H_S-CRP IS A POTENTIAL PROINFLAMMATORY BIOMARKER IN POSTMENOPAUSAL WOMEN

Sheikh Shafatin Mushtaree[1*] Hafizul Islam[2] Mahmudul Haque[3] Nayeema Tasnim[2] Nahida Afrin[1] Shantunu Dutta[4]

Abstract

Background: Menopause is a normal physiological process of permanent cessation of menstruation resulting from the eventual atresia of almost all oocytes in the ovaries. Risk of inflammation may be increased in menopause due to estrogen deprivation. Inflammatory markers are thought to be associated with development of atherosclerosis & coronary heart diseasein postmenopausal women. Several inflammatory markers like High Sensitivity C-reactive Protein (H_S-CRP) is held responsible for cardiovascular events in the post-menopausal women. In menopause production of H_S-CRP are increased from different cell types. The study was undertaken to estimate the serum high sensitive C-reactive protein (H_S-CRP) level for prediction of Atherosclerotic Cardiovascular Disease (ASCVD) risk due to inflammation in postmenopausal women.

Materials and methods: A hospital based observational study was carried out in outpatient Department of Gynecology & Obstetrics (CMCH) & Department of Biochemistry of Chittagong Medical College (CMC) from January 2019 to December 2019. One hundred (100) postmenopausal women aging 50-65 years was included in the study by non-probability consecutive sampling technique. Postmenopausal women were enrolled as cases by their given H/O of cessation of regular menstrual cycle for last one year. Serum H_S-CRP and fasting serum lipid profile were estimated in enrolled cases. Post-menopausal women considered as cases are grouped by the values of serum H_S-CRP according AHA (American Heart Association).Blood pressure and waist circumference were also recorded.

Results: In this studyLow risk, Average risk, and High risk group of H_S-CRP (according AHA) were identified with their frequencies of 9, 44, and 47 respectively. Other parameters of ASCVD risk factors such as lipid profile, Waist circumference, Hypertension were also significantly associated with different risk group of serum H_S-CRP in cases. Mean H_S-CRP were (2.13 ± 0.08 mg/L) and (5.5 ± 0.44 mg/L) in average and high risk group respectively in postmenopausal women.

Conclusion: This study established increased serum H_S-CRP as a sensitive proinflammatory biomarker for prediction of Atherosclerotic Cardiovascular Disease (ASCVD) risk in postmenopausal women.

Key words: H_S-CRP; AHA; PM; BMI; CVD; ASCVD.

Introduction

Coronary Artery Disease (CAD) is the leading cause of morbidity and mortality in developing countries both in male and female. According to WHO estimates, 16.7 million people around globe die of cardiovascular diseases each year-among them 8.6 million are women.[1] The cardiovascular disease accounts for more than 50% of all death in women over 50 years of age.[2] Studies have shown that women are at a lesser risk for developing coronary artery disease compared to males. But this advantages is abolished after menopause.[3] Menopause is defined by the WHO as 'the permanent cessation of menstruation as a result of the loss of ovarian activity'.[4] This transition is normally not sudden, tends to occur over a period of years. Menopause develops due to low estrogen production by disturbed hormonal cycle of ovulation.[5] Some medical literature suggest that menopause may be associated with an increased risk of cardiovascular disease. Inflammatory markers shown to be important & independent risk factors for prediction of Coronary Heart Disease (CHD) in postmenopausal women. Several circulating inflammatory marker like High Sensitivity C-reactive Protein (H_S-CRP) is associated with increased CVD risk in this population.[6] Process of inflammation is thought to be the key mechanism in atheroma formation, in pathogenesis of atherosclerosis, development of HTN, progression of plaque till its rupture & stent restenosis.[7]

It seems to have created a false impression that H_S-CRP and CRP are different which is incorrect. Actually both are same but in the mid-1990s, a new method enzyme linked immunosorbentassay

1. Lecturer of Biochemistry
Chittagong Medical College, Chattogram.
2. Associate Professor of Biochemistry
Chittagong Medical College, chattogram.
3. Professor of Biochemistry (Retired)
Chittagong Medical College, Chattogram.
4. Student of MPhil, Department of Biochemistry
Chittagong Medical College, Chattogram.

