Relationship among Serum Level of Butyrylcholinesterase , Oxidative Stress and Lipid Profile in Premenopausal and Postmenopausal Iraqi Women

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Abstract - The study was conducted on fifty healthy women .Twenty five premenopausal women and twenty five postmenopausal women .Twenty five premenopausal age range (32- 42) years and twenty five postmenopausal women age range (47- 72) years.

Blood samples were taken from premenopausal women and postmenopausal women from Babylon maternity and pediatric hospital in Hilla city, the sera obtained from the blood were used to determine correlation butyrylcholinestrase enzyme with oxidative stress (Malondialdehyde),lipid profile and Superoxide Dismutase.

In results present study,There were significant increase ($p<0.05$) in malondialdehyde, low density lipoproteinand and Butyrylcholinestrase concentration in sera of postmenopausal women when compared to premenopausal women while there was non significant decrease ($p>0.05$) in high density lipoprotein concentration in sera of postmenopausal women when compared to premenopausal women and significant decrease ($p>0.05$) in superoxide dismutase concentration in sera of postmenopausal women when compared to premenopausal women .

The linear regression analysis demonstrated significant positive correlation ($r=0.483^*,p=0.020$) between serum malondialdehyde and butyrylcholinestrase concentration in sera of premenopausal women versus a highly significant positive correlation ($r=0.699^{**},p=0.000$) between serum malondialdehyde and butyrylcholinestrase concentration in sera of postmenopausal women,also there are significant positive correlation ($r=0.498^*,p=0.013$) between serum low density lipoprotein concentration and butyrylcholinestrase concentration in sera of premenopausal women versus significant positive correlation ($r=0.643^{**},p=0.001$) between serum low density lipoprotein concentration and butyrylcholinestrase concentration in sera of postmenopausal women. It also showed a highly significant negative correlation ($r=-0.809^{**},p=0.000$) between serum high density lipoprotein concentration and butyrylcholinestrase concentration in sera of postmenopausal women versus significant negative correlation ($r=-0.598^{**},p=0.002$) in premenopausal women, also the results demonstrated significant negative correlation ($r=-0.630,p=0.021$) between serum superoxide dismutase concentration and butyrylcholinestrase concentration in sera of postmenopausal women while non significant negative correlation ($r=-0.553,p=0.272$) between serum superoxide dismutase concentration in sera of premenopausal women and butyrylcholinestrase concentration.

Index Terms- BCHE, HDL ,LDL, MDA, SOD.

1-INTRODUCTION

The term "menopause" comes from two Greek words 'menos' (month) and 'pausis' (cessation) [1].Menopause is a physiologic process in women that occurs around 45-55 years old, which is defined as permanent cessation of menstruation by one year [2]. The age of menopause depends on multiple factors such as number of ovules from the female at birth, the frequency of loss of these ovules through her life and the number of ovarian follicles required maintaining the menstrual cycle. The diagnosis of natural menopause can usually be made from the characteristic history of vasomotor symptoms of hot flushes and night sweats and prolonged episodes of amenorrhoea (after a year without menses). Measurement of plasma hormone levels [oestradiol, follicle stimulating hormone (FSH) and luteinizing hormone (LH)] in women in their late 40s onwards with classical symptoms are not essential [3].This process is characterized by gradual decrease of estrogen (E) secretion and changes related with sex hormones, so that estradiol levels ranging from 5 to 25 pg/mL, while increasing titre of gonadotrophins, so that the values of follicle stimulating hormone (FSH) between 40 and 250 mU/mL and luteinizing hormone (LH), from 30 to 150 mU/mL [4,5].

Menopause is a stage that favors weight gain and development or worsening of obesity,and causes of this problem are many; some are clearly related to hypoestrogenism and conditioning increased intake and decreased energy expenditure [6,7].During this period there is an abnormal atherogenic lipid profile characterized by increased lipoprotein cholesterol, low density (LDL-C), triglycerides (TG) with reduced HDL-C [8] ,perhaps as a direct result of ovarian failure or indirectly as a result of central redistribution of body fat, and this favors the formation of atheromatous plaques and progression of coronary atherosclerosis and therefore cardiovascular disease incidence increases substantially in postmenopausal women [9],Where estrogen deficiency leads to insulin resistance,itself an independent risk factor for coronary heart disease[10].Thus giving the insulin-sensitizing agents(clinical trials) as treatment against coronary heart disease for postmenopausal women[11].

