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ELECTROPHORETICALLY RESOLVED SERUM PROTEIN FRACTIONS IN POSTMENOPAUSAL WOMEN

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Abstract: Blood samples of naturally postmenopausal women and healthy control cycling females were collected at different places in Lahore by the expert persons. The study concerns on the physiological adaptations after the natural menopause. Sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) based on the method of Laemmli (1970) was employed for studying protein profile of postmenopausal and control subjects. Gels were photographed and their images were stored for quantification of various protein fractions by Gene Genius Bio-imaging Gel Documentation System that provided the data of molecular weights and percent raw volumes by each of the fractions. The data was analyzed statistically using Student t-test in finding the enhancement or reduction and appearance or disappearance of particular protein fractions for comparisons among the postmenopausal and the control subjects. The six low molecular weight protein fractions were detected that were ranging between 26-11 kDa. Non significant elevations were observed in 26, 20 and 11 kDa protein fractions. Highly significant elevations were observed in 17, 15 and 14 kDa protein fractions in postmenopausal subjects when compared with control subjects.

Key words: Gel electrophoresis, postmenopausal women, protein fractions

INTRODUCTION

Menopause refers to the period of climactic, which encompasses the transition from reproductive years up to and beyond the last episode of menstrual bleeding (Carr, 1998). The average age at the onset of menopause is 51, however, it can vary (Richardson *et al.*, 1987). The age is retrospectively defined after 1 year of no menses (Bromberger *et al.*, 1997). Throughout a women's reproductive life about 400 of primordial follicles grow into vesicular follicles and ovulate, while literally hundreds of thousands of ova degenerate (Guyton and Hall, 2000). Some years before the cessation of menstruation, levels of gonadotropins increase, while ovarian hormones begin to decrease (Sherman *et al.*, 1976; Chakravartis *et al.*, 1976).

Schlessinger (2001) has reported that a mutation in the newly identified gene $FOXL_2$ causes premature menopause which usually occurs at the age 30 years. A wide array of symptoms is often associated with menopause including vaginal dryness, heart palpitations, urinary tract infections, emotional changes and social dysfunction (McKinlay

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and Jeffry, 1998). Hot flushes are the most common objective symptoms of menopause (McKinlay *et al.*, 1987). These are caused by decline in estrogen blood concentrations associated with menopause (Lauritzen, 1973).

There is a close relationship between estrogen deprivation and development of osteoporosis in postmenopausal women. The susceptible women may lead to vertebral, hip and wrist fractures (Lindsay, 1978). Hormone replacement therapy (HRT) is effective in preventing the bone loss and decreasing fracture rate (Lufkin *et al.*, 1992). The principal cause of death in postmenopausal women is cardiovascular disease of which the most common form is the coronary artery disease (Henderson *et al.*, 1986).

It was reported by Ross *et al.* (1981) that estrogen replacement therapy reduces the incidence of cardiovascular disease in postmenopausal women by raising the HDL-cholesterol and lowering the LDL-cholesterol. Estrogen replacement therapy has certain risk factors like endometrial carcinoma in postmenopausal women. Therefore, the addition of progestins either cyclically or continuously reduces the risk of estrogen induced carcinoma (Carr *et al.*, 1998).

Phytoestrogens are the type of plant estrogens which serve as an alternate of hormone replacement therapy. They not only prevent the bone loss but also provide cardioprotective benefits to postmenopausal women (Polkowski, 2000).

Polyacrylamide gel electrophoresis (PAGE) of serum proteins has an important role as diagnostic investigation. Magruiness *et al.* (1993) separated the tubal epithelial proteins (TEP-1 and TEP-2) from tubal mucosa and endometria by one dimensional gel electrophoresis. These two protein bands were present throughout the ovarian cycle but were absent from the tubal mucosa obtained from postmenopausal women.

Tchernof *et al.* (2002) reported that weight loss decreased the plasma C-reactive protein levels in postmenopausal women which may mediate part of its cardioprotective effects in obese postmenopausal women. The diameter of LDL-protein was determined by gradient gel electrophoresis in postmenopausal women. The plasma concentrations of LDL-particles were increased after menopause. Lower levels of endogenous estrogen appeared to cause the size of LDL particles to be reduced (Ikenoue *et al.*, 1999).