***Correspondence:** **Dr. Sheikh Shafatin Mushtaree**
E-mail: dr.mushtareeshafatin@yahoo.com
Cell : 01717 14 83 45

Submitted on : 10.12.2020
Accepted on : 26.12.2020

was established to evaluate the level of H_S-CRP (High Sensitivity C-reactive Protein) with greater sensitivity (Detecting CRP concentration <5mg/l) than those classic method used previously.[8] The Hs-CRP is the measurement of CRP level with greater accuracy. These sensitive measurement of CRP reflect low-grade inflammation and have a predictive value of future risk for CHD events.

C-reactive protein, the classical acute-phaseinflammatory biomarker belongs to the pentraxin protein family, is a nonspecific systemic marker of inflammation & tissue damage. CRP produced predominantly from hepatocytes in response to several cytokines.[9] Synthesis of CRP is mainly regulated by Interleukin-6 (IL-6) which in turn is up-regulated by other inflammatory cytokines such as IL-1 and Tumor Necrosis Factor (TNF)-a. CRP also produced locally in atherosclerotic lesions by SMCs lymphocytes and monocytic cells.[10] High sensitivity assays of C-reactive protein help to quantify low grades of systemic inflammation, in the absence of overt systemic inflammatory or immunological disorders.[11] The most widely evaluated biomarker in the quest for an ideal biomarker for global cardiovascular disease risk prediction ishigh sensitivity C-reactive protein.[12] H_S-crp is a sensitive marker of increased inflammatory activity of arterial wall.[13]

The possible mechanistic role of CRP in plaque deposition is highly complex, exerting pro-atherogenic effects in many cells involved in atherosclerosis, it may facilitate monocyte adhesion & transmigration into the vessel wall-a critical steps in atherosclerotic process.[14] Futhermore, M1 macrophage polarization, catalyzed by CRP, is also a pro-inflammatory trigger in plaque deposition, leading to macrophage infiltration of both adipose tissue & atherosclerotic lesion.[15] Beyond this inflammatory mechanism of plaque deposition, another studies have also shown an association among CRP, inhibition of endothelium nitric oxide synthase & impaired vasoreactivity.[16] In case of chronic low intensity inflammation CRP damage the glycocalyx of vascular endothelium, causing its dysfunction & making it more susceptible to pro-atherogenic factor.[17] Increased concentrations of high sensitivity C-reactive protein is well associated with an impairment of endothelial vasoreactivity and normalization of this level is associated with improvements in regional blood flow. A recent study showed prevalence of high H_S-CRP in postmenopausal women was 31%.[18]

Estrogen exerts cardio-protective action by maintaining high level of HDL-C (High Density Lipoprotein) lowering the LDL-C (Low Density Lipoprotein) & TG (Triglycerides) it also maintain the vascular tone by increasing nitrous oxide production. Loss of this protection after menopause may therefore be responsible for increased risk of developing CVD (Cardio Vascular Disease) inpostmenopausal women.[3] Evaluation of H_S-CRP along with serum lipid profile may increase the specificity of ongoing inflammation.

Vascular inflammatory changes can hardly be evaluated by using imaging method. From the available data, the centers for disease control & prevention, the American Heart Association scientific statement on markers of inflammation & cardiovascular disease has recommended that H_S-CRP may be measured in asymptomatic people with an intermediate risk of coronary heart disease (Class IIa recommendation) to optimize the global assessment of cardiovascular risk.[19] Cardiovascular risk assessment cut-offs have been recommended by American Heart Association (AHA) as low risk: (< 1mg/l), average risk: (1.0 ~ 3.0 mg/l) & high risk (>3.0 mg/l).[20] Therefore, the present study was aimed to estimate the serum high sensitivity C-reactive proteinin postmenopausal women. So that estimation of serum H_S-CRP can be used as a strong biomarker for prediction of future cardiovascular disease risk in postmenopausal women of Bangladesh.

Materials and methods

This hospital based observational study was carried out in the Department of Biochemistry (CMC) and outpatient Department of Gynecology & Obstetrics of Chittagong Medical College Hospital (CMCH) from January 2019 to December 2019. After taking proper permission from the concerned departments and ethical review committee, One hundred (100) postmenopausal women aging 50-65 years was included in the study by non-probability consecutive sampling technique. Post-menopausal women were enrolled as cases by their given H/O of cessation of regular menstrual cycle for last one year.

