Effect of hormone replacement therapy on hemostatic variables in post-menopausal women

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ABSTRACT

Objective: Hormone replacement therapy (HRT) has been increasingly promoted over the last 40 years to improve quality of life, and to reduce the risks of osteoporotic fractures and coronary heart disease (CHD). Recent randomized controlled clinical trials reported that HRT usage is associated with an increased risk of myocardial infarction (MI), stroke, and venous thrombosis. We conducted this study to evaluate the mean levels of some hemostatic parameters among groups that differ in estrogen levels and age.

Methods: We studied 150 healthy women in an observational comparative study, divided into 3 groups. Forty women were post-menopausal using HRT for a period of 6 months to 17 years. Fifty-five women were post-menopausal and were not using HRT. Fifty-five women were younger pre-menopausal women with an age range of 20-54 years. The HRT group women were recruited from gynecologist private clinics while the other 2 groups were recruited in a random way from the society in Damascus, Syria between August 2002 and January 2003. We determined estradiol, fibrinogen, antithrombin III (AT III) and protein C in all women.

Results: When compared with post-menopausal non-users group, current HRT users had higher mean levels of estradiol, but lower mean levels of AT III and protein C, and similar mean levels of fibrinogen. When compared with pre-menopausal group, current users had similar mean levels of estradiol, AT III and protein C, but higher mean levels of fibrinogen. However, post-menopausal non-users women had higher mean levels of fibrinogen and lower mean levels of AT III and protein C when compared with pre-menopausal women.

Conclusion: Hormone replacement therapy treatment did not change fibrinogen mean levels, but it caused a decrease in AT III and protein C mean levels.

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From the endocrine point of view, menopause is considered an estrogen deficiency state. Therefore, hormone or estrogen replacement therapy is regarded as therapy restoring the pre-menopausal endocrine milieu.[1] For many years, observational studies have shown that estrogen therapy is a very effective treatment for vasomotor symptoms,[2] which can begin when hormone levels are still fluctuating.[3] Estrogen can also reduce vaginal dryness and urethritis.[4] When used for a long term, HRT is effective in preventing fractures,[5,6] heart disease,[7] and Alzheimer's disease.[8] Hormone-replacement therapy studies focused on the cardioprotection aspect of estrogen in HRT users,[9-11] while the effects of estrogen administration on the hemostatic system have been less studied.[12] On the other hand, thrombosis results from in-appropriate initiation and propagation of the hemostatic response and may occur as a result of activation of coagulation or inhibition of fibrinolysis.[13] Clinical studies suggest that estrogen has a complex

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effect on the mechanisms underlying thrombosis. This means that although it produces a dose-dependent increase in plasma markers of thrombin and fibrin generation,[14] it also decreases plasma fibrinolytic inhibitory activity.[15] Recently, published randomized controlled trials show no evidence of benefit of HRT in women with established vascular disease or in apparently healthy women. The increased risks of breast cancer and thromboembolic disease have been confirmed in the above mentioned controlled trials.[16,17] This suggests that the risks of HRT outweigh the benefits.[18] Rossouw et al[17] made some comments as a response to these trials, particularly with regard to the relevance of the Women's Health Initiative (WHI) study results to the traditional use of HRT at the beginning of menopause, since the age distribution and late start of HRT in the women in the WHI study do not correspond to the traditional use of HRT.[19] Our study contributes in clearing the effect of estrogens on hemostatic parameters in a group of women using HRT and compared it with post-menopausal non-HRT users and pre-meno-pause women groups.

Methods. One hundred and fifty healthy women were studied in an observational comparative study, and they were divided into 3 groups. Forty women were post-menopausal using HRT (continuous combined HRT with 2 mg of the natural human estrogen 17ß-estradiol with 1 mg of norethisterone acetate [NETA]). All women were using this preparation for a period of 6 months to 17 years. Fifty-five women were post-menopausal and were not using HRT. The 2 groups were matched for age (mean: 57.23±6.5 versus 52.5±6.2 years) and the menopausal age was more than one year in each (mean: 4.85±4.6 versus 9±5.3 years). Fifty-five women were younger pre-menopausal women with an age range of 20-54 years and their estradiol level was >40 pg/ml. Inclusion criteria for all subjects were: apparently healthy women by medical examination, non-smoker and non-alcoholic. Exclusion criteria were: presence of medical history of heart disease, vascular disease, usage of hormonal drugs (other than HRT) and the usage of anticoagulation drugs. Inclusion criteria for post-menopausal women: menopause duration of >1 year and 17ß-estradiol levels was <40 pg/ml. Inclusion criteria for HRT users group: usage duration of >6 months and the preparation used was 2 mg of 17ß-estradiol + 1 mg NETA.