The studies regarding the electrophoretic protein profile in response to ovarian hormones pathophysiology are meagre and non-existent. By keeping in view of the importance of proteins in so many physiological phenomena, the present study is planned to investigate the alterations in low molecular weight protein fractions in postmenopausal women sampled, in Lahore.

MATERIALS AND METHODS

Blood samples of naturally menopausal (N=18) and healthy control (N=9) cycling subjects in luteal phase of menstrual cycle were collected at different places in Lahore.

Polyacrylamide gel (14%) for low molecular weight protein fractions was prepared using the method of Laemmli (1970). Serum samples were denatured in loading

dye and diluted with distilled water to prepare the working dilutions for loading on to the gel. Each of the serum samples and low molecular weight protein markers were loaded in separate wells and gel was electrophoresed at a current supply of 30mA and voltage of 200V, in a cooling chamber maintained at 4°C until the dye reached the lower end of the gel. Following electrophoresing run the gels were stained with coomassie blue for one hour with constant agitation and destained afterwards until the clearance of blue background. Protein fractions of different molecular weights were visible in the form of blue bands on a transparent background.

Stained gels were photographed afterwards and their images were saved on a floppy disk with Gene Genius Bio-imaging Gel Documentation System. Quantification of separated protein fractions was carried out by the same system that provided the data of molecular weights and percent volumes covered by each of the fractions.

The data was analyzed statistically using Student t-test and employed in finding the enhancement or reduction and appearance or disappearance of particular protein fractions for comparison among the postmenopausal and the control subjects.

RESULTS

The electrophoretic results in the form of percent raw volumes covered by the resolved low molecular weight serum protein fractions were analyzed in comparable groups categorized as control (subjects 1-9) and postmenopausal (subjects 10-27) groups.

Control group

An overall view of protein profile resulted in the detection of six low molecular weight serum protein fractions ranging between 26-11 kDa (Figs. 1-6) during the luteal phase of normal regular menstrual cycle (subjects 1-9).

Among these, protein fraction of 26 kDa exhibited a dominant expression in all subjects with raw volume percent ranging between 22.75-44.62%.

Protein fraction of 20 kDa was next in expression. The fraction exhibited the raw volume percent ranging between 2.09-5.76%.

The protein fraction of 17 kDa indicated its considerable presence with percent raw volume ranging between 0.58-1.60%.

The protein fraction of 15 kDa was missing in subjects No. 4, 7, 8 and 9, whereas the fraction ranged between 0.19-1.27%, in rest of the subjects of control group.

The 14 kDa fraction which ranged between 0.28-0.70%, in control subjects No. 1, 2, 3 and 5 was also unexpressed in subjects No. 4, 6, 7, 8 and 9.

The last resolved fraction of 11 kDa ranged between 0.66-2.64% and was expressed in all of the subjects of the control group.

Postmenopausal group

The analysis of serum protein profile in the postmenopausal subjects (subjects 10-27) resulted in the detection of six low molecular weight fractions ranging between 26-11 kDa (Figs. 1-6).

