosterone, and lipoprotein in women, estradiol was negatively associated with lipid accumulation product and positively associated with HDL-c, whereas testosterone was positively associated with lipid accumulation product and negatively associated with HDL-c and SHBG (Maturana et al., 2011). The relationship between the E2/ TT ratio and CVD, however, is still uncertain. The association between the cerebral vasculature and the E2/TT balance was investigated. Adjusting estrogen and androgen levels in the brain has been discovered to have an impact on vascular tone, endothelial function, oxidative stress, and inflammatory responses (Krause et al., 2011). This demonstrated that keeping the balance of endogenous estrogen and androgen is critical for maintaining internal homeostasis.

Changes in lipoprotein profile, body fat distribution, vascular endothelium dysfunction, coagulation, and fibrinolysis occur as estrogen production from the ovaries reduces during menopause (Bales, 2000). The association between endogenous sex hormones and CVD and related risk factors in women might be clarified by a few molecular mechanisms. By reducing angiotensin-converting enzyme transcription, estrogen can enhance plasma concentrations of the endothelium-derived relaxing factor nitric oxide and inhibit the renin-angiotensin system (Miller and Duckles, 2008). Estrogens can lower blood pressure via boosting endothelial vasodilator activity and altering autonomic function, in addition to their beneficial effects on lipids. By up-regulating thromboxane, testosterone, on the other hand, can cause vasoconstriction and enhanced platelet aggregation (Wehr et al., 2011).

Androgens have been linked to increased visceral fat storage, lipid levels, and cardiometabolic risk factors in population studies (Maturana et al., 2008). In this study, there was a significant difference ($p<0.05$) between menopausal women with CVD and healthy women in the mean value of serum E2 levels (case: 20.066±1.773g/ml, control: 29.454±3.806Pg/ml) (Table3). Similar results have been reported by (Dai et al., 2012) showing that E2 levels concentration is lower in subjects with significant cardiovascular disease. Furthermore, these findings were supported by many previous studies (Rexrode et al., 2003) (Chen et al., 2011) and (Das et al., 2019). The decreased conversion of testosterone by an aromatase enzyme complex to E2 in menopausal women may be the reason for decreasing estradiol in this study. The beneficial effects induced by estrogen are well described and include reductions in LDL, an increase in HDL, and decreased LDL oxidation (Mendelsohn and Karas, 1999). Estrogen influences vascular tone, resulting in vasodilation, in addition to having a positive effect on lipid profile. In the vascular system, estrogen and testosterone affect vascular tone and endothelial function. These modifications may modify vasculature and increase atherogenicity in vessels, putting people at risk for cardiovascular disease (Khanna et al., 2021).

In the present study, E2 was negatively associated with cholesterol TC, and LDL-c (r= -1.76, p=0.041) (r= -0.219, p=0.011) (table 4). Progesterone's effects often counteract estrogens in a variety of physiological systems, including cardiovascular health. The E2 receptor in the artery wall can be downregulated by progesterone. LDL levels are increased in plasma as a result of down-regulation of LDL receptor activation, LDL oxidation is enhanced, and fibrinogen levels tend to rise when P is present (Zhu et al., 1999). In addition, the results of this study illustrated lower mean values of P level in menopausal women with CVD compared to healthy women (table 3), (case: 0.233±.010 ng/ml, control: 0.451±0.136 ng/ml) and (p-value <0.05), which was supported with other studies (Nickenig et al., 2000) , (He et al., 2007) and (Maturana et al., 2011).

In females, SHBG a serum protein that modulates free circulating hormone levels was found to be inversely associated with CVD (Sowers et al., 2003). As a result, a significant rise in TT in menopausal women with CVD in the current study compared to the healthy group could