The HRT group of women were recruited from gynecologist private clinics while the other 2 groups were recruited in a random way from the society. Written informed consent was obtained for each subject. Body mass index (BMI) was calculated as weight (kg) divided by height (m) squared. The pre-menopausal women were sampled on day 10 of their menstrual cycle to avoid the effect of mensual changes of estradiol on hemostatic parameters. All subjects were sampled after an overnight fast. Seven ml of blood was collected by venepuncture into plain tubes (2.5 ml) for estradiol determination, and in trisodium citrate (4.5 ml- final concentration 0. 11 M) tubes for the determination of hemostatic factors. Serum and plasma were harvested for 10 minutes by high speed centrifugation (2500 g). Aliquots of serum and plasma were stored immediately at -80°c until analysis.

All laboratory tests were carried out in the Pharmacy College, Damascus University. 17ß-estradiol was determined by radio immuno assay-ria assay (Coat-a-Count, DPC, USA). The assay sensitivity expressed as the detection limit was 8 pg/ml. The antiserum of the assay is highly specific for 17ß-estradiol with a relatively low cross-reactivity to other naturally occurring steroids. Fibrinogen was determined by a kinetic method on Hitachi 911 apparatus (Roche diagnostics GmbH, Mannheim, Germany). Antithrombin III (AT III) and protein C were determined by a chromogenic method on Hitachi 911 apparatus (Roche diagnostics GmbH, Mannheim, Germany).

Results were expressed as mean ± SD and range. Statistical calculations were performed by using Stat-Soft Statistica version 6.0 system. Correlation studies were based on Pearson's (r) coefficient. Inter-group variations were evaluated by student's t-test. Significance was accepted at a level of probability of $p<0.05$. Multiple regression analysis was performed to define independent factors which are influencing hemostatic parameters.

Results. 17ß-estradiol levels varied among the 3 groups. The mean value in HRT users group was 155.5 ± 95 pg/ml, while it was 9.43 ±12 pg/ml in the post-menopausal group who were not using HRT. This value raised to 143.6 ± 47 pg/ml in the pre-menopausal group who are from a younger age (**Table 1**). Therefore, the difference was significant between post-menopausal women and HRT women while there was no significant difference between HRT women and pre-menopausal women (**Table 1**). In hemostatic parameters, there was no significant difference in fibrinogen levels between the HRT users group and the post-menopausal non-users group ($p=0.49$), but the fibrinogen mean levels in HRT users group was significantly higher than its value in pre-menopausal group ($p=0.000129$). We found also a significant difference in fibrinogen levels between the second and the third group (non-HRT users and pre-menopausal) ($p=0.000238$) (**Table 1**). Mean values of AT III increased significantly in HRT users group compared with post-menopausal non-HRT users ($p=0.0032$). We found no significant difference in AT III levels between the HRT users group and the pre-menopausal group ($p=0.915$) (**Table 1**). There was also a significant difference in AT III levels between the non-users group and pre-

Table 1 - Characteristics of selected subject.

Variables	HRT users group (n=40)		Non-users group (n=55)		Pre-menopausal group (n=55)	
	Mean±SD	Range	Mean±SD	Range	Mean±SD	Range
Age (years)	52.5 ± 6.2	42 - 73	57.3 ± 6.5	38 - 70	33.4 ± 7.5	20 - 54
Weight (kg)	66 ± 9.5	56.5 - 75	69.4 ± 11	48 - 100	64.8 ± 10	45 - 97
Height (cm)	159 ± 5.5	166 - 154	157 ± 5.6	140 - 170	161 ± 6.7	148 - 175
Body mass index (kg/m^2)	26.10 ± 5.5	20.5 - 37	27.9 ± 4	19.4 - 38.2	25.8 ± 6.9	17.7 - 38.5
Menopause duration(years)	4.9 ± 4.6	1 - 18	9 ± 5.3	1- 20		
Usage duration (years)	2.75 ± 3.3	0.5 - 17				
Fibrinogen 150-450 (mg/dl)	385.5 ± 108	178 - 706	370 ± 125	349 - 637	249.2 ± 92.5	120 - 350
Antithrombin III 80-120%	90.67 ± 12.7	69.8 - 117.4	99.75 ± 10.6	78.8 - 122	90.29 ± 11	66.1 - 118
Protein C 70-140%	96.15 ± 23	63.8 - 185	111 ± 26.12	66.6 - 181	95.22 ± 21	64.1 - 163
Estradiol pg/ml	155.5 ± 95	26.3 - 388.9	9.43 ± 12	0 - 35	143.6 ± 47	76.5 - 251

Table 2 - Simple regression correlation among compounding variables.