Inclusion criteria

Postmenopausal women aging 50-65 years attending outpatient Department of Gynecology & Obstetrics of Chittagong Medical College Hospital (CMCH).

Exclusion criteria

Women with the following chronic disorder (Diabetes mellitus, Cardiovascular disease Renal failure Malignancy) Women who are on medications that effects the immunological status HRT (Hormone Replacement Therapy) Cardiogenic drugs Anti-inflammatory drugs, Postmenopausal women who have undergone-Hysterectomy & oophorectomy.

Informed written consent was taken from all the participants. Patient-profiles were completed by relevant history, physical examination and anthropometric measurements. Serum H_S-CRP was measured by nephalometry in (Siemens BN pro-Spec) analyzer. Serum H_S-CRP was measured in mg/L. Concentration of overnight fasting serum lipid profile was measured by enzymatic kinetic method using an auto-analyzer. Serum H_S-CRP in cardiovascular risk assessment cut-offs have been recommended by American Heart Association (AHA) as low risk: (< 1mg/l) average risk: (1.0 ~ 3.0 mg/l) & high risk (>3.0 mg/l). Data were analyzed using computer based statistical software. Continuous data were expressed as mean ± SEM and categorical data as frequency and percentage. The confidence level was fixed at 95% and p-value of < 0.05 was considered significant.

Table I: Distribution and mean values of serum Hs-CRP in cases (n=100).

Serum Inflammatory Marker		n	Frequency	Percentage (%)	Mean	± SEM
Serum Hs-CRP (mg/L)	Low risk	100	09	9.0	0.57	0.11
	Average risk		44	44.0	2.13	0.08
	High risk		47	47.0	5.5	0.44

Table demonstrates that trend of elevated H_S-CRP were 44% in average risk group and 47% in high risk group in cases and the mean values of Hs-CRP were 0.57mg/l, 2.13mg/l, and 5.5 mg/l in Low average high risk group respectively in cases (Table I).

Table II : Distribution and mean values of fasting serum lipid profile in cases (n=100).

Serum Lipid Profile Status (n=100)		Frequency	Percentage (%)	Mean	± SEM
Serum Total cholesterol (mg/dl)	Normal	33	33.0	256.73	8.09
	Increased	67	67.0		
Serum Triglyceride (mg/dl)	Normal	15	15.0	235.26	9.36
	Increased	85	85.0		
Serum LDL (mg/dl)	Normal	13	13.0	156.41	6.39
	Increased	87	87.0		
Serum HDL (mg/dl)	Normal	36	36.0	33.67	0.56
	Increased	64	64.0		

Table shows that most of the cases of this study were dyslipidemic. And the mean values of serum total cholesterol, serum triglyceride and serum LDL level were 256.73 mg/dl, 235.26 mg/dl, 156.41mg/dl and serum HDL was 33.67 mg/dl in cases (Table II).

Table III : Association of serum Hs-CRP with serum Total Cholesterol (TC), serum Triglycerides (TG) status among the study subject with chi-square (χ^2)test significance (n=100).

Serum Hs-CRP Status (n=100)	Serum Total Cholesterol (TC) Status		p Value*
	Normal (n = 33)	Increased (n = 67)	
Low Risk	7 (21.2)	2 (3.0)	p < 0.05 Significant
Average Risk	15 (45.5)	29 (43.3)	
High Risk	11 (33.3)	36 (53.7)	
Serum Hs-CRP Status (n=100)	Serum Triglycerides (TG) Status		p Value*
	Normal (n = 15)	Increased (n = 85)	
Low Risk	4 (26.7)	5(5.9)	p < 0.05 Significant
Average Risk	8 (53.3)	36 (42.4)	
High Risk	3 (20.0)	44 (51.7)	

- Figures within parentheses indicate percentages

Table demonstrated that there were significant association between serum Total Cholesterol (TC) and serum Triglycerides (TG) with serum Hs-CRP, in different risk group of serum H_S-CRP in cases as (p< 0.05) (Table III).