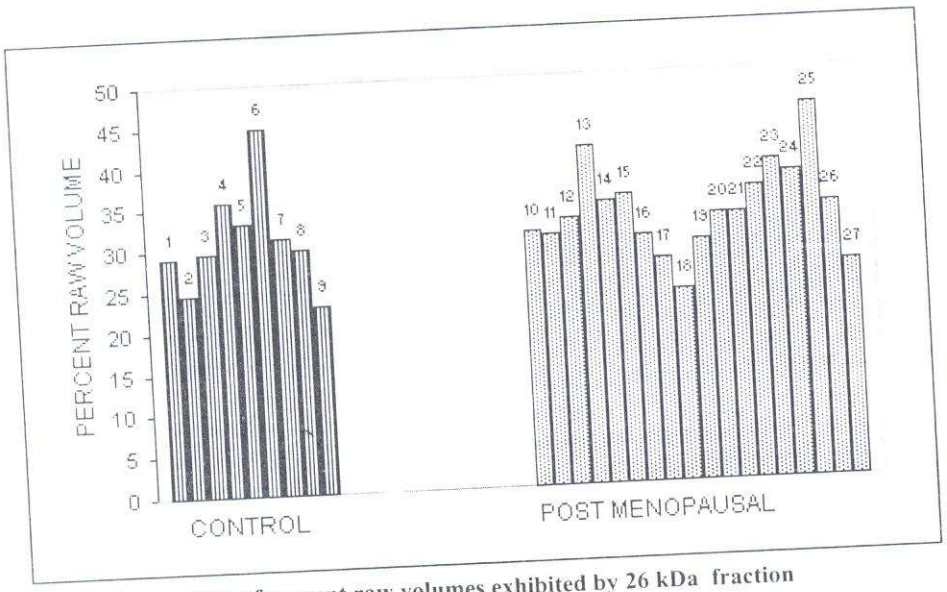

Fig. 1. Comparison of percent raw volumes exhibited by 26 kDa fraction in control (1-9) and post menopausal (10-27) women.

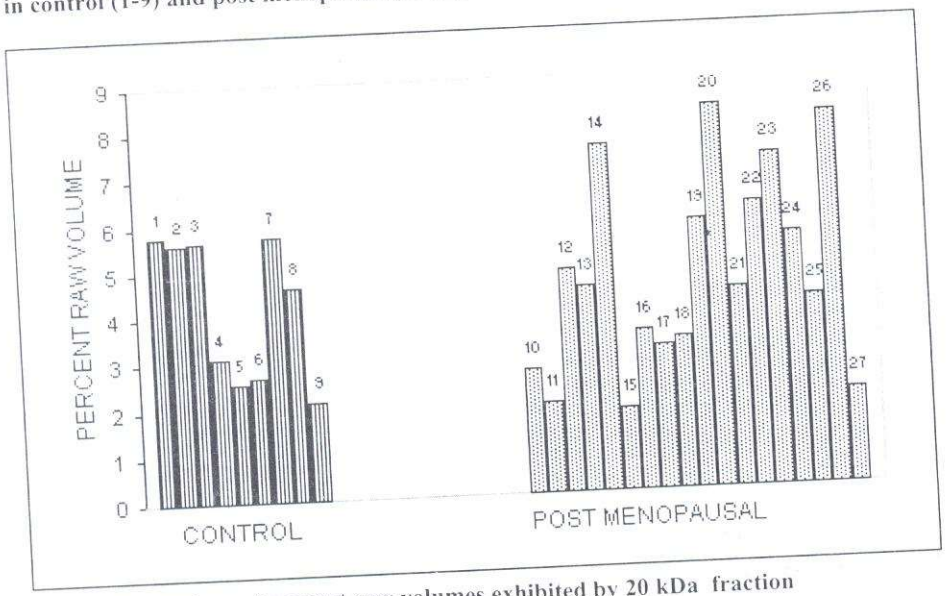

Fig. 2. Comparison of percent raw volumes exhibited by 20 kDa fraction in control (1-9) and post menopausal (10-27) women.

Among these, the dominantly expressed fraction of 26 kDa exhibited the raw volumes percent ranging between 23.36-45.54%, in various subjects of the group, indicating a slight elevation in most of the postmenopausal subjects as compared to the control group (Fig. 1).

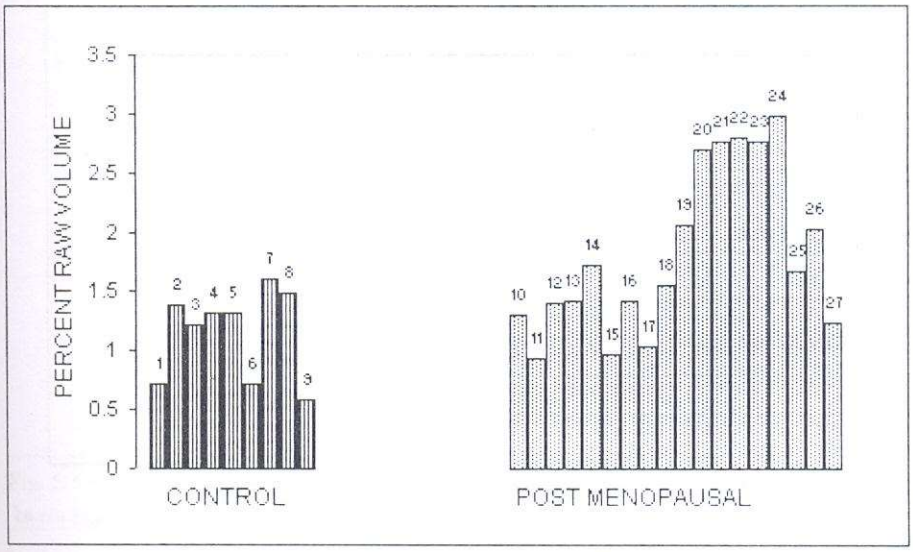

Fig. 3. Comparison of percent raw volumes exhibited by 17 kDa fraction in control (1-9) and post menopausal (10-27) women.