Variables	Duration of HRT			Year since the onset of menopause			Body mass index			Estradiol level		
	r^2	ß	*p*-value	r^2	ß	*p*-value	r^2	ß	*p*-value	r2	ß	*p*-value
Age	0.064	0.253	0.002	0.593	0.771	0.00	0.132	0.367	0.000	0.128	-0.36	0.000
Duration of HRT				0.053	0.232	0.004	0.003	-0.06	0.453	0.067	0.259	0.001
Duration of menopause							0.063	0.252	0.002	0.178	-0.42	0.000
Body mass index										0.029	-0.17	0.000

r^2 - coeffecient of determination, ß - estimated parameters, HRT - hormone replacement therapy

Table 3 - Multiple regression analysis for antithrombin III, fibrogen and protein C.

Variables	Antithrombin III			Fibrinogen			Protein C		
	r^2	ß	*p*-value	r^2	ß	*p*-value	r^2	ß	*p*-value
Duration of hormone replacement therapy	0.0759	-0.06			0.266			-0.04	
Body mass index		0.141	0.0119	0.0846	0.072	0.0060	0.0938	0.294	0.0031
Estradiol		-0.19			-0.17			-0.03	

r^2 - coeffecient of determination, ß - estimated parameters, HRT - hormone replacement therapy

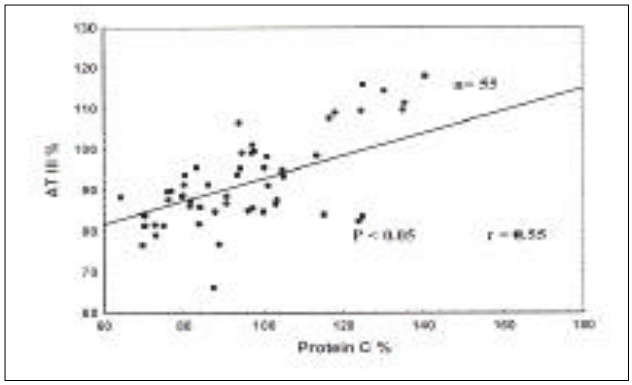

Figure 1 - Correlation between protein C and antithrombin III (AT III) in pre-menopausal group.

menopausal group (p=0.0004). Protein C mean values increased significantly in post menopausal non-users group compared with HRT users group (p=0.0075). There was no significant difference in protein C levels between HRT users and pre-menopausal women (p=0.851) (**Table 1**).

Protein C mean values in post-menopausal non-users group was significantly higher than its value of pre-menopause group (p=0.0012). **Table 1** shows the characteristics of the 3 groups. Associations between hemostatic parameters within each group were studied. We found a weak statistically significant correlation between protein C and fibrinogen in HRT users group (r = 0.32, p<0.05). We also found a moderate significant correlation between protein C and AT III (r=0.32, p<0.05). A similar but stronger correlation was found between protein C and AT III in pre-menopausal women (r=0.55, p<0.05) (**Figure 1**). Since there were several variables contributing in the differences among the studied groups, we considered a multiple regression analysis to define the variable, which is the most influencing hemostatic parameters. As a beginning, we performed a simple regression correlation to investigate whether the compounding variables are independent or not. **Table 2** presents the findings of this analysis. As a result of this analysis we found several correlations between the variables. So, to deal with the multi-collinearity among the above mentioned variables we selected a subset of them to avoid repetition of variables of the same nature. 17ß-estradiol was the strongest independent variable for either AT III and fibrinogen. Body mass index was found to have a strong effect on protein C, while duration of HRT has a strong effect on fibrinogen. **Table 3** presents the finding of the multiple regression analysis of the selected variables.