be attributed to a low level of SHBG in menopausal women with CVD, which leads to an increase in TT. A negative correlation was observed between TT and serum HDL in menopausal women with CVD was observed (r= -0.207, p=0.016), (table 4), and a positive correlation between TT and TG in menopausal women with CVD (r=0.107, p=0.213). Similar results have been reported by (Maturana et al., 2011), (Das et al., 2019) and (Khanna et al., 2021). SHBG the main circulating protein that binds to and transports steroid sex hormones is produced by the liver. According to the free hormone hypothesis, SHBG-bound sex hormone is not available to target tissues. SHBG is a traditional indirect measure of androgenicity because it has a higher affinity for testosterone than E2 and its concentrations are negatively related to total testosterone (Rastrelli et al., 2018). Reduced SHBG levels have been linked to a negative lipid profile, including lower HDL-C levels (Noyan et al., 2004). The current study looked at serum SHBG to see if there was a link between CVD and SHBG. The results in this study indicated that the concentration of SHBG was lower in menopausal with CVD compared with the healthy group (case: 31.705±1.085 nmol/L, control: 37.191±1.804 nmol/L) with significant differences ($p<0.001$), as listed in (table 3). Similar results have been reported by other studies (Khanna et al., 2021) and (Buttari et al., 2022). Additionally, SHBG correlated positively with HDL cholesterol and significant negative correlation with total cholesterol, TG, LDL-c, and AIP in menopause women with CVD ($p<0.05$) as shown in (table 4). This result is supported by (Khanna et al., 2021). Also, a significant difference between SHBG and BMI in menopause with CVD was observed in (table 6). Apart from its relationship with a favorable risk profile, SHBG may protect against atherosclerosis through one of two mechanisms: 1) modulating bioavailable androgen and estrogen levels, or 2) direct effects at the cellular level. SHBG has a strong affinity for testosterone, dihydrotestosterone, and E2, allowing it to regulate their free concentrations (Bolognese et al., 2009). Because SHBG is stable and estradiol is bound with less affinity than testosterone (Mikhael et al., 2019).

The E2/TT ratio has a definite negative relationship with TC, LDL-C, and AIP, and a positive relationship with TG and HDL-c, as well as HDL-C/LDL-C (Dobiásová, 2006), confirming the AIP's importance as an atherogenic marker. These findings showed that the E2/TT ratio may play an essential role in lipid profile modulation. HDL's protective mechanism could be related to its function in reverse cholesterol transport, which results in cholesterol redistribution away from the arterial wall, as well as its suppression of monocyte adhesion and anti-oxidative activity, which could prevent LDL oxidation (Reddy Kilim and Chandala, 2013)

Additionally, menopause duration was significant negatively correlated with E2, TT and P in menopausal women with CVD as seen in (table 6) the study (Mirza et al., 2010) supported the outcome of this study and reached similar results. Also, a definitive consensus on the negative association between E2 and menopausal duration was found in different epidemiological research (Reddy Kilim and Chandala, 2013). As menopause duration increases, sex hormone and SHBG levels decrease faster, so menopause was one of the risk factors for heart disease. Bogers et al (2007) According to the findings, for every 5-unit increase in BMI, the risk of coronary heart disease (CHD) rises by 29% but lowers to 16% if blood pressure and cholesterol levels are adjusted. These results showed a statistically significant positive correlation between E2 and BMI in CVD menopause women (r=0.214, p=0.013). These results were in agreement with the result of (Chen et al., 2011) as shown in (table 6).

The outcome of this study noticed a highly significant difference ($p < 0.001$) between case and control groups in the mean values of serum AIP levels (case: 0.651±0.191, control: 0.401±0.223) (table 2). These results were in agreement with prior reports (Dai et al., 2012), (Khazaál, 2013). As a result of the high association between AIP and lipoprotein particle size found in several investigations, AIP could be used as a predictor of atherogenic lipoprotein status (Dobiášová and Frohlich, 2001). AIP has been shown to have a predictive value for atherosclerosis. (Njajou et al., 2009). Indeed, AIP levels of 0.3 to 0.1 have been linked to moderate CV risk, 0.1 to 0.24 with medium CV risk, and above 0.24 with high CV risk (M Dobiásová, 2006). Menopausal women in this study are thus at a medium risk of getting CAD, according to data estimated in this study. Although the differences in AIP did not demonstrate a statistically significant rise as menopause

continued, the absolute values suggest that the first decade of menopause is linked with low risk for CAD and the second decade is related to medium risk (Zhang et al., 2016)

5. CONCLUSIONS

In menopausal women, there was an imbalance of E2 and TT which might be associated with CVD, SHBG may be utilized as a predictor of CVD. High testosterone levels with low E2, and low SHBG levels raise the risk of CVD in menopausal women, either directly or indirectly, by affecting lipid profiles and BMI markers. E2 was shown to be negatively related to LDL-C and positively associated with HDL-C, while testosterone was found to be positively associated with LDL-C and negatively associated with HDL-c in menopausal women with CVD. The relationship between E2, SHBG, TT, and body mass index in menopausal women with CVD, E2, and SHBG was negatively associated with BMI, while testosterone was positively associated with BMI.

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Conflict of Interest

The authors declared that they have no conflicts of interest.

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