Oxidative stress is defined as an imbalance between production of free radicals and reactive metabolites, so-called oxidants, and their elimination by protective mechanisms, referred to as antioxidative systems. This imbalance leads to damage of important biomolecules and organs with potential impact on the whole organism [12].Oxidative stress, which is induced by the imbalance between the production and removal of reactive oxygen species ROS, is regarded as a primary factor in various degenerative diseases, such as cancer, atherosclerosis, and aging [13]. Free radicals, with an unpaired electron in one of their orbits, are chemical species produced in many different ways, such as activation of phagocytes and the general immune system, lipid peroxidation, electron transport system in mitochondria, ischemia and trauma [14]. MDA measurement is an indicator of lipid peroxidation and is used as a biomarker of oxidative stress [15]. Malondialdehyde plays a crucial role in the development and prognosis of atherogenic events .MDA is a product of lipid peroxidation [16].

Cholinesterase represents a group of enzymes that hydrolyze acetylcholine and other choline esters. There are two main types of cholinesterase with different biochemical properties: true or specific cholinesterase or acetylcholinesterase found in all excitable tissues (central and peripheral nervous system and muscles) and in erythrocytes. It is a high-turnover enzyme with high affinity for acetylcholine,inhibited at high concentrations of acetylcholine, and with low affinity for noncholine esters.The other one is the nonspecific or pseudocholinesterase or butyrylcholinesterase which hydrolyses both choline and aliphatic esters. Butyrylcholinesterase (BChE) is an α glycoprotein found in the central and peripheral nervous system, in most tissues, and in the liver[17].

2-Materials and Methods

2.1 Materials

Fifty normal female volunteers Babylon governorate participated in this study which was carried out from 1st of November till 1st of June 2014 .Twenty five healthy postmenopausal women with a history of natural menopause were selected for this study, the age group ranged from 47 - 72 years.Twenty five healthy premenopausal women with regular menstruation were selected for this study, the age group ranged from 32- 42 years.

Women with cardiovascular disease, hypertension, diabetes mellitus, hepatic, smokers, alcoholics, metabolic and renal disease, and those who were on exogenous hormones , hormone replacement therapy, or lipid lowering drugs, were excluded from this study.

The samples were collected from Babylon maternity and pediatric hospital in Hilla city. The practical side of the study was performed at the laboratory of Biochemistry Department in College of Medicine / Babylon University.A questionnaire was taken for each female volunteers.

2.2 Blood Sampling

Venous blood samples were drawn from pre and postmenopausal women after fasting by using disposable syringes (5mL).A volume 5mL of blood were obtained from each Woman by vein puncture .It were put into disposable tubes containing separating gel.Blood in the gel containing tubes was allowed to clot at room temperature for 10-15 minutes and then centrifuged at (4000 rpm) for approximately 10-15 minutes then the sera were obtained and stored at -20° C until analysis.

2.3 Methods

Serum malondialdehyde (MDA) concentration was determined according to the procedure published by Carl A. and Edward R.[18]. Extracellular SOD (EC-SOD) activity was determined by using a simple and rapid method ,based on the ability of the enzyme to inhibit the autoxidation of pyrogallol [19,20]. BCHE level was determined by human BCHE ELISA kit [21]. HDL-cholesterol level was determined by Biolabo SA (France) kit[18] .LDL-C level was calculated by using Friedewald formula: [22].

$$\text{LDL-cholesterol (mmol/L)} = \text{Total-cholesterol} - \text{HDL-cholesterol} - \frac{\text{TG}}{2.22}$$

Serum malondialdehyde (MDA),Extracellular SOD (EC-SOD) activity and HDL-cholesterol level were measured by using spectrophotometer. BCHE level was measured by enzyme labeled immunoassay include enzyme –linked method.

3 Results

The results in table(1) revealed a significant increase in serum malondialdehyde BCHE and LDL-C while these were insignificant decrease in HDL-cholesterol and significant decrease in extracellular SOD (EC-SOD) levels in Postmenopausal women in comparison with premenopausal cases.

Table 1: Show Serum Level of Serum malondialdehyde (MDA), Extracellular SOD (EC-SOD), BCHE, HDL-cholesterol and LDL-C in premenopausal Women and postmenopausal Women.

