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Role of biochemical markers in pre, peri and postmenopausal women with comorbidities to assess the risk of Atherosclerotic cardiovascular disease.

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Abstract

Women experience hormonal changes in their life time, in addition, comorbidities also may affect them. Diabetes, hypertension, obesity, dyslipidemia dominate in postmenopausal period and several inflammatory markers, female specific risk factors influence risk of Atherosclerotic Cardiovascular disease (ASCVD) at postmenopausal age. Though a few studies have been carried out on selected biochemical markers of CVD in postmenopausal women with comorbidities, no detailed research has so far been focused on women in pre and perimenopausal stages. In this study, 210 women in the age group of 20-65 years who attended the Gynecology Out- Patient Department of a tertiary care hospital were recruited and stratified into pre, peri and postmenopausal groups (70 subjects in each group) based on STRAW classification. Those women with comorbidities, blood was collected and and biochemical markers such as high sensitivity C-reactive protein (hs-CRP), interleukin-6 (IL-6) and fibrinogen besides homocysteine (Hcy), lipid parameters, Apolipoprotein-B and estradiol were estimated. From among 70 subjects in each of pre, peri and postmenopausal groups, women with comorbidities were 27%, 28% and 43% respectively. Significant Higher total cholesterol, triglycerides, LDL-C and HDL-C found in peri and postmenopausal women with comorbidities. There was significant elevation in homocysteine ($p<0.000$), fibrinogen ($p<0.05$) IL-6 and hs-CRP in comorbid postmenopausal women with comorbidities when compared to pre and peri groups. Hyperhomocystienemia (HHcy) and higher hs-CRP levels found in peri and postmenopausal women. Hence an early screening of these biochemical markers to identify cardiac risk and aggressive risk factor lowering strategies with positive lifestyle patterns in peri and postmenopausal women are suggesting together was recommended along with appropriate management to overcome the development of ASCVD in their later age.

Keywords: Comorbidities; Atherosclerotic Cardiovascular diseases; Perimenopause.

Introduction:

Women experience hormonal changes in their life time in addition, to the diseases also may affect them. The term 'comorbidities' indicates the presence of more than one disease in the same person (Dervic *et al.*, 2021). Traditional risk factors such as diabetes, hypertension, obesity, dyslipidemia dominate in postmenopausal period and several inflammatory markers, female specific risk factors influence women's CVD risk at pre and peri menopausal stages (Maas *et al.*, 2021). The worldwide prevalence of diabetes mellitus is more than 500 million worldwide which is expected to rise in the following years. Diabetics have double risk of all-cause mortality with CVD being the major cause (Kaiser *et al.*, 2018; Preis *et al.*, 2009). The estimates in 2019 showed that 77 million individuals had diabetes in India, which is expected to rise to over 134 million by 2045 (Pradeepa & Mohan., 2021). Women with diabetes have high risk of cardiac failure than men (Sucharitha *et al.*, 2021).

Hypertension is another major risk factor of CVD. Menopause is associated with a two-fold increase in risk

of hypertension with a prevalence of 75% in postmenopausal women in the U.S.A. (Barton *et al.*, 2009). Post-menopausal women are at high risk for developing hypertention than men (Hyattsville *et al.,* 2011). Estrogen has potential benefits on vascular functions. As estrogen level falls the risk of developing hypertension in women rises shaply (Usoro *et al.*, 2006). Estrogens deficiency in post-menopause plays a crucial role in hypertension development due to adaptations of the sympathetic nervous system (SNS), renin-angiotensin-aldosterone system (RAAS), body mass (BM), endothelial function, oxidative stress, and salt sensitivity (Sabbatini *et al.*, 2020).

While considering the mortality rate due to coronary causes, women with diabetes are at a higher risk than men. In a meta-analysis of 37 studies (including 447.064 T2DM patients), the relative risk (RR 95% CI) for fatal CHD between patients with and without diabetes was greater among women 3.50 (2.0–4.53) than in men 2.06 (1.81–2.34) (Huxley *et al.*, 2006). Thus, in women with diabetes, the relative risk for a fatal coronary event is 50% higher than in men. This was probably explained by a less favorable cardiovascular risk profle in women linked to hypertension and hyperlipidemia (Bertoluci *et al.,* 2017). The women with diabetes seem to loose the protection greatly increasing the incidence of CHD with a greater mortality due to Acute myocardial infarction as compared to men (Huxley *et al.*, 2006).