Table IV : Association of serum Hs-CRP with serum Low Density Lipoprotein (LDL-C) cholesterol and High Density Lipoprotein (HDL-C) status among the study subject with chi-square (χ^2) test significance (n=100).

Serum Hs-CRP Status (n=100)	Serum Low Density Lipoprotein (LDL-C) Status		p Value*
	Normal (n = 13)	Increased (n = 87)	
Low Risk Significant	5 (38.5)	4 (4.6)	p < 0.05
Average Risk	2 (15.4)	42 (48.3)	
High Risk	6 (46.1)	41 (47.1)	
Serum Hs-CRP Status (n=100)	Serum High Density Lipoprotein (HDL-C) Status		p Value*
	Normal (n = 36)	Decreased (n = 64)	
Low Risk Significant	6 (16.7)	3 (4.7)	P < 0.05
Average Risk	18 (50)	26 (40.6)	
High Risk	12 (33.3)	35 (54.7)	

● Figures within parentheses indicate percentages

Table demonstrated that there were significant association between serum LDL-C and HDL-C with serum Hs-CRP, in different risk group of serum H_S-CRP in cases as ($p< 0.05$) (Table IV).

Table V : Association of serum Hs-CRP with Hypertension and Waist Circumference (WC) among the study subject with chi-square (χ^2) test significance (n=100).

Serum Hs-CRP Status (n=100)	Blood pressure status		p Value*
	Normal (n = 52)	Increased (n = 48)	
Low Risk Non-significant.	7 (13.46)	2 (4.2)	p > 0.05
Average Risk	34 (65.38)	10 (20.8)	p < 0.05 significant
High Risk	11 (21.15)	36 (75)	p < 0.05 Significant
Serum Hs-CRP Status (n=100)	Waist circumference (WC) Status		p Value*
	Normal (n = 15)	Increased (n = 85)	
Low Risk	3 (20)	6 (7.06)	p < 0.05 significant
Average Risk	5 (33.33))	39(45.88)	
High Risk	7 (46.67)	40 (47.06)	

Table demonstrated that there were significant association between increased blood pressure and serum Hs-CRP in average & high risk group of cases as ($p< 0.05$).there were significant association between serum Hs-crp and waist circumference (wc) in cases as ($p< 0.05$) (Table V).

Table VI : Pearson's Correlation between serum Hs-CRP and Lipid profile, Waist Circumference and HTN among the study subjects (n= 100).

Serum H_S-CRP and Serum Total Cholesterol (TC)	+0.191	p<0.05 significant
Serum H_S-CRP and Serum Serum Triglycerides (TG)	+0.379	p<0.001 Very Highly Significant
Serum H_S-CRP and Serum LDL Cholesterol (LDL-C)	+0.481	p<0.001 Very Highly Significant
Serum H_S-CRP and Serum HDL Cholesterol (HDL-C)	-0.385	P<0.001 Very Highly Significant
Serum H_S-CRP and Hypertension (HTN)	+0.345	p<0.001 Very Highly Significant
Serum H_S-CRP and Waist Circumference (WC)	+0.362	p<0.001 Very Highly Significant

Table shows positive significant correlations of serum Hs-CRP with serum lipid profile in cases. Hypertension, waist circumference also showing significant correlations with serum Hs-CRP in cases (Table VI).

Disscussion

This study is designed to evaluate the serum Hs-CRP in postmenopausal women. In addition to its other conventional cardiovascular risk factors were assessed to evaluate the association of this with the pro-inflammatory markers. The number of the postmenopausal women were hundred (n=100). This study revealed that there were significant association & correlation of serum Hs-CRP with other conventional CVD risk factors in postmenopausal women. Increased level of serum Hs-CRP classified into Low risk, Average risk and High risk group (AHA) were found in 9%, 44%, 47% cases respectively (Table I).

The mean ± SEM of serum Hs-CRP level in different risk group (Low risk, average risk, high risk) were 0.57± .11 mg/l, 2.13±.08 mg/l and 5.5±.44 mg/l respectively (Table I).