Parameters	Type of Group	Mean ±SD	p-Value
MDA mmol/L	Premenopausal Women	0.0016±0.0007	0.0001**
	Postmenopausal Women	0.0033±0.001	
SOD U/ml	Premenopausal Women	3.142±2.082	0.012
	Postmenopausal Women	1.990±1.270	
BCHE ng/ml	Premenopausal Women	2.353±2.02	0.048
	Postmenopausal Women	4.132±3.63	
LDL-C mmol/L	Premenopausal Women	2.557±1.39	0.0001**
	Postmenopausal Women	4.967±2.34	
HDL mmol/L	Premenopausal Women	1.262±0.79	0.679
	Postmenopausal Women	1.182±0.68	

The results of linear regression analysis shown in figures (1,2,3,4,5,6,7,8) revealed a significant positive correlation between serum MDA conc.with BCHE activity ,a negative correlation between serum SOD activity and BCHE activity with the corresponding dyslipidemia represented by the changes in the pattern of lipid profile gives a novel idea about the role of this enzyme in dyslipidemia and subsequent cardiovascular changes in postmenopausal women .It is worthy to mention that the activity of BCHE may be used as a good indicator of oxidative stress in the corresponding women.

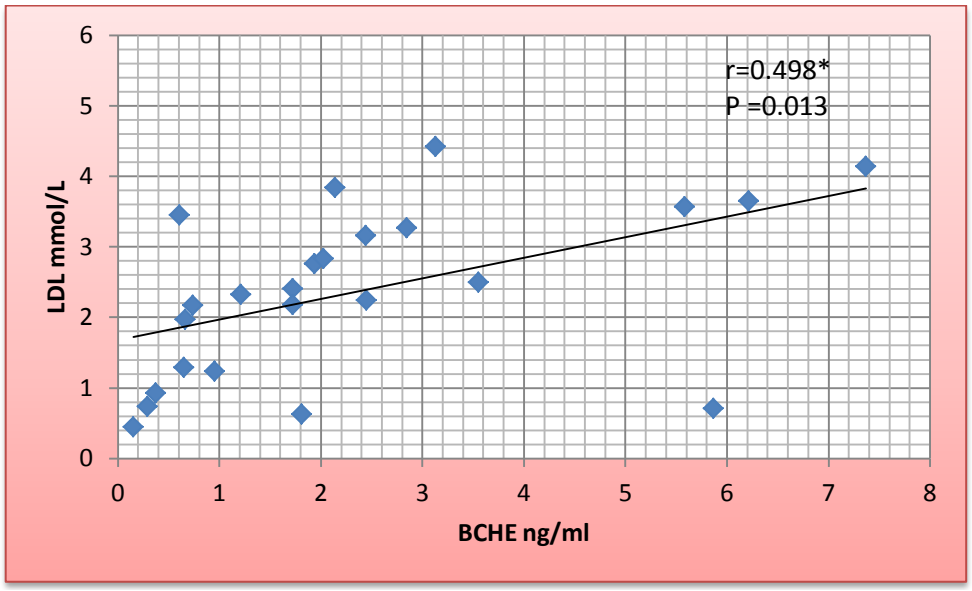

Fig. (1):The correlation between BCHE levels and LDL levels in premenopausal womens' serum.