As discussed in the earlier chapter, dyslipidemia is a strong predictor of coronary artery disease (Parinita *et al.*,2012) which is highly prevalent among women, with menopause still worsening the situation with lipid profile (Chandrakala & Desai., 2017). Menopause is associated with altered lipid metabolism due to hormonal changes, such as decreased level of estrogen and elevated FSH level that lead to the development of CVD (KO SH et.al, 2020). In women the metabolism is affected by alterations in hormonal levels throughout their lifetime during pre or postmenopausal period (William *et al*., 2019). Insulin resistance is also associated with dyslipidemia and metabolic syndrome which are linked to atherosclerosis. (Grundy, 2016).

Obesity is an independent risk factor for cardiovascular disease (CVD) and one of the main causes of the increased risk factors such as dyslipidemia, insulin resistance, hypertension both in adults and children (Barroso *et al.*, 2017; Akil *et al.*, 2011). Obesity enhances early atherosclerotic changes through several mechanisms including insulin resistance and inflammation (McGill et al., 2002). Between 1980 and 2015, the global prevalence of a body mass index was $\geq$ 25 kg/m^2 which rose from 27.8 to 39.4% in women, and 25.4 to 38.5% in men in later years. In parallel, the global prevalence of obesity increased from 8.9 to 14.8% in women and 5 to 10.1% in men (Chooi *et al.*, 2019). Obesity and several related downstream metabolic cardiovascular risk factors including elevated blood pressure, dyslipidemia and hyperglycemia, have been linked to atherosclerosis in autopsy studies of young adults (Berenson *et al.*, 1998. McGill *et al.*, 1995). Visceral adiposity promotes systemic and vascular inflammation, which is fundamental to all aspects of the atherosclerotic process from fatty streak development to atherothrombosis. (Rocha *et al.*, 2009; Ross,1999). Inflammation induced by obesity increases the low-density lipoprotein oxidation, which in turn promotes atherogenesis (Couillard *et al.*, 2005). Endothelial dysfunction in obesity, principally caused by diminished bioavailability of nitric oxide in the setting of inflammation and oxidative stress is also fundamental to atherosclerosis progression (Engin *et al.*, 2017). Multiple studies have established that obesity is the major risk factor for the development of Heart Failure. In Framingham Heart Study with 5881 participants, incidence of HF increased by 5% in men and 7% in women for every 1-unit BMI increase after the adjustment for other risk factors. The risk of HF increased across the entire spectrum of BMI (Kenchaiah *et al.*, 2002). This was subsequently confirmed in several studies (Hu *et al.*, 2010; Bozkurt *et al.*, 2016).

Biochemical markers such as homocysteine, hs-CRP, fibrinogen, interleukin-6, apo-B, and lipid profile play important role in prediction of ASCVD. Studies on assessment of these biochemical markers in different menopausal stages of women with comorbidities to assess the atherosclerotic risk are scanty. Hence the present study carried out to understand the role of biochemical markers in pre, peri and postmenopausal stages that could help in assessing ASCVD risk in women with comorbidities like diabetes, hypertension, dyslipidemia and obesity. This could help in the early detection of ASCVD risk and timely intervention to enhance their cardiovascular health

Section: Research Paper
ISSN 2063-5346

at postmenopausal age.

Materials and methods

Study subjects: In this cross sectional study, 210 women who satisfied the inclusion criteria were enrolled and are divided into pre, peri and post-menopausal groups as per Stages of Reproductive Aging Workshop (STRAW) classification (Harlow et al, 2012) with each group containing 70 subjects. The study was carried out in NRI Medical College Hospital, a tertiary care institution situated at Chinakakani in Guntur district of Andhra Pradesh (India) during the period July 2021 to February 2022. The ethical clearance was obtained for the study from Institutional Ethics Committee (IEC/NRIMC/168) and the Informed Consents were taken from each participant.

