

**A STUDY TO INVESTIGATE THE EFFECT OF HORMONE  
REPLACEMENT THERAPY (HRT) ON PLATELET ACTIVATION  
MARKERS (CD62P & PAC-1) IN HEALTHY POSTMENOPAUSAL  
WOMEN.**

**A PROSPECTIVE CASE CONTROL STUDY**

**By**

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**A STUDY TO INVESTIGATE THE EFFECT OF HORMONE REPLACEMENT THERAPY (HRT) ON PLATELET ACTIVATION MARKERS (CD62P & PAC-1) DETERMINED BY FLOW CYTOMETRY IN HEALTHY POSTMENOPAUSAL WOMEN.**

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**Introduction:** In healthy postmenopausal women increased platelet activation has been associated with the adverse cardiovascular events including unstable angina, myocardial infarction, stroke and other thrombotic states. There is much debate about the relationship between platelet function and serum estradiol levels in such postmenopausal women. It is postulated that estrogen may result in decreased platelet activation.

**Objectives:** The aim of the study was to determine the effect of HRT on the platelet activation markers (CD62P & PAC-1) in healthy postmenopausal women and to determine the correlation between platelet activation markers and serum estradiol, BMI and Age of postmenopausal women.

**Patients and Methods:** A prospective case control study on 48 postmenopausal women was conducted at Hospital Universiti Sains Malaysia (HUSM). Group-A consisted of 48 women NOT on HRT (Control Group) and Group-B comprised of same 48 women who were given HRT (Conjugated equine estrogen 0.625 mg orally once daily) for two weeks (Study group). Platelet activation was evaluated at baseline and after two weeks of treatment by flow cytometric analysis using CD62P and PAC1 as activation markers. Comparisons within groups (before and after HRT) were analyzed using the paired *t*-tests.

**Results:** The expressions of CD62P and PAC1 showed a decreased platelet activation status in postmenopausal women who were given HRT. Platelet activation markers (CD62P & PAC-1) among healthy postmenopausal women in the group-A were 7.00%  $\pm$ 5.91 (CD62P) and 41.75%  $\pm$ 26.85(PAC-1) respectively (increased platelet activation in this group) which were reduced to 3.05%  $\pm$ 2.47 (CD62P) and 20.86%  $\pm$ 19.02 (PAC-1) respectively in the group-B after two weeks of HRT administration (p-value <0.001).

**Conclusion:** HRT decreases the platelet activation markers (CD62P & PAC-1) in healthy post menopausal women. Platelet activation markers (CD62P & PAC-1) are found to be increased in healthy postmenopausal women as compared to the postmenopausal women who were treated with HRT. There is a significant negative fair correlation between estradiol and platelet activation markers (CD62P & PAC-1) however; there was no significant relation among BMI, age and platelet activation markers.

It is concluded that short term use of HRT has a favorable effect on reduction of platelet activity in post-menopausal women and thus it is postulated to be cardio protective. Further study on the long term effect of HRT on platelets is needed.

Professor Dr.Nik Mohamed Zaki Nik Mahmood: Supervisor

Associate professor Shah Reza Johan Noor: Co-Supervisor

Dr.Tariq Mahmood Roshan: Co-Supervisor

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## **Presentations, Publications and awards.**

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*A study to investigate the effect of hormone replacement therapy (HRT) on platelet activation markers (CD62P & PAC-1) determined by flow cytometry in healthy postmenopausal women.*

**Presented by:** Dr. Shabbir Ahmad Sheikh.

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1. Rosline H, Shah Reza JN, Adzah AM, Rapiaah M, Abdul Aziz B, Shabbir AS, W Soriany WMZ, Tariq M Roshan.

*Cardio-protective effect on postmenopausal women of HRT based on platelet activation markers.*

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### List of Abbreviations

<b>5-HT</b>	5-hydroxy tryptamine
<b>ADP</b>	Adenosine Diphosphate
<b>ATP</b>	Adenosine Triphosphate
<b>BMI</b>	Body mass Index
<b>Ca<sup>2+</sup></b>	Calcium
<b>cAMP</b>	Cyclic Adenosine Monophosphate
<b>CD</b>	Cluster Of Differentiation
<b>CEE</b>	Conjugated Equine Estrogen
<b>cGMP</b>	Cyclic Guanosine Monophosphate
<b>CRF</b>	Case Report Form
<b>CVD</b>	Cardiovascular Disease
<b>DM</b>	Diabetes Mellitus
<b>EDTA</b>	Ethylenediaminetetra acetic Acid
<b>ELISA</b>	Enzyme Linked Immunosorbant Assay
<b>eNOS</b>	Endothelial Nitric Oxide Synthase
<b>ER</b>	Estrogen Receptors.