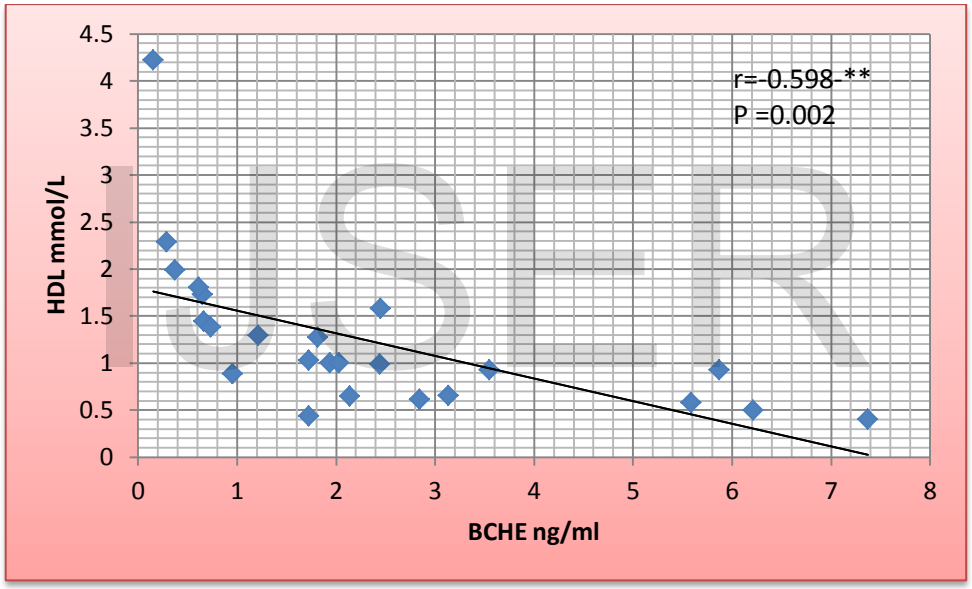

Fig. (2): The correlation between BCHE levels and HDL levels in premenopausal womens' serum.

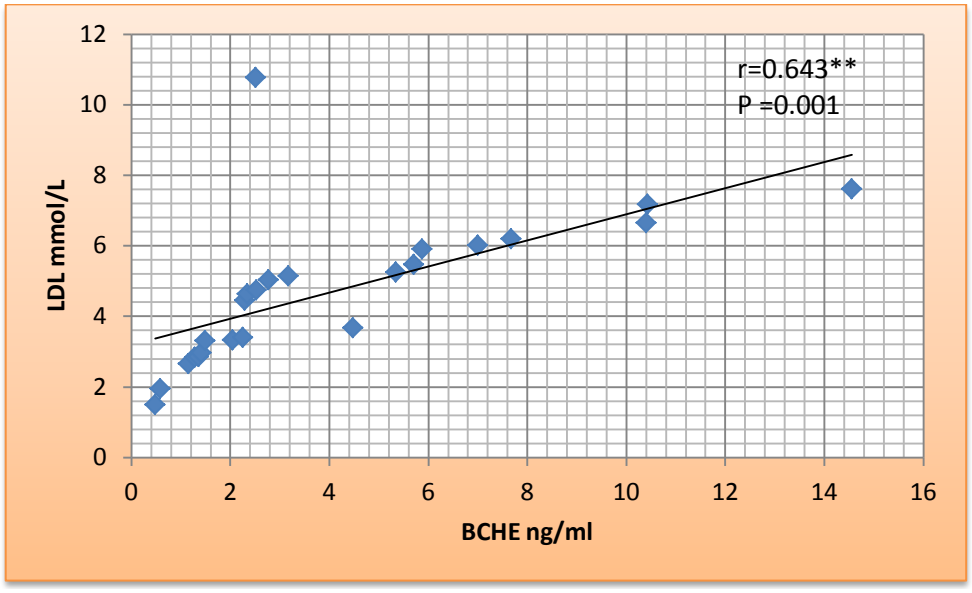

Fig. (3): The correlation between BCHE levels and LDL levels in postmenopausal womens' serum.

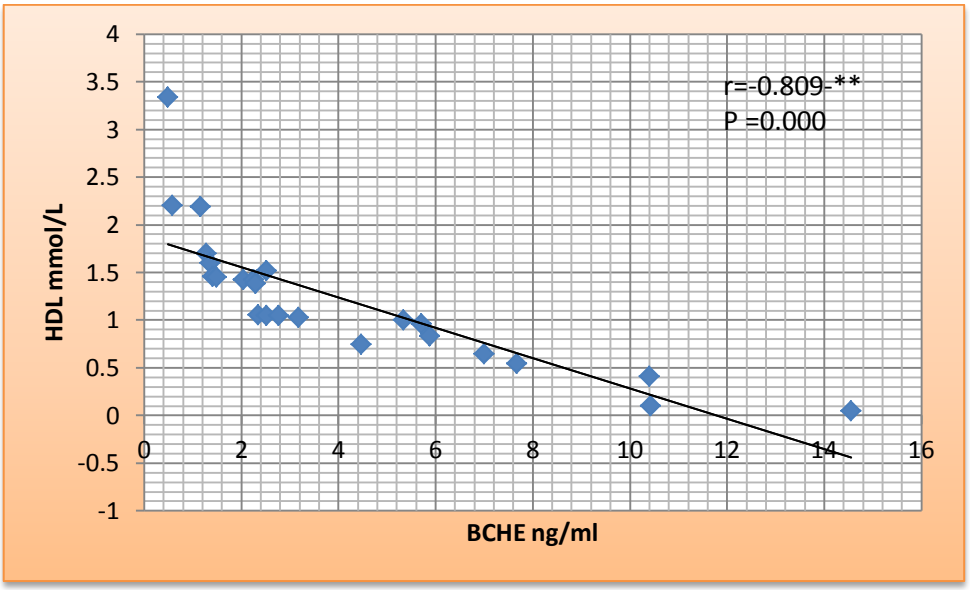

Fig.(4): The correlation between BCHE levels and HDL levels in postmenopausal womens' serum.

