

Altered Liver Function and the Status of Calcium in Postmenopausal Women in and Around Mangalore

Suchetha Kumari N¹, Smitha B.Rosario² and Damodara Gowda K.M

¹Department of Biochemistry, K.S.Hegde Medical Academy, Deralakatte, Mangalore, Karnataka, India, ²Department of Biotechnology, Shree Dharmasthala Manjunatheshwara College, Ujire, D.K., Karnataka, India and ³Department of Physiology, K.S.Hegde Medical Academy, Deralakatte, Mangalore, Karnataka, India

Abstract

At the time of menopause female sexual cycle ceases and female sex hormones diminish rapidly. It is characterized by various physiological, psychological and biochemical changes, and also metabolic bone disorders. Therefore in the present study we analyzed the level of AST, ALT, serum proteins, total bilirubin, direct bilirubin and the level of calcium in pre and postmenopausal women. The study involves 80 subjects, of which 40 pre-menopausal and 40 postmenopausal women. Statistical analysis was done using one-way ANOVA. In pre-menopausal women the concentration of AST was elevated significantly as compared to postmenopausal women ($p < 0.001$) but, the concentration of ALT was increased non-significantly. The concentration of total serum protein increases in post menopausal women non significantly as compared to pre menopausal women's whereas, the albumin level was decreased significantly in post menopausal women ($p < 0.0001$). The level of total bilirubin and direct bilirubin increased significantly in post menopausal women as compared to pre menopausal women's ($p < 0.01$). The serum calcium level was declined significantly in post menopausal women as compared to pre menopausal women's ($p < 0.0001$). Thus our study demonstrated that, as age advances liver gets irritated and the liver functions gets disturbed. The decreased concentration of calcium in postmenopausal women indicates that they are more prone to fractures and osteoporosis.

Key words: Alanine aminotransferase, Aspartate aminotransferase, Total bilirubin, Direct bilirubin

Introduction

The period during which the female sexual cycle ceases and female sex hormones diminish rapidly to almost none at all is called Menopause. It occurs between 45-55 years of age. It is characterized by hot flushes, night sweats and various other psychological and biochemical changes occur. It also leads to metabolic bone disorders. With the onset of menopause, rapid bone loss occurs which is believed to average approximately 2% to 3% over the following 5 to 10 yrs, being greatest in the early postmenopausal years [1-2]. Life time losses may reach 30% to 40% of the peak bone mass in women. The pathogenesis of postmenopausal osteoporosis involves the interplay of many factors- Nutritional, Environmental, and Genetic[3-4]. Biochemical markers of bone turnover have been shown to provide valuable information for the diagnosis and monitoring of metabolic bone disease [5-6]. The occurrence of Osteoporosis in postmenopausal women is very common problem especially in India who are exposed to many of the risk factors like prolonged amenorrhea, low calcium diet, lack of exercise, Vitamin D deficiency. But there are very few Indian studies regarding the prevalence of osteoporosis in postmenopausal women and also regarding the biochemical markers which indicate bone turnover.