Discussion. The main finding of this observational study is that HRT users group had increased plasma levels of fibrinogen (compared with pre-menopausal women), an effect which is due to age- and decreased plasma levels of AT III and protein C levels (compared with post-menopausal non-users women). Hormone-replacement therapy users group and post-menopausal non-users group had almost similar mean ages (52.5 ± 6.7 years and 57.23 ± 6.5 years). This eliminates the effect of age on hemostatic parameters. Measurements of estradiol values in the 3 groups revealed that there was a convergence in estradiol levels between the HRT users group (155.5±95 pg/ml) and the pre-menopausal women group (143.6±47 pg/ml) (p=0.528). This enabled us to focus on the effect of age on hemostatic parameters when comparing these 2 groups and to focus on hormone effect on hemostatic parameters when comparing HRT users with postmenopausal non-users women. Fibrinogen is the major determinant of plasma viscosity and induces platelet aggregation. High plasma fibrinogen precipitate the event of thrombosis. In this study, we did not observe an alteration in fibrinogen levels between the HRT users group and the post-menopausal non-users group (p=0.49). However, we found a significant difference in fibrinogen levels between HRT users and pre-menopausal women (p=0.000129), and between post-menopausal non-users and pre-menopausal women (p=0.000238). We also found that 8 women out of 40 women in HRT users group (20%) had fibrinogen levels over the upper limit of the reference range, while there were 16 women out of 55 women in post-menopausal non-users group (29.9%) had fibrinogen levels over this value. In contrast, there were only 3 women in the pre-menopause group (5.2%) who had fibrinogen levels over the upper limit. This suggests that fibrinogen increase was due to age and not to HRT usage. In cross-sectional studies,[20] there was a negative association between estradiol and plasma concentration of fibrinogen. In a longitudinal study, however, HRT for 6 months did not reduce the level of plasma fibrinogen.[21,22] This is in agreement with our study, but in other studies achieved this reduction.[23-27] This finding is not in agreement with Perry et al[28] reports that HRT therapy causes elevation in fibrinogen levels in addition to elevation in other hemostatic parameters. Deficiencies of AT III and protein C predispose individuals to venous thrombosis, but high levels may reflect a response to thrombogenesis.[29] In this study, current users of hormones had lower levels of AT III and protein C than non-users. We found a statistically significant decrease in AT III in HRT users group in comparison with post-menopausal non-users group (p=0.0032), while no significant difference was found between HRT users and pre-menopausal non-users women (p=0.915). However, there was a significant increase in AT III levels in post-menopausal when compared with pre-menopausal women (p=0.0004). When gathered, these findings suggest that AT III decrease is due to

the effect of estrogen and not age. This is a well-known effect of estrogen on AT III but it varies between significant to mild decrease.[30] Twenty percent of HRT users group had AT III levels lower than reference range (8 of 40 women) versus 3.4% of post-menopausal non-users (2 of 55 women), while 9.1% of pre-menopausal women (5 of 55 women) had this value. These ratios support the idea that estrogen lowers AT III levels. The association between the use of hormones and protein C levels mirrored that between hormones and AT III levels. Protein C levels in HRT users were significantly lower than those of post-menopausal non-users (p=0.0045), while there was no difference between HRT users and pre-menopausal group (p=0.851), accordingly, levels in pre-menopausal women were significantly lower than post-menopausal women (p=0.0012). This suggests that estrogen has a lowering effect on protein C levels. This is in agreement with Perry et al[28] study and Lobo et al[24] study. Other studies refer to unchanged protein C values,[29] or to elevation in protein C values.[31] The correlation found in this study between protein C and AT III either in HRT group or in pre-menopausal women is concordant with low values of the 2 parameters in HRT group and in pre-menopausal group. These low values become extremely important in the presence of other risk factors for thrombosis including high fibrinogen levels, genetic deficiency of either AT III or protein C, other genetic disorder such as V-Leiden mutation,[32] prothrombin mutation, surgery, obesity and others. The findings of multiple regression analysis summarize our results as follows: 17-ß estradiol has a strong and independent influence on the studied hemostatic parameters, but there are other compounding variables which also affect these parameters such as BMI and age. Most of HRT women in our study had continued their education beyond high school, did not smoke, were not obese, and moderately practiced sport. Although an observational study may be biased by unknown selection factors influencing the use of HRT, and may reflect differences between hormone users and non-users, rather than the effects of the hormones themselves, we had to carry out this study as an observational comparative study and not a longitudinal prospective one. This was carried out for several reasons, it was more convenient than following the same group of women before and after HRT usage. Even if it was prescribed by a specialist, most women do not adhere to HRT therapy as they are concerned on the side effects of HRT usage in long-term therapy such as bloating, weight gain, vaginal bleeding, breast tenderness and others. So, the short-term treatment is not enough to monitor HRT effects on hemostatic parameters. On the contrary, this will be misleading. This made us comparing different groups to get larger number of HRT users, and to reflex the affirmative situation of hemostatic parameters in long-term HRT users group. This preliminary study, however, shows the need for long-term prospective trials of large numbers of patients to reflect the correct situation of hemostatic parameters in elderly women in general, and in HRT users women. Specially younger and healthy ones who really need this kind of treatment to improve their lives.

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