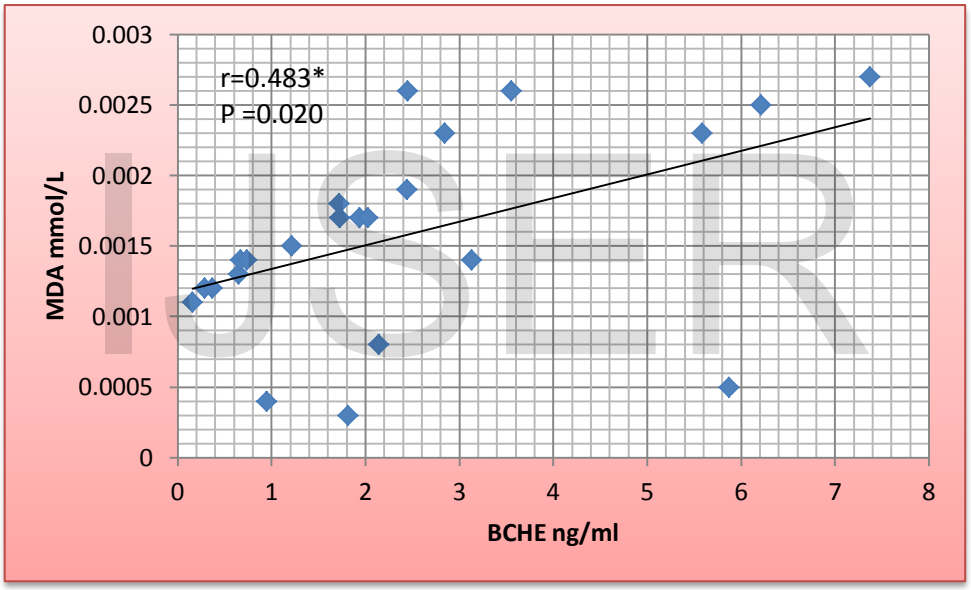

Fig.(5):The correlation between BCHE levels and MDA levels in premenopausal womens' serum.

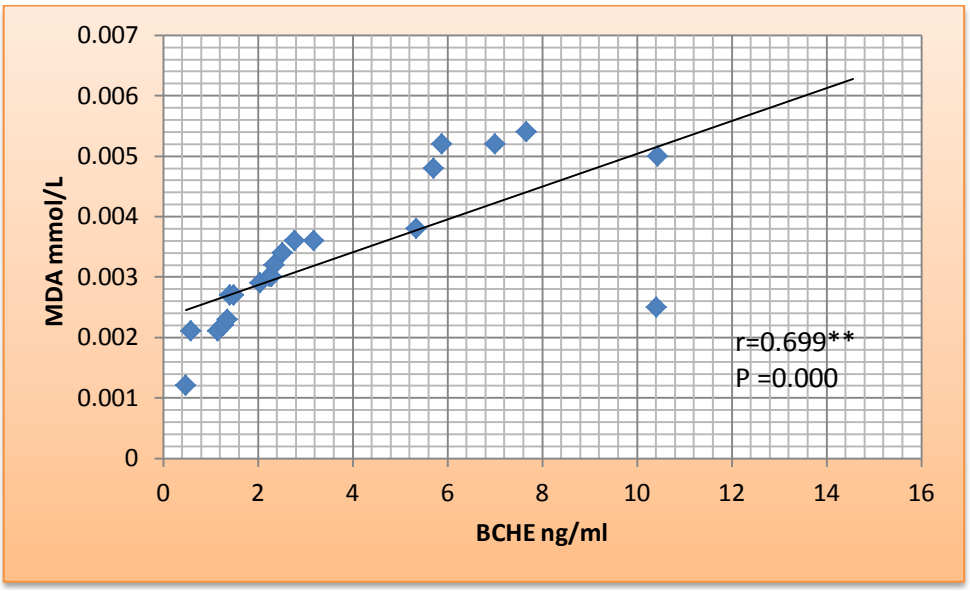

Fig.(6):The correlation between BCHE levels and MDA levels in postmenopausal womens' serum.