Onset of menopause also alters the liver functions. The liver performs diverse functions, essential for life. Liver function tests (LFT) are effective modalities to detect hepatic dysfunction. The most common tests include testing for Alanine aminotransferase (ALT), Aspartate aminotransferase (AST), Bilirubin and Albumin. A total serum protein test measures the total amount of protein including albumin and globulin in the blood. The elevated total serum protein levels indicate dehydration, high protein and high calorie diet, Liver/biliary dysfunction etc. Albumin, a major serum protein synthesized exclusively by the liver. In some inflammatory conditions the release of tumor necrosis factor inhibits albumin synthesis but induces the synthesis of proteins of the acute phase response. When the liver has been chronically damaged, the albumin level decreases. Bilirubin, a breakdown product of hemoglobin, derived from red cells that have outlived their natural life and subsequently have been removed by the spleen. It is extracted and bio transformed in the liver and excreted in bile and urine. As the liver becomes irritated, the total bilirubin may rise. Hence it is important to understand the difference between total bilirubin and direct bilirubin. Aspartate aminotransferase (AST) formerly called serum glutamic- oxaloacetic transaminase or SGOT, is another enzyme necessary for energy production. This enzyme also reflects damage to the hepatic cell. It may be elevated in other conditions such as myocardial infarction. Although AST is not a specific for liver as the ALT, ALT and AST are useful in assessing the etiology of liver enzyme abnormalities. AST is elevated in liver and heart disease. Alanine aminotransferase (ALT), formerly called serum glutamate pyruvate transaminase or SGPT, is another enzyme necessary for energy production. ALT, produced by the liver, abnormally increased in conditions where cells of the liver have been inflamed or undergone cell death. As the cells are damaged, the ALT leaks into the bloodstream leading to a rise in the serum levels. Any form of hepatic cell damage can result in an elevation in the ALT. It is the most sensitive marker for liver cell damage. Calcium, a component of skeleton, soft tissues, and extra cellular fluid is the fifth most common element and the most prevalent cation found in the body. The skeleton contains 99% of the body's calcium. Soft tissues and extra cellular fluid contain about 1% of the body's calcium. Of the protein bound calcium fraction, approximately 80% is associated with albumin and 20% with globulins. With all these background we have undertaken this study to analyze the liver function test and the level of calcium in pre and post menopausal women.

Materials and Methods

The present work was carried out in the central research laboratory of A.B.Shetty Memorial Institute of Dental Sciences, Deralakatte, Mangalore, after taking the written consent from the subjects. About 5ml of venous blood sample was collected and the serum was obtained and stored at -4°C and was used for the estimation as explained below. The total protein level was estimated by Biuret method[7-8]. In this method the peptide bonds of the protein bind with copper ions in alkaline solution to form a blue – violet complex. Tartarate is added as a stabilizer while potassium iodide is used to prevent auto-reduction of the alkaline copper complex. The intensity

of the blue-violet complex is proportional to the protein concentrations. Serum albumin level was estimated by using bromocresol green indicator method[7-8]. Direct bilirubin level was estimated by Van Den Bergh method [9]. Here the Conjugated bilirubin (direct bilirubin) reacts with diazotized sulfanilic acid to form a red colored compound. The intensity of the color is proportional to bilirubin concentration in the sample. Total bilirubin was estimated by Dimethyl sulfoxide method[9]. In this method the total bilirubin reacts with diazotized sulfanilic acid in presence of dimethyl sulfoxide (DMSO) to form a colored compound. The intensity of the color is proportional to the bilirubin concentration in the sample. ALT (SGPT) and AST (SGOT) level was estimated by IFCC Method [10]. Serum calcium level was estimated by using Ortho– Cresolphthalein complex [11].

STATISTICAL ANALYSIS: The data were expressed as Mean \pm S.D. and are analyzed for statistical significance using one way ANOVA. $P < 0.05$ was considered as the level of significance.

Results

The level of serum protein and the concentration of liver enzymes mainly ALT and AST indicate the functional ability of liver. In the present study, we estimated the serum level of ALT, AST, albumin, total protein, direct bilirubin, total bilirubin and calcium level in pre menopausal and post menopausal women (Table-1, Fig-1). From this study we found that, the concentration of AST was elevated significantly in post menopausal women as compared to pre menopausal women's ($p < 0.001$). But, the concentration of ALT was increased none significantly in post menopausal women. The concentration of total serum protein increases in post menopausal women nonsignificantly as compared to pre menopausal women's whereas, the albumin level was decreased significantly in post menopausal women ($p < 0.0001$). The level of Total Bilirubin and direct bilirubin increased significantly in post menopausal women as compared to pre menopausal women's ($p < 0.01$). The serum calcium level was declined significantly in post menopausal women as compared to pre menopausal women's ($p < 0.0001$).

Table-1: Average values of various parameters in pre-menopausal and post-menopausal women. The values are expressed as mean \pm SD, $n=40$ each).