In this current study mean values of serum Total cholesterol, Triglycerides, LDL-Cholesterol were increased and serum HDL-Cholesterol were decreased in postmenopausal women as values are away from the desired level of lipid profile.(Table II) Similar observation were cited in the previous

studies done by Srinivas, Maulik et al Rajesh K Jambhulkar et al.[21-23] Decreased level of serum HDL-Cholesterol in postmenopausal women was found in this study and was also reported in the previous studies done by other researchers.[24,25]

There were significant association of increased serum total cholesterol, serum Triglycerides (TG) LDL-Cholesterol and decreased serum HDL-Cholesterol of different risk groups serum Hs-CRP in cases (Table III, IV). This observation was similar to the study done by Suma M Natarajet al and Sultan N et al.[26,27]

There were positive correlation of serum Total Cholesterol (TC) Triglycerides (TG), LDL-Cholesterol (LDL-C) with serum H_S-CRP in cases (Table VI). These observations were supported in another study.[28] Table VI also showed significant negative correlation between serum H_S-CRP and HDL-Cholesterol. This similar finding was in agreement with several other studies.[28,29]

Hypertension was significantly associated & correlated with average and high risk group of serum Hs-CRP in postmenopausal women in this study (Table V, VI). It is an established strong risk factor for CVD in postmenopausal women. This observation was supported in another study.[30]

In addition significant association of different risk group of serum Hs-CRP with Waist Circumference (WC) in cases were observed in this study (Table V). Significant correlation of Waist Circumference (WC) observed with serum H_S-CRP in cases (Table VI). This finding is documented by previous study done by other researchers.[31]

Cardiovascular events in postmenopausal women are specially addressed in this study as atherosclerotic risk factors are assumed to be increased in postmenopausal women. Greater CRP level identify persons at risk of progression to CVD. Inflammation plays a central role in the development of CVD. Human macrophage derived monocyte are stimulated with oxidized lipoprotein and hence Hs-CRP is expressed. Withdrawal of estrogen is documented to be responsible for such change in Hs-CRP level and causative lipid micro environment in early menopausal and postmenopausal women.[32] Hormonal changes after menopause is responsible for dyslipidemic changes causing cardiovascular disease in postmenopausal women. So the observation of the present study may be believed to be applicable for the development of CAD risk factor in postmenopausal women.

In all of the cited studies in this context, there was menopausal influence on the inflammatory markers such as Hs-CRP, lipid microenvironment, hypertension and waist circumference. Withdrawal effects of estrogen due to menopause is thought to have a role in pathogenesis of atherosclerosis and its consequences causing ASCVD in postmenopausal women.[32] Impaired endothelial integrity with the expression of Hs-CRP is responsible in development of microvascular in flammation. So use ofinflammatory markers such as Hs-CRP may assess the specificity for ongoing microvascular inflammation & predict patients at higher risk of atherosclerotic cardiovascular disease.

The shape of the association among the proinflammatory markers & cardiovascular risk were explained. The extent of the association could partially be explained by the difference in the conventional risk factors (Variables) with H_S-CRP. The postmenopausal women might benefit from close monitoring of such parameter along with the other risk factors for the development of Atherosclerotic Cardiovascular Diseases (ASCVD).

Limitations

This study has certain limitations, which include:-

- Sample size in the present study was small that may not reflect generalization of the findings to reference population.
- Observational type of the study may have lowered its strength.
- The study was conducted in a single Hospital.
- Gold standard method for diagnosis of ASCVD such as biopsy of the arterial wall and color Doppler were not done here.

Conclusion

From this study it can be concluded that H_S-CRP is significantly correlated with other variables of cardiovascular risk assessment; Except HTN & WC have no correlation with low risk group of H_S-CRP. H_S-CRP is conventional, cost effective & more frequently practiced method for prediction of inflammatory status. So estimation of serum H_S-CRP can be a beneficial approach for prediction of Atherosclerotic Cardiovascular Disease (ASCVD) risk in postmenopausal women.

Recommendations

i) Further studies are indicated to establish the serum H_S-CRP as early ASCVD risk predictor tool for Bangladeshi postmenopausal women.

ii) Multicenter study with large sample size should be done to get the national scenario.

iii) Community based intervention should be aimed to convey awareness regarding measurement of inflammatory marker serum H_S-CRP as early risk prediction tool for ASCVD in postmenopausal women.

Acknowledgement

Authors are grateful to Professor Dr. Pradip Kumar Dutta former Head, Department of Nephrology for his valuable advice and enthusiastic encouragement to complete the research works. The authors gratefully acknowledge the contribution of all the colleagues and staff of the Department of Biochemistry of Chittagong Medical College.