Inclusion criteria: Women aged between 20-65 years.

Exclusion criteria: Women on hormonal therapy, with carcinoma, renal failure, congestive heart failure, chronic disorders and pregnancy.

Methodology: From the overnight fasted participants, 5ml of venous blood was collected and centrifuged for the separation of serum. The serum samples were analyzed for hs-CRP, IL-6, and homocysteine, estradiol, total cholesterol, triglycerides, HDL-C, LDL cholesterol by standard methods (immuno turbidometry, immunometric immunoassay, competitive immunoassay, cholesterol oxidase peroxidase, 4-aminophenazone (CHOD-PAP) (Allain *et al.*,1974 clinical chem), glycerophosphate oxidase-peroxidase-4aminophena-zone (GPO-PAP) (Spayd *et al.*, *Clinical Chemistry*), Non –HDL precipitation method (Burstein *et al*, 1970 and cholesterol esterase and oxidase method). other markers such as Apo-B, FSH, FBS, plasma fibrinogen were estimated respectively by immuno turbidometry, immunometric immunoassay, Glucose oxidase peroxidase method (GOD-POD) (Curme *et al.*,1978) and clotting based assay in Sysmax fully automated analyzer (Clauss *et al.*, 1957).

Statistical analysis: The data obtained from the study was analyzed by One-way Analysis of variance (ANOVA) and for comparison among three groups using SPSS software 20.0 version. Data is represented as Mean ± SD, median and interquartile range. Values with $P < 0.05$ were considered as significant.

Results:

From among 70 subjects in each of pre, peri and postmenopausal groups, women with comorbidities were 27%, 28% and 43% respectively. Table -1 shows Mean ± SD of lipid markers, estradiol and homocysteine in pre, peri and postmenopausal women with comorbidities. The total cholesterol, TG, LDL-C levels are elevated in peri and postmenopausal women with comorbidities in comparison with premenopausal group and the difference is statistically significant with p value <0.05. Apo-B is also elevated in similar way with p<0.001. The alterations in lipid profile are high in peri menopausal women among all three groups where abrupt hormonal changes occur. Table -2 shows Mean ±SD of inflammatory markers and homocysteine in pre, peri and postmenopausal women with comorbidities. There was significant elevation in homocysteine (p<0.000), fibrinogen (p<0.05) IL-6 and hs- CRP in postmenopausal women with comorbidities when compared to pre and peri groups. When compared to premenopausal women, the levels of inflammatory markers and homocysteine are elevated in peri group.

Section: Research Paper
ISSN 2063-5346

Table -1 shows distribution of lipid markers, homocysteine and fibrinogen in pre, peri and postmenopausal women with comorbidities.

PARAMETER	Pre menopausal group (n=27)	Peri menopausal group (n=28)	Post menopausal group (n=43)	F	P
Total cholesterol	177.67±40.29	203.04±34.97	187.02±38.09	3.192	0.046
Triglycerides	144.48±54.86	190.36±84.65	177.44±83.21	2.631	0.077
HDL-C	40.63±8.62	38.25±7.65	35.86±7.86	2.98	0.056
LDL-C	103.14±33.51	125.66±28	114.53±32.40	3.506	0.034
Apo-B	100.79±24.70	127.96±28.49	126.16±29.03	8.66	0.000
Fibrinogen	232.94±69.22	270.83±72.47	283.86±75.46	4.12	0.019
Homocysteine	9.93±4.22	11.32±4.22	14.39±5.01	8.67	0.000

n= Number of subjects, $p<0.05$ is considered as significant.