The fraction of 20 kDa was next in expression, ranging from 1.76-8.05%, indicating a significant elevation in postmenopausal subjects No. 14, 20, 22, 23 and 26 as compared to controls. The fraction, however, remained in the range of controls in the rest of the postmenopausal subjects (Fig. 2).

The protein fraction of 17 kDa ranged between 1.04-2.98%, indicating an elevation in postmenopausal subjects No. 14, 19, 20, 21, 22, 23, 24 and 26 as compared to controls. The fraction remained in the control range in rest of the postmenopausal subjects (Fig. 3).

The protein fraction of 15 kDa was unexpressed in postmenopausal subjects No. 11, 13, 16, 18, 19, 20, 21, 22, 24 and 26. However, it was found significantly elevated in subjects No. 12, 17, 23, 25 and 27 as compared to the controls (Fig. 4).

The fraction of 14 kDa appeared only in postmenopausal subjects No. 26 and 27. The fraction was also unexpressed in almost half of the control subjects (Fig. 5).

The last resolved fraction of 11 kDa exhibited its considerable presence in the group with percent raw volumes ranging from 1.00-2.71%. The fraction indicated a non significant elevation in post menopausal compared to control subjects (Fig. 6).

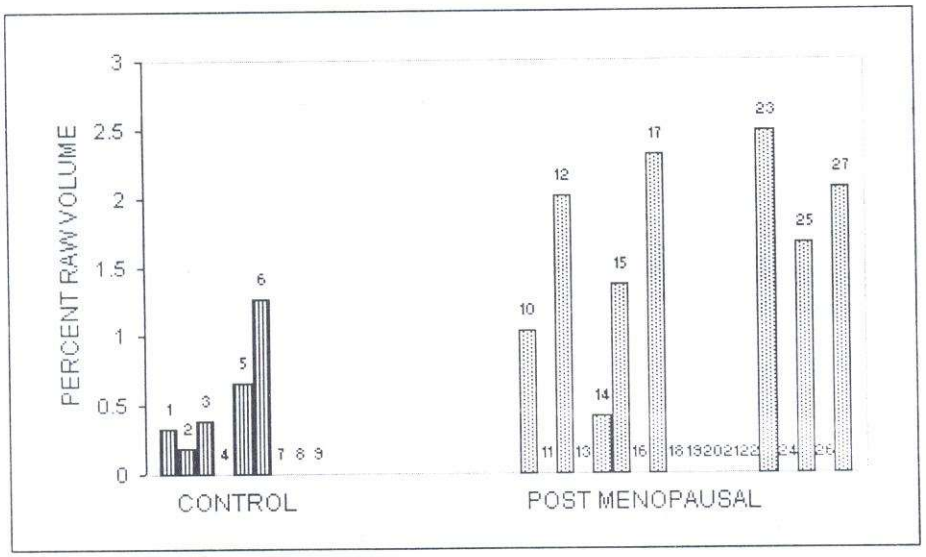

Fig. 4. Comparison of percent raw volumes exhibited by 15 kDa fraction in control (1-9) and post menopausal (10-27) women.

Average group comparison

Average raw volumes covered by 26 kDa were found to be 33.11±1.26% and 31.15±2.13, in postmenopausal and control subjects, respectively. A non significant increase of 6% was, therefore, observed in postmenopausal subjects compared with control subjects.

The 20 kDa protein fraction, with non significant elevation of 12%, exhibited average raw volumes of 4.68±4.98 and 4.19±0.524%, in postmenopausal and control subjects, respectively.

Protein fractions of 17, 15 and 14 kDa showed highly significant elevations of 58%, 198% and 331%, with average raw volumes of 1.82±0.164, 1.67±0.24, 2.15±1.74%, in postmenopausal subjects with average % raw volume of 1.15±0.125, 0.56±0.191, 0.50±0.09 in control subjects, respectively.