<b>ERT</b>	Estrogen Replacement Therapy
<b>FBC</b>	Full Blood Count
<b>FBS</b>	Fasting Blood Sugar
<b>FTTC</b>	Fluorescein Isothiocyanate
<b>FSH</b>	Follicle Stimulating Hormone
<b>GP</b>	Glycoproteins
<b>GP-V</b>	Glycoprotein-V
<b>HDL</b>	High Density Lipoproteins
<b>HERS</b>	Heart and Estrogen Replacement Study
<b>HLA</b>	Human Leukocyte Antigen
<b>HPLC</b>	High Pressure Liquid Chromatography
<b>HRT</b>	Hormone Replacement Therapy
<b>HUSM</b>	Hospital Universiti Sains Malaysia
<b>IHD</b>	Ischemic Heart Disease
<b>IL1</b>	Interleukin 1
<b>LDL</b>	Low Density Lipoproteins
<b>LFT</b>	Liver Function Test
<b>LH</b>	Leuteinizing Hormone

<b>MI</b>	Myocardial Infarction
<b>MPA</b>	Medroxy progesterone Acetate
<b>NO</b>	Nitric Oxide
<b>NOS</b>	Nitric Oxide Synthase
<b>NSAID</b>	Non-steroidal anti-inflammatory drugs
<b>O&amp;G</b>	Obstetrics and Gynaecology
<b>OD</b>	Once Daily
<b>PAF</b>	Platelet Activation Factor
<b>PAI</b>	Plasminogen Activator Inhibitor
<b>PDGF</b>	Platelet Derived Growth Factor
<b>PE</b>	Phycoerythrin
<b>PECAM 1</b>	Platelet-Endothelial Cell Adhesion Molecule
<b>PF4</b>	Platelet Factor 4
<b>PGI<sub>2</sub></b>	Prostacyclin
<b>PRP</b>	Platelet Rich Plasma
<b>RFT</b>	Renal Function Test
<b>SD</b>	Standard deviation
<b>TNF</b>	Tissue Necrosis Factor

<b>tPA</b>	Tissue type Plasminogen Activator
<b>TxA2</b>	Thromboxane A2
<b>VNTR</b>	Variable Number Tandem Repeat
<b>vWF</b>	von Willebrand Factor
<b>VLA</b>	Very Late Antigen



## **ABSTRACT**

### **A STUDY TO INVESTIGATE THE EFFECT OF HORMONE REPLACEMENT THERAPY (HRT) ON PLATELET ACTIVATION MARKERS (CD62P & PAC-1) DETERMINED BY FLOW CYTOMETRY IN HEALTHY POSTMENOPAUSAL WOMEN.**

#### **Objectives:**

The aim of the study was to determine the effect of HRT on the platelet activation markers (CD62P & PAC-1) in healthy postmenopausal women. In such women platelet activation has been associated with the adverse cardiovascular events including unstable angina, myocardial infarction, stroke and other thrombotic states. There is much debate about the relationship between platelet function and serum estradiol levels including concerns about the influence of low estradiol in menopausal women. It is postulated that estrogen may result in decreased platelet activation.

#### **Methodology:**

A prospective case control study on 48 postmenopausal women was conducted at Hospital USM. Group-A consisted of 48 women NOT on HRT (Control Group) and Group-B comprised of same 48 women who were given HRT (Conjugated equine estrogen 0.625 mg orally once daily) for two weeks (Study group). Platelet activation was evaluated at baseline and after two weeks of treatment by flow cytometric analysis using CD62P and PAC1 as activation markers. Comparisons within groups (before and after HRT) were analyzed using the paired *t*-tests.