Parameter	Pre-menopausal women	Post-menopausal women	P value
AST (U/L)	12.54 \pm 1.28	15.97 \pm 3.27	<0.001
ALT (U/L)	7.18 \pm 2.18	7.57 \pm 2.63	0.472
Albumin (gm/dl)	4.07 \pm 1.34	3.21 \pm 0.42	<0.0001
Total Protein (gm/dl)	6.38 \pm 5.86	6.17 \pm 2.94	0.840
Total bilirubin (mg/dl)	0.58 \pm 0.94	1.46 \pm 1.61	<0.01
Direct bilirubin (mg/dl)	0.17 \pm 0.067	1.01 \pm 0.677	< 0.001
Calcium (gm/dl)	9.38 \pm 1.03	7.15 \pm 1.10	< 0.0001

Note: AST=Aspartate amino transferase,ALT=Alanine aminotransferase

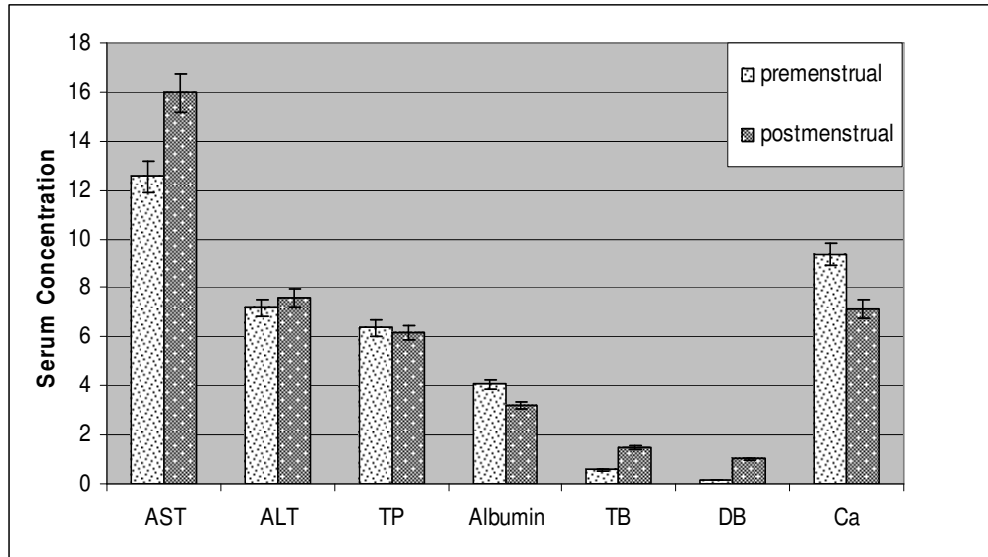


Fig-1: Level of various parameters in pre-menopausal and postmenopausal women. AST=Aspartate amino transferase,ALT=Alanine amino transferase,TP=Total protein,TB=Total bilirubin,DB=Direct bilirubin,Ca=Calcium.

Discussion

Biochemical parameters can give an idea as to the rates of bone formation and resorption. The results of the present study indicated that the level of serum calcium was declined significantly in post menopausal women as compared to pre menopausal Women's. In general women lose about 1% of their bone density per year during and after Menopause. However, nearly 35% of women lose bone at a faster rate during the late pre- menopausal period. Biochemical markers can detect women who are considered "rapid losers" that is, those who lose 3% to 5% of bone per year [12-14]. Our study also demonstrates that the concentration of AST, total bilirubin, and direct bilirubin was elevated significantly in post menopausal women whereas, albumin, and calcium level decline significantly in post menopausal women as compared to pre menopausal women. The level of serum protein and the concentration of liver enzymes mainly ALT and AST indicate the functional ability of liver. The serum level of ALT, AST, albumin, total protein, direct bilirubin, total bilirubin and calcium level in pre menopausal and post menopausal women, and we found that, the concentration of AST was elevated significantly in post menopausal women as compared to pre menopausal women's . When the liver becomes irritated, the total bilirubin may increases and direct bilirubin level also increases. ALT is the enzyme produced within the cells of the liver. The level of ALT abnormally is increase in conditions where cells of the liver have been inflamed or undergone cell death. As the cells are damaged, the ALT leaks into the bloodstream leading to a rise