A non significant elevation of 7% was noticed in protein fraction of 11 kDa, with average raw volumes of 1.68±0.130 and 1.57±0.195%, in postmenopausal subjects compared with the control subjects (Fig. 7).

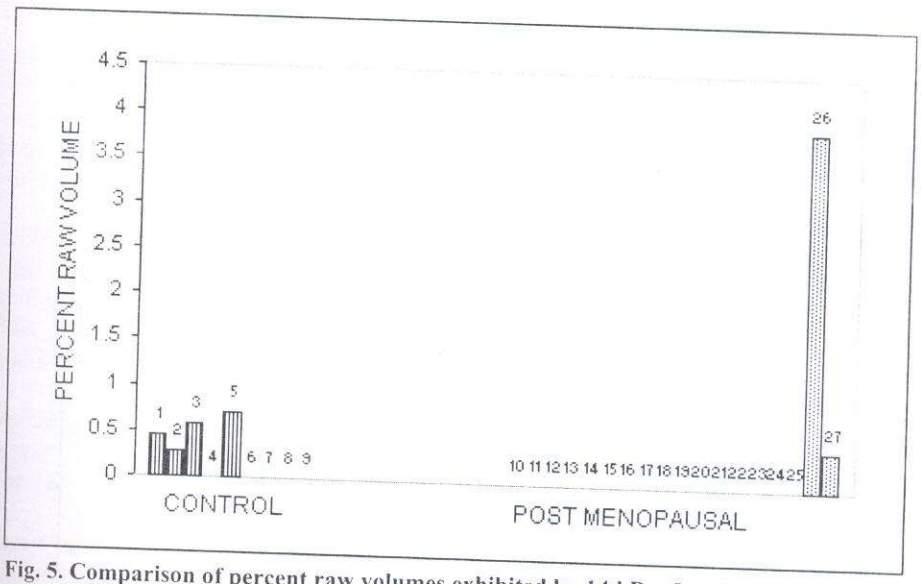

Fig. 5. Comparison of percent raw volumes exhibited by 14 kDa fraction in control (1-9) and post menopausal (10-27) women.

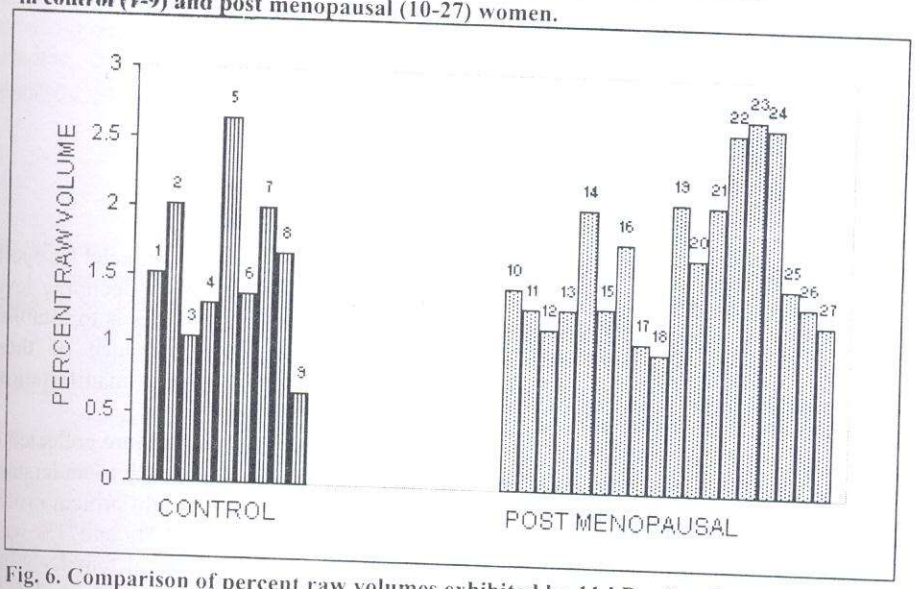

Fig. 6. Comparison of percent raw volumes exhibited by 11 kDa fraction in control (1-9) and post menopausal (10-27) women.