### **Results:**

The expressions of CD62P and PAC1 showed a decreased platelet activation status in postmenopausal women who were given HRT. Platelet activation markers (CD62P & PAC-1) among healthy postmenopausal women in the group-A were 7.00%  $\pm$ 5.91 (CD62P) and 41.75%  $\pm$ 26.85(PAC-1) respectively (increased platelet activation in this group) which were reduced to 3.05%  $\pm$ 2.47 (CD62P) and 20.86%  $\pm$ 19.02 (PAC-1) respectively in the group-B after two weeks of HRT administration (p-value <0.001).

### **Conclusion:**

HRT decreases the platelet activation markers (CD62P & PAC-1) in healthy post menopausal women. Platelet activation markers (CD62P & PAC-1) are found to be increased in healthy postmenopausal women as compared to the postmenopausal women who were treated with HRT. There is a significant negative fair correlation between estradiol and platelet activation markers (CD62P & PAC-1) however; there was no significant relation among BMI, age and platelet activation markers.

It is concluded that short term use of HRT has a favorable effect on reduction of platelet activity in post-menopausal women and thus it is postulated to be cardio protective. Further study on the long term effect of HRT on platelets is needed.

## **ABSTRAK**

### **KAJIAN UNTUK MENGENAL PASTI KESAN TERAPI HORMON KEATAS PETANDA (MARKERS) AKTIVASI PLATELET MELALUI KAEDAH “FLOW CYTOMETRY” KEATAS WANITA PUTUS HAID YANG SIHAT.**

#### **Objektif:**

Tujuan kajian ini adalah untuk menentukan kesan terapi hormon terhadap petanda aktivasi platelet di kalangan wanita yang telah putus haid. Ini kerana aktiviti pengaktifan platelet dikaitkan dengan kesan negatif terhadap kardiovaskular termasuk ketidakstabilan angina, sakit jantung, angin ahmar dan pembekuan saluran darah. Terdapat banyak kontroversi berkaitan dengan fungsi platelet dan tahap serum estradiol termasuk pengaruh serum estradiol yang rendah dikalangan wanita yang telah putus haid. Ramai yang berpendapat oestrogen mungkin mengurangkan fungsi pengaktifan platelet.

#### **Kaedah:**

Kajian secara prospektif kontrol telah dijalankan melibatkan 48 wanita yang telah putus haid di Hospital USM. Kumpulan A merangkumi 48 peserta yang tidak diberikan terapi hormon (kumpulan control) dan Kumpulan B melibatkan 48 orang peserta yang sama yang diberikan terapi hormon (Conjugated equine estrogen 0.625mg-satu kali sehari) untuk 2 minggu (Kumpulan Kajian). Pengaktifan platelet telah dianalisa pada permulaan kajian dan selepas 2 minggu rawatan menggunakan terapi hormon dengan menggunakan kaedah “flowcytometry” untuk CD62P dan PAC1 sebagai pengaktifan markers. Perbandingan diantara kumpulan (sebelum dan selepas terapi hormon) dianalisa menggunakan ujian-*t*.

**Keputusan:**

Penghasilan CD62P dan PAC1 menunjukkan pengurangan status pengaktifan platelet di kalangan wanita yang telah putus haid yang telah diberikan terapi hormon. CD62P dan PAC1 di kalangan wanita yang telah putus haid pada kumpulan kontrol telah mengurang secara signifikan dari  $7.00\% \pm 5.91$  kepada  $3.05\% \pm 2.47$  dan  $41.75\% \pm 26.85$  kepada  $20.86\% \pm 19.02$  selepas diberikan terapi hormon (kadar-p < 0.001).

**Kesimpulan:**

Terapi hormon menurunkan petanda aktivasi platelet (CD62P & PAC1) di kalangan wanita sihat yang telah putus haid. Petanda aktivasi platelet (CD62P & PAC1) meningkat di kalangan wanita putus haid yang sihat berbanding dengan wanita yang telah putus haid yang diberikan terapi hormon. Terdapat “korelasi signifikan secara negatif” diantara estradiol dan petanda aktivasi platelet, walau bagaimanapun tidak terdapat kaitan yang signifikan terhadap indeks isipadu tubuh (BMI), umur dan petanda aktivasi platelet.

Kesimpulannya, penggunaan terapi hormon secara sementara memberikan kesan positif terhadap pengurangan aktiviti platelet di kalangan wanita yang telah putus haid dan ini mungkin memberikan kesan positif terhadap kardiovaskular. Kajian yang lebih lanjut diperlukan untuk menentukan kesan jangka panjang terapi hormon terhadap platelet.