Contribution of authors

SSM- Conception, design, acquisition of data, drafting and final approval.

HI- Design, data analysis, interpretation of data, critical revision and final approval.

MH-Interpretation of data, critical revision and final approval.

NT- Acquisition of data, data analysis, drafting and final approval.

NA-Data analysis, interpretation of data, drafting and final approval.

SD- Acquisition of data, drafting and final approval.

Disclosure

All the authors declared no competing interests.

References

1. Bulletin of the World Health Organization 2013.

2. Muhil M Sembian U. Babitha, Meena T.A duration based study of Lipid profile status and associated changes in the Left Ventricular Function of Postmenopausal women.Journal of Clinical & Diagnostic Research. 2011;5(1): 45-47.

3. Wasir JS, Misra A, Vikram NK, Pandey RM, Luthra K. C-reactive protein, obesity, and insulin resistance in postmenopausal women in urban slums of North India. Diabetes and metabolic syndrome: Clinical Research and Reviews. 2007; 1(2):83-89.

4. Anita Deshpande, Sumangala Patil et al. A Study of Atherosclerotic Risk Factors in Postmenopausal Women. International Journal of Biomedical and Advance Research (IJBAR). 2012;03(08):645-647.

5. Judith Wylie-Rosett. Menopause, micronutrients, and hormone therapy. Am J ClinNutr. 2005;81:1223–1231.

6. Ridker PM, Hennekens CH, Buring JE, Rifai N. C-reactive protein and other markers of inflammation in the prediction of cardiovascular disease in women. N Engl J Med. 2000;342(12):836-843.

7. Yousuf O, Bibhu D, Mohanty BD, Martin SS, Joshi PH, Blaha MJ et al. High-sensitivity C-reactive protein and cardiovascular disease. A resolute belief or an elusive link? JACC. 2013;62(5):397-408.

8. Bucova M, Bernadic M, Buckingham T. C-reactive protein, cytokines and inflammation in cardiovascular diseases. BratislLek Listy.2008;109:333–340.

9. Norata GD, Marchesi P, PulakazhiVenu VK et al. Deficiency of the long pentraxin PTX3 promotes vascular inflammation & atherosclerosis. Circulation. 2009;120:699-708.

10. Paffen E, DeMaat MP. C-reactive protein in atherosclerosis: A causal factor? Cardiovasc Res. 2006;71:30–39.

11. Kamath DY, Xavier D, Sigamani A, Pias P. High sensitivity C-Reactive Protein (hs-crp) & cardiovascular disease: An Indian Perspective. Indian J Med Res. 2015;142(3):261-268.
Available from: doi: 10.4103/0971-5916.166582.

12. Ridker PM, Buring JE, Rifai N, Cook NR. Development and Validation of Improved Algorithms for the Assessment of Global Cardiovascular Risk in Women: The Reynolds Risk Score. JAMA. 2007; 297(6):611–619. doi:10.1001/jama.297.6.611.

13. Pfutzner A, Frost T. High-sensitivity C - reactive protein as cardiovascular risk marker in patients with diabetes mellitus. Diabetes TechnolTher. 2006;8(1):28-36.

14. Libby P, Nahrendorf M, Pittet MJ, Swirski FK. Diversity of denizens of the atherosclerotic plaque: Not all monocytes are created equal. Circulation. 2008;117:3168-170.

15. Kones R. Primary prevention of coronary heart disease: Integration of new data,evolving views, revised goals, and role of rosuvastatin in management. A comprehensive survey. Drug Des Devel. Ther 2011;5:325-380.

16. Jialal I, verma S, Devaraj S. Inhibition of endothelial nitric oxide synthase by C-reactive protein: Clinical relevance. Clinchem. 2009;55:206-208.

17. Koenig W. High -sensitivity C-reactive protein and atherosclerotic disease: From improved risk pridiction to risk guided therapy. Int J Cardiol. 2013;168(6):5126-5134.