Section: Research Paper
ISSN 2063-5346

Figure 1: Proportion of pre, peri and postmenopausal women with comorbidities

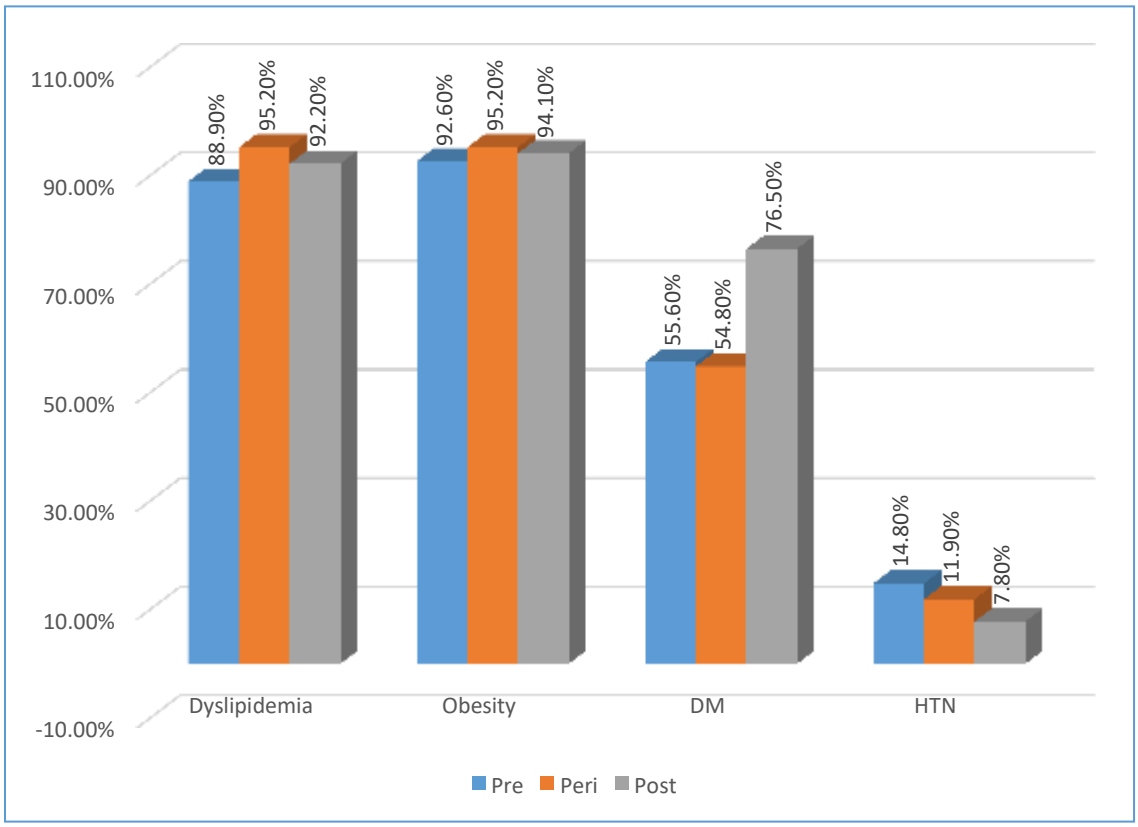

Table -2: Median and interquartile range of Inflammatory markers and estradiol in pre, peri and postmenopausal women with comorbidities.

Parameter	Premenopausal group (n=27)	Perimenopausal group (n=28)	Postmenopausal group (n=43)
IL-6	6.0	8.8	9.9
hs -CRP	5.2	5.15	6.7
Estrogen	160.33	46.36	18.63

Discussion:

In peri and postmenopausal women with comorbidities, elevated levels of TC (p=0.046), TG (p=0.090), LDL-C (p=0.034) were found with decreased HDL-C in comparison with premenopausal group suggesting increased ASCVD risk in peri and postmenopausal groups. An interesting observation of the present study was the higher apo-B levels in perimenopausal women with comorbidities than pre and postmenopausal counterparts. This may be due to abrupt hormonal changes and alteration in lipid metabolism taking place at peri menopausal stage which might progress further in postmenopausal age leading to atherosclerosis. According to the Study of Women's

Health Across the Nation (SWAN), significant increase in LDL-C, TC, Apo-B concentrations were seen within a year of the final menstrual period (Matthews et al, 2009). It has been reported that the reported that presence of comorbidities such as dyslipidemia, obesity, hypertension and diabetes accelerates CVD risk in menopausal women (Anagnostis *et al.*, 2021). A study conducted on 47 pre and 77 postmenopausal healthy Indian women by Shenoy et al., (2015) reported that postmenopausal women had higher cardiometabolic risk factors like TC, TG/HDL ratio, LDL-C than premenopausal women. The complications occur due to comorbidities and direct effect of diabetes on cardiovascular system are hypercoagulabity, oxidative stress and endothelial dysfunction which keep the patients with diabetic patients at a higher risk of CVD (Leon *et al.*, 2015). Another mechanism which links diabetes and obesity to CVD is low grade inflammation as the former promotes inflammation and accumulation of lipids by causing overproduction of cytokines impacting blood vessels causing myocardial infarction (Shoelson *et al.*, 2006).