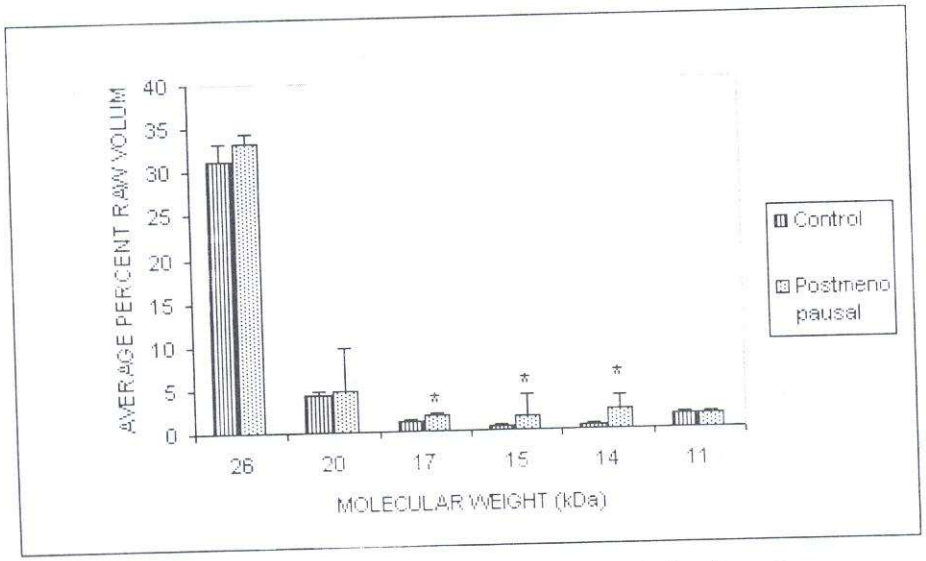

Fig. 7. Average raw volumes (%) exhibited by various protein fractions resolved by SDS-PAGE in control and post menopausal women.

Values are mean±SEM. *** Significance at P<0.05**

DISCUSSION

Menopause begins with the last episode of menstrual bleeding induced by cyclic endogenous secretion of ovarian hormones (Goldfien, 2001). It results in declining level of ovarian hormones specifically the estrogens. The role of these hormones is to establish a physiological homeostasis in cycling pattern. Therefore, disappearance of these hormones brings altered physiological state accompanied by clinical manifestations (Jonathan and Wright, 1996).

In the present study, serum samples of postmenopausal females were collected at different places and have been analyzed for protein fractions in an approach to understand their regulatory role in postmenopausal females. The low molecular weight protein profile was ranging between 26-11 kDa. Non-significant elevations of 6%, 12% and 7% were found in 26 kDa, 20 kDa and 11 kDa protein fractions in postmenopausal females when compared with controls. Similarly, highly significant elevation of 58% was observed in 17 kDa protein fraction, respectively in postmenopausal females when compared with control subjects. The 15 and 14 kDa fractions were non uniformly expressed in control and

postmenopausal subjects, however, on the average, highly significant elevations of 198 and 331% were expressed, respectively, by 15 and 14 kDa protein fractions.

Yildiri *et al.* (2002) has reported elevated levels of homocysteine in postmenopausal women but hormone replacement therapy reduces the plasma homocysteine levels thus providing cardioprotective benefits. Further investigations also revealed that production of interleukin-1 (IL-1), interleukin-6 (IL-6) and tumor necrosis factor-α (TNF-α) increases in estrogen deficient oesteoporotic postmenopausal females (Heiss *et al.*, 1995).

In the present study, apolipoprotein A-II has shown highly significant elevation of 158% in postmenopausal females when compared with controls. This result reflects the significance of 17 kDa protein fraction for its greater appearance in postmenopausal state. Ikenoune *et al.* (1999) have reported that plasma levels of apolipoprotein A-II did not differ significantly between naturally and surgically induced menopausal females. Thus, absence of ovarian hormones probably affects apolipoprotein A-II.

The protein fraction of 14 kDa appeared only in two postmenopausal females. Similarly, 15 kDa protein fraction is unexpressed in half of the postmenopausal subjects. Further investigations, on these protein fractions, may contribute in understanding the molecular mechanisms in postmenopausal females.

The present study is initial or preliminary study regarding the role of low molecular weight protein fractions in menopause. It is assumed that declining levels of ovarian hormones as well as obesity and aging are responsible for alterations in these low molecular weight protein fractions. The clear picture may be seen on further investigation on menopause and its various short and long term symptoms with large population samples. The larger sample population will provide clear situation for the judgments in assessing the protein fractions as marker proteins.

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