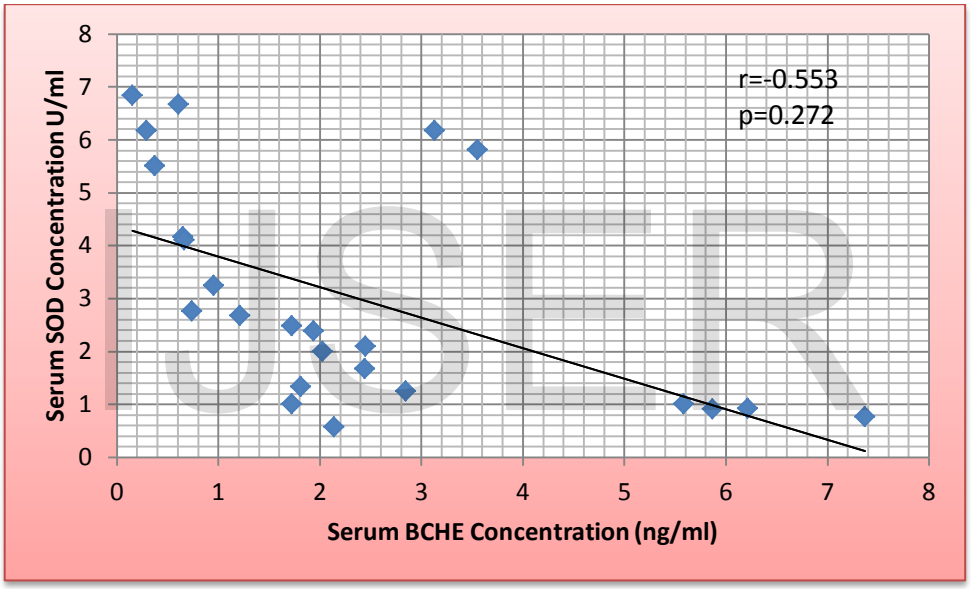

Fig.(7):The correlation between BCHE levels and SOD levels in premenopausal womens' serum.

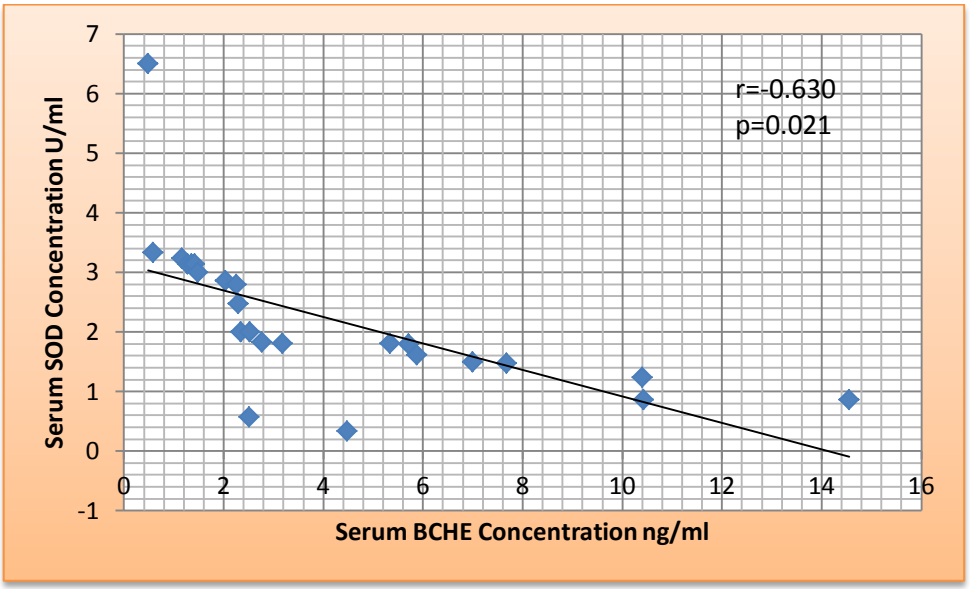

Fig.(8): The correlation between BCHE levels and SOD levels in postmenopausal womens' serum.