18. Tandon VR, Mahajan A, Sharma S, Sharma A. Prevalence of cardiovascular risk factors in postmenopausal women: A rural study. J Midlife Health. 2010;1(1):26-29. doi:10.4103/0976-7800.66993

19. Thomas A. Pearson, MD, PhD; George A.Mensah, MD, R Wayne Alexander et al. Markers of Inflammation and Cardiovascular Disease. Circulation. 2003; 107:499.

20. Pearson TA, Mensah GA, Alexander RW, Anderson JL, Cannon 3rd RO, Criqui M et al. Markers of inflammation and cardio-vascular disease: Application to clinical and public health practice: a statement for healthcare professionals from the Centers for Disease Control and Prevention and the American Heart Asso-ciation.Circulation. 2003;107:499–511.

21. Srinivas RK, Srinivas RC. A comparative study of lipid profile and oestradiol in pre and post menopausal women; Journal of clinical and diagnostic research. 2013;7(8): 1596- 1598.

22. Maulik SV, Annp MV, Hitesh AJ, Chirag VS, Varsha SJ. A comparative study of serum lipid profile between pre- menopausal and postmenopausal women, National Journal of Integrated Research in Medicine. 2011; 3(1): 43-45.

23. Rajesh K Jambhulkar, TruptiRamteke , Ashish Anjakar, PankajKamble.Study of Comparison of Lipid Profile in premenopausal and postmenopausal women. International Journal of Recent Trends in Science & Technology. 2015:14(3):631-635.

24. Usoro CAO, Adikwuru CC, Usoro IN and Nsonwu AC. Lipid profiles of postmenopausal women. Pakistan Journal of Nutrition. 2006; 5(1): 79-82.

25. Nwagha UI, Ikekpeazu EJ, Ejezie FE, Neboh EE, Maduka IC. Atherogenic index of plasma in postmenopausal women in Enugu, Nigeria. Africa Health Science. 2010;10(3): 248-252.

26. Devaki, RN &Gowdappa, H & Nataraj, Suma & Prashanth, V & Akila, Prashant& Devi, B.D. &Deepa, K & Goud, Manjunatha & Nayal, Bhavna. A study of C- Reactive protein and its relationship with CHD and lipid metabolism. International Journal of Pharmaceutical Sciences Review and Research. 2011;6:125-127.

27. Sultan N, Nawaz M, Sultan A, Fayaz M, Baseer A. Effect of menopause on serum HDL-cholesterol level. J Ayub Med Coll Abbottabad (JAMC). 2003;15(13).

28. Rehnuma, B., Hassan, Z., Ibrahim, M. and Ali, L. Serum levels of high sensitivity C-Reactive protein and its association with lipidemic status in Bangladeshi healthy adults. Journal of Pathology of Nepal. 2014;4(8):644-648. DOI:https://doi.org/10.3126/jpn.v4i8.11591.

29. Aleksandra N. Klisic, MD, Nadja D. Vasiljevic, MD, PhD, Tatjana P. Simic, MD, PhD, Tatjana I. Djukic, MD, Milos Z. Maksimovic, MD, PhD, Marija G. Matic, MD, PhD, Association Between C-Reactive Protein, Anthropometric and Lipid Parameters Among Healthy Normal Weight and Overweight Postmenopausal Women in Montenegro, Laboratory Medicine. 2014;45(1):12–16. https://doi.org/10.1309/LMI6I2RN7AMPEUUL

30. Ebong, Imo A. MD, MS, Schreiner, Pamela PhD, Lewis, Cora E. MD, MSPH, Appiah, Duke MPH, PhD, Ghelani, Azmina MPH, Wellons, Mellissa MD, MHS. The association between high-sensitivity C-reactive protein and hypertension in women of the CARDIA study, Menopause: 2016;23(6): 662-668.
doi: 10.1097/GME.0000000000000609.

31. Marques-Vidal P, Bochud M, Bastardot F. et al. Association between inflammatory and obesity markers in a Swiss population-based sample (CoLaus Study). Obes Facts. 2012;5(5):734-744.
doi:10.1159/000345045.

32. Mohammed FM, Shafiq NA, Abdulhameed E. High Sensitive C - Reactive Protein Levels in Pre and Post Menopausal Healthy Women in Kirkuk City-Iraq. Int J Vaccines Vaccin. 2016;2(1): 00021.
DOI: 10.15406/ijvv.2016.02.00021.