in the serum levels. Any form of hepatic cell damage can result in an elevation in the ALT. AST also reflects damage to the hepatic cell. AST level was elevated in liver and heart diseases. Albumin decreases in chronic liver disease, particularly if the disease is getting worse. The elevated level of total protein in the serum causes increase in the cholesterol, biliary/liver dysfunction. Calcium level decreases in postmenopausal women hence, after the age of 50, there is a general tendency for osteoporosis. AST, ALT, Total bilirubin, direct bilirubin, total protein level is increased in postmenopausal women where as albumin and calcium level decreases in postmenopausal women compare to premenopausal women. The level of total bilirubin and direct bilirubin increased significantly in post menopausal women as compared to pre menopausal women's. This indicates that postmenopausal women are more prone to liver damage and exhibit altered liver function, as the age advances. The decreased concentration of calcium in postmenopausal women indicates that they are more prone to fractures and osteoporosis.

References

1. Susan A. Calcium Supplementation in Postmenopausal Women. From Medscape Ob/Gyn & Women's health, 2003;8(2) <http://www.medscape.com/viewarticle/460438>
2. Gupta A. Osteoporosis in India-the nutritional hypothesis. *Natl Med J Ind* 1996;9(6):268-74
3. Indumati.V, Vidya.S.Patil, Rama Jaikhani. Hospital based preliminary study on osteoporosis in postmenopausal women, *J Clinl Biochem* 2007;22 (2): 96-100.
4. Delmas PD. Biochemical markers of bone turnover for the clinical investigation of osteoporosis. *Osteoporos Int* 1993;3(1):81-6.
5. Woitge HW, Nave CS, Kissing C, Bruckner GL, Meyer K, Grauer A, et al. Seasonal Variation of Biochemical Indexes of Bone Turnover; Results of a population-based Study. *J.Clin Endocrinol Metab* 1998;92(1): 68-75.
6. Delmas PD, Eastell R, Garnero P, Seibel MJ, Stepan J. The Use of Biochemical Markers of Bone Turnover in Osteoporosis. *Osteoporos Int* 2000; Suppl 6:2-17.
7. McMurray JR, Plasma Proteins. In Gowenlock AH, editor. Varley's Practical clinical Biochemistry, 6th Ed, CBS publishers (Indian Reprint), New Delhi, 2002, 407-8.
8. Varley H, editor. The Plasma Proteins: In Practical clinical Biochemistry, 4th ed, CBS publishers (Indian Edition), New Delhi, 1988, 243-4.
9. Griffiths WJ, Kaye G. A study of the bile pigments in relation to the van den bergh reaction. *Biochem J.* 1930;24(5):1400-1407.
10. Henry RJ, editor, Enzymes: In Clinical Chemistry Principle and techniques, Harper and Row publishers, New York, 1974, pp 815.
11. Gitelman HJ. An Improved automatic procedure for the determination of Calcium in biologic specimens, *Anal Biochem* 1967;18: 521-31.
12. Rosen CJ, Tenenhouse A. Biochemical markers of bone turnover. *Postgraduate Med* 1998;104(4):101-18.
13. De Rosa G, Testa A, Giacomini D, Carrozza C, Astazi P, Caradonna P. Prospective study of bone loss in pre- and post- menopausal women on L-thyroxine therapy for toxic goiter. *Clin Endocrinol* 1997;47(5):529-35.
14. Loffman O, Magnusson P, Toss G, Larsson L. Common biochemical markers of bone turnover predicts future bone loss:a 5-year follow-up study. *Clin. Chim. Acta* 2005;356:67-75.

All correspondences to: Dr.Suchetha Kumari M.Sc; PhD. Professor, Dept. of Biochemistry, K.S.Hegde Medical Academy, Nithyanandanagar, Deralakatte, Mangalore Pin code: 575018 Phone Number 9448451318 E-mail: suchetha.shetty@rediffmail.com, suchetha49@gmail.com