4 discussion

Circulating estrogen is a regulator of lipoprotein lipase (LPL). LPL catalyzes the hydrolysis of VLDL to form IDL and later LDL. After menopause due to estrogen deficiency, there will be increased plasma LPL and hepatic TG lipase activity causing plasma LDL accumulation and also leads to down-regulation of LDL receptors [23,24].

Due to estrogen deficiency,postmenopausal women will have highest activity of postheparin hepatic lipase that enhances the uptake of HDL and also increases the catabolism of HDL thus decreasing plasma HDL concentration [25]. Also in postmenopausal women there is increased LDL accumulation, so more and more HDL gets esterified for the metabolism of those accumulated LDL.

Estrogens have free radical scavenging activity structures[26] .Where estrogens are a powerful antioxidants which prevents lipid peroxidation due to the phenolic structure in their molecules,it may donate hydrogen atom from their phenolic hydroxyl group to lipid peroxy radicals terminating chain reactions[27].Therfore deficiency of estrogen in postmenopausal women develops oxidative stress.

Hence menopausal women develop oxidative stress (OS) because of estrogen deficiency.Also oestrogens stabilize the endothelial cells and enhance antioxidant effects[28].In end oestrogens deficiency in Postmenopausal Women lead to increased free radicals generation,this condition lead to an imbalance between oxidative stress and antioxidant system.Thus and according to results of the present study,MDA concentration increase in postmenopausal women and decreased SOD concentration in postmenopausal women compared with premenopausal women.

This increase in butyrylcholinesterase (BCHE) concentration in sera of postmenopausal women may be due to a decrease in sex steroid hormones,which could result in reduced hepatic synthesis or in the release of enzyme[29].An increased flux of free fatty acids from adipose tissue to the liver stimulated the hepatic synthesis of serum BChE[30].

The results of the present study are in agreement with Sharanabasappa, et al. who showed a significant increase in serum BChE of postmenopausal women compared with premenopausal women. [31].

The results of the present study are in agreement with Mamta, Varun. and Gupta [32]. who found a significant decrease in superoxide dismutase (SOD) concentration in sera of postmenopausal women .

The results of the present study are in agreement with Geetha, et al. [27].who found a significant increase in malondialdehyde concentration in sera of menopausal women .

5 Conclusion

It can be concluded that increase in oxidative stress in postmenopausal women leads to a concomitant increase in serum butyrylcholinestrase compared with premenopausal women. The latter enzyme leads to dyslipidemia in the corresponding women.

6 References

1. Keith D.(2012).Dewhurst's Textbook of Obstetrics and Gynaecology; 18th ed .p.553.
2. Reddish S. (2011). Menopausal transition- assessment in general practice. Australian Family Physician; 40(5): 266-72.
3. International Menopause Society Consensus Statement.Ageing ,menopause , cardiovascular disease and HRT.(2009).Climacteric;12:368-377.
4. Guthrie J., Dennerstein L., Hopper J., et al. (1996). Hot flushes,menstrual status and hormone levels in a population-based sample of midlife women.Obstetrics and Gynecology; 88: 437-441
5. Zarate A., Austria E., Saucedo R., et al. (2011).Diagnosis of premature menopause measuring circulating anti-Mullerian hormone. Ginecologia Obstetricia Mexicana; 79(5): 303-7.
6. Pavon P., Alameda H. and Olivar R. (2006). Obesidad menopausia. Nutricion Hospitalaria; 21: 633-637
7. Zillikens M., Uitterlinden A., Berends A., et al. (2010). The role of body mass index, insulin, and adiponectin in the relation between fat distribution and bone mineral density. Calcified Tissue International; 86(2): 25-116.
8. Cho E., Min Y., Kim J., et al. (2011). Effects of the transition from premenopause to postmenopause on lipids and lipoproteins: quantification and related parameters. Korean Journal Internal Medicine; 26(1): 47-53.
9. Kallikazaros I., Tsioufis C., Zambaras P., et al. (2008). Estrogen-induced improvement in coronary flow responses during atrial pacing in relation to endothelin-1 levels in postmenopausal women without coronary disease. Vascular Health Risk and Management; 4(3):14-705.

