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% ±5.91 (CD62P) and 41.75% ±26.85(PAC-1) respectively (increased platelet activation in this group) which were reduced to 3.05% ±2.47 (CD62P) and 20.86% ±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.

Awards

Best oral presentation award for the second candidate, 19th Congress of Obstetrical & Gynecological Society of Malaysia (OGSM), 4-7th June, 2009, Kota kinabalu, Sabah, Malaysia.

Presentations

Oral presentation

Shabbir AS, Roshan TM, Shah Reza JN, Rosline H, Khatak MN, Zaki NM.

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.

Venue: 19th Congress of Obstetrical & Gynecological Society of Malaysia, held in Kota kinabalu, Sabah, Malaysia.

Date: 4-7th June, 2009.

Poster presentations.

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.

   Venue: 8Th Annual Scientific meeting & Annual general meeting of the college of pathologists, Academy of medicine Malaysia, held in Renaissance Hotel, Kota Bharu, Kelantan, Malaysia.

   Date: 6-7 June, 2009.

2. Rosline H, Shah Reza JN, Rapiaah M, Shabbir AS**,** Adzah AM, Tariq M Roshan.

   ***Evidence of platelet activation by flowcytometry following Hormone replacement therapy in Postmenopausal women*.**

   Published in abstract book in Journal of Clinical Cytometry, in affiliation with European Society for clinical cell analysis, Vol 76B:6, 400-401, 2009.

   Venue: 24th clinical Autometry meeting & course, Florida, United States of America.

   Date: 16-21st October, 2009.

Publication.

This study was published in "Menopause International" which is the official Journal of the British Menopause Society. Menopause International is abstracted or indexed by Medline/Pub Med and EMBASE.

Title:

Short term estrogen replacement therapy reduces platelet marker levels in Malaysian postmenopausal women.

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List of contents

Contents **Page no**

CHAPTER NO. 1

CHAPTER NO. 2

CHAPTER NO. 3

CHAPTER NO. 4

CHAPTER NO. 5

CHAPTER NO. 6

List of Tables

Tables **Page no**

List of Figures

List of Abbreviations

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

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

MI	Myocardial Infarction
MPA	Medroxy progesterone Acetate
NO	Nitric Oxide
NOS	Nitric Oxide Synthase
NSAID	Non-steroidal anti-inflammatory drugs
O&G	Obstetrics and Gynaecology
OD	Once Daily
PAF	Platelet Activation Factor
PAI	Plasminogen Activator Inhibitor
PDGF	Platelet Derived Growth Factor
PE	Phycoerythrin
PECAM 1	Platelet-Endothelial Cell Adhesion Molecule
PF4	Platelet Factor 4
PGI2	Prostacyclin
PRP	Platelet Rich Plasma
RFT	Renal Function Test
SD	Standard deviation
TNF	Tissue Necrosis Factor

tPA Tissue type Plasminogen Activator

TxA2 Thromboxane A2

VNTR Variable Number Tandem Repeat

vWF von Willebrand Factor

VLA 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.

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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 menunjukan pengurangan status pengaktifan platelet di kalangan wanita yang telah putus haid yang telah diberikan terapi hormon. CD62P dan PAC1 dikalangan wanita yang telah putus haid pada kumpulan kontrol telah mengurang secara signifikan dari 7.00% ±5.91 kepada 3.05% ±2.47 dan 41.75% ± 26.85 kepada 20.86% ±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 kardiovascular. Kajian yang lebih lanjut diperlukan untuk menentukan kesan jangka panjang terapi hormon terhadap platelet.