In present study with comorbid women homocysteine and inflammatory markers IL-6 hs-CRP and fibrinogen were elevated in peri and postmenopausal groups in comparison with pre subjects. Kengne *et al.*, (2012) conducted pooled analysis from 4 prospective cohort studies that included 25,979 participants in the United Kingdom and followed them for 93 months. They found hs-CRP had link with 53% elevated risk of CVD. Females with diabetes are at increased CVD risk when compared to males. A meta-analysis of 37 studies and observed that 50% of diabetic women had higher risk of heart disease (Huxley et al., 2006.)

In diabetics, high blood glucose promotes formation of ROS that neutralizes the effects of NO which are vasorelaxant and anti-inflammatory (Meyer *et al.*, 2011). In menopausal women with hyperglycemia, the ratio of α and β estrogen receptor ratio decline with the decreased secretion of estradiol on endothelial estrogen receptor expression, that might lead to vascular injury (Chakrabarti *et al.*, 2009). Diabetes enhances oxidative stress and pro-inflammatory response which leads to the formation of advanced glycation end products and protein Kinase C activation. This causes vascular injury at the molecular level that promotes the progression of CVD. These combine together promote the development of CVD (Takenaka *et al.*, 2006; Razmjou *et al.*, 2018). Higher CVD risk observed among diabetic postmenopausal women (Simkhada et al., 2021). The related factors are increased LDL-C and fasting plasma glucose levels. Thus, menopause significantly increases the circulatory levels of lipids and lipoproteins with increased LDL-C levels (de Aloysio *et al.*, 1999). A study comparing pre and postmenopausal women , found higher levels of lipid profile parameters in the latter group (Shenoy et al., 2015). chronic inflammation is associated with diabetes and is characterized by increased pro-inflammatory cytokines, acute-phase proteins, and other mediators of inflammation which aggravate cardiovascular disease (Pandey *et al.*, 2015; Paneni *et al.*, 2013; Wang *et al.*, 2018). Therefore, diabetes in a menopausal female increases the risk of cardiovascular disease (zhou et al., 2019).

Hs- CRP is elevated in systemic inflammation and is identified as independently causing a significant risk of CVD. Cardiovascular risk assessment cut-offs of hs-CRP have been recommended by American Heart Association (AHA) as Low risk: 1.0 mg/L; moderate risk, 1.0 - 3.0 mg/L; and high risk: >3.0mg/L (Myers et al., 2004). The state of inflammation is affected by obesity altered levels of IL-6 in addition to the condition of diabetes. A study was conducted by Mohammed (2019) on 164 hypertensive diabetics aged 38 to 60 years among whom 118 women and 46 men had the highest tertile of hs-CRP was strongly associated in women with higher obesity indices, hypertension, altered lipid profile, elevated FBS and higher IL-6. In urban adults with hypertension, hypertriglyceridemia and hyperglycemia CRP levels were significantly elevated (Santos *et al.*, 2005) Subjects with FBG≥100 (mg/dl) had significantly elevated CRP levels (Kawamoto *et al.*, 2011). Peri menopausal females with diabetes had higher risk of CVD when compared to those without it (Cowie *et al.*, 2018).

Conclusion:

Peri and postmenopausal women with comorbidities were found to have hyperhomocystienemia (HHcy) and higher inflammatory markers such as hs –CRP. Hence an early screening for cardiac risk and aggressive risk

factor lowering strategies with positive lifestyle patterns in peri and postmenopausal women are suggesting together was recommended along with appropriate management to overcome the development of ASCVD in their later age.

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