10. Lejskova M.,Alusik S.,Suchanek M., et al. (2011).Menopause: clustering of metabolic syndrome components and population changes in insulin resistance.Climacteric;14:83-91.
11. Nardo L.,Christodoulou D.,Gould D., et al. (2007).Anti-mullerian hormone levels and antral follicle count in women enrolled in in vitro fertilisation cycle:Relationship to lifestyle factors ,chronological age and reproductive history .Gynecol Endocrinol;24:1-8.
12. Durackova Z. (2010). Some current insights into oxidative stress. Review. Physiol. Res.; 59: 459-469.
13. Shinde A., Ganu J. and Naik P.(2012). Effect of free radicals & antioxidants on oxidative stress:A Review Article.Journal of Dental & Allied Sciences;1(2):63-66.
14. Gutteridge J. (1995). Lipid peroxidation and antioxidants as biomarkers of tissue damage. Clinical Chemistry; 41: 1819–1828.
15.
16. Nelson F., Custovic A., Simpson A. , et al. (1997). Clin. Chem.; 43 (7): 1209.
17. Rosenfeld M.,Palinki V. and Hetuala Y. (1990).Distribution of oxidation specific lipid protein adducts and apolipoprotein B in atherosclerotic lesions of varying severity from N/HHL rabbits.Atherosclerosis ;10:49-336.
18.
19. Davis L., Britten J. and Morgan M.(1997).Cholinesterase. Its significance in anaesthetic practice. Anaesthesia;52:60–244.
20. Carl A. and Edward R. (1999). Tietz text book of clinical Biochemistery; 3rd ed. p.1034–1054 and 819–861.
21. Luc M., Gaydou M. and Jean C.(2000) .Spectrophotometric measurement of antioxidant properties of flavones and flavones against superoxide anoin . Anal. Chim . Acta .; 16 – 209 .
22. Marklund .(1974). Involvement of the superoxide anion radical in the autooxidation of pyrogallol and a convenient assay for superoxide dismutase . Eur. J. Biochem ; 47 : 74 – 469.
23. Creative diagnostics (CD) Company(USA) specification sheet Rev.102618A (2013).
24.
25. Tornvall P., Bavenholm P., Landou C., et al. (1993).Relation of plasma levels and composition of apolipoprotein B containing lipoproteins to angiographycially defined coronary artery disease in young patients with myocardial infarction. Circulation; 88(5): 2180-2189.
26. Muzzio M., Berg G., Zago V., et al.(2007) . Circulating small dense LDL, endothelial injuring factors and fibronectin in healthy postmenopausal women. Clin. Chim. Acta ; 381: 157-163.
27. Wakatsuki A. and Sagara Y. (1995). Lipoprotein metabolism in postmenopausal and oophorectomized women. Obstet Gynecol; 85(4): 523-528.
28. Arora S., Jain A. and Chitra R. (2006).Effects of short-term hormone replacement on a atherogenic indices in Indian postmenopausal women. Indian J Clin. Biochem.; 21(1): 41-47.
29. Ruiz M., Martin C. and Martinez R. (2000). Antioxidant activities of estrogens against aqueous and lipophillic radicals; differences between phenol and catechol estrogens. Chem. Phys. Lipids;105: 88-179.
30. Geetha H., Chitra H., Pranathi. M, et al. (2014). Quality of life after menopause: Effects of hormone replacement therapy,vitamin E and sudarhana kriya yoga practice: A comparative study. Biomedical Research ; 25 (2): 240-242.
31. Igweh J. (2005).The effects of menopause on the serum lipid profile of normal females of south east Nigeria. Nigerian Journal of Physiological Sciences; 20 (1-2): 48-53.
32. Sidell F. and Kaminskis A.(1975).Influence of age,sex and oral contraceptives on human blood cholinesterase activity.Clin. Chem. ;21: 1393-1395.
33. Randell E., Mathews M., Zhang H., et al. (2005). Relationship between serum butyrylcholinesterase and the metabolic syndrome. Clin. Biochem.;38:799–805.
34. Sharanabasappa M ., Jagadish B ., Moin S., et al. (2014). Serum butyrylcholinesterase and lipid profile in pre and "post-menopausal" women.Research Journal of Pharmaceutical, Biological and Chemical Sciences; 2 (2):364-369.
35. Mamta M., Varun K. and Gupta R.(2014). Antioxidant status in peri-menopausal and post-menopausal women